Scientia

IMPROVING HEALTH ACROSS THE GLOBE: INNOVATIONS FOR A NEW DECADE

EXCLUSIVES:

- **Association of Medical Research Charities**
- **Sparks Children's Research Charity**

HIGHLIGHTS:

- **Unlocking the Sweet Secrets of the Microbiota**
- **Bile Acids Are Not Just for Digestion**
- **A New Approach to Tuberculosis Treatment**
- **Innovative Strategies for Imaging Cancers**

Issue: #128

Do you want to become a regular **READER OF SCIENTIA?**

Scientia's mission is to connect people: scientists and educators, policy-makers and researchers, and the public and private sectors. We communicate science into the wider world, providing **high-quality, engaging,** and **relevant information** to our audience of thinkers and explorers.

Register today for your **FREE** subscription at:

www.scientia.global/subscribe/

WELCOME...

We open 2020 and this critical issue of Scientia by celebrating a diverse range of scientific breakthroughs and achievements that are driving forward health and well-being worldwide. In this new decade, we will undoubtedly bear witness to the promise of medical care that is more efficient, effective and patient-centred than ever before. Thus, it is with excitement that we showcase the work of researchers who are challenging and changing the ways in which we diagnose, treat, and even prevent disease.

In our first section, we meet the researchers who embrace a basic science approach to understanding how biological processes impact on health and disease. Far from the pursuit of science in the metaphorical ivory towers, we read how this laboratory-based research holds important theory-driven implications for improving human health.

In our second section of this issue, we showcase technological and methodological advancements that are transforming the diagnosis and treatment of disease. We read how innovations ranging from medical imaging to facial reconstructive surgery hold real potential for improving patient care as they progress through the journey from novel innovation to clinical practice. We conclude the section by meeting Aisling Burnand, Chief Executive Officer of the Association of Medical Research Charities in our exclusive interview. We read of their important work in supporting UK-based research as well of the current challenges facing medicine and health care.

Recent advances in drug development are the focus of our third section. We read of the translation of innovation in the laboratory to the clinic from a pharmaceutical perspective and discover how serious diseases including diabetic retinopathy, drug-resistant tuberculosis, and chronic kidney disease are being targeted across the world in the latest research. In our exclusive interview, we meet Kiki Syrad, Director of Grants at Sparks Children's Medical Research Charity, and read about their vital work in transforming the care of children with rare and potentially fatal diseases.

In our fourth section, we meet the researchers who are committed to challenging cancer in its numerous forms. From the benefits of a whole food diet as a preventative intervention to techniques to minimise side effects of routine cancer treatment, we read about diverse and multi-faceted approaches with the shared goal of overcoming this ever-increasing global threat.

In our fifth and last section, we meet the researchers who take their scientific pursuits outside the laboratory and clinic by exploring health and well-being in society more broadly. This final section provides a pertinent reminder of the importance of science in the real world, and the implications for health and well-being from societal, public health, and individual perspectives.

Science Diffusion

CONTACT

Published in the UK, by Science Diffusion ltd

ISSN 2059-8971 (print)
ISSN 2059-898X (online)

E: info@sciencediffusion.com
W: www.sciencediffusion.com
W: www.scientia.global

@scientia_social
www.facebook.com/socialscientia
www.linkedin.com/company-beta/11065635

Meet The Team...

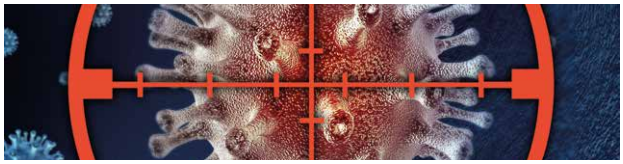
CONTENTS

ISSUE : #128

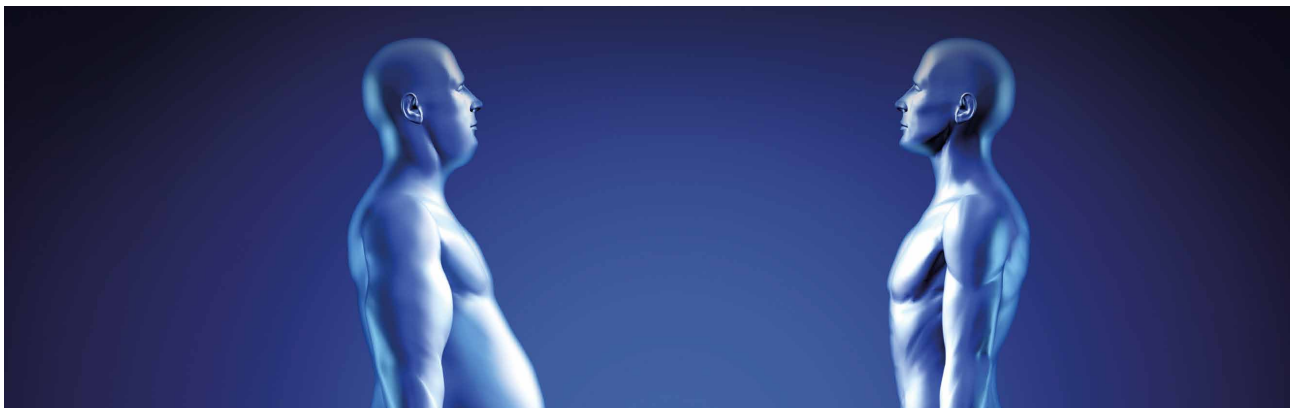
BASIC MECHANISMS

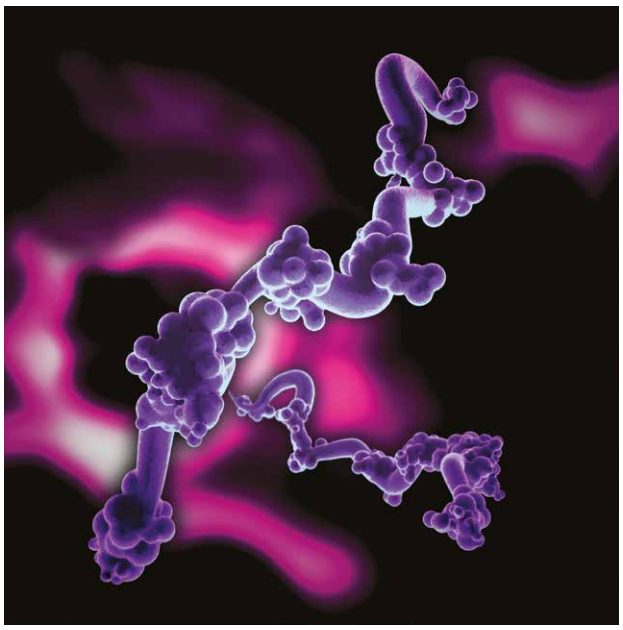
TECHNOLOGICAL AND METHODOLOGICAL ADVANCES

INNOVATION IN TREATING DISEASE



ADVANCES IN CANCER RESEARCH





HEALTHCARE, SCIENCE AND SOCIETY

BASIC MECHANISMS

CRITICAL ADVANCEMENTS IN THE BASIC SCIENCES

The basic sciences underpin progress in healthcare and medicine. With a focus on understanding the intricacies of biological processes, such efforts are often driven by scientific curiosity. But from understanding how cells communicate with their environment to the function of microorganisms in the body, advancements in basic science are also often the first step in the development of successful interventions for disease. We open this exciting issue of Scientia by showcasing the critical work of researchers in the basic sciences, highlighting the broader implications for improving healthcare and medicine.

We open this section with the work of Professor Jeffrey Becker at the University of Tennessee, who explores how cells, the basic building blocks of life, communicate with their external environment. Cells are fundamental to many physiological processes, and the malfunctioning of cells is associated with numerous diseases. We discover how by using yeast as a model, Professor Becker has advanced both theory and methodology in his field.

We then turn to the work of Professor Thomas Bosch at the Christian-Albrechts-University of Kiel. Professor Bosch takes a basic science approach to his investigation of the multi-organismic nature of life. We read how by using the tiny freshwater organisms known as hydra as a model, Professor Bosch is illuminating our understanding of how the complex interplay of genetics and physiology both mediates and is mediated by the body's microbiome, and how these processes are related to disease.

The alternative oxidase, the enzyme that provides an alternative route for molecular oxygen in the pathways of energy production, is the key focus of research for Professor Anthony Moore and his team at the University of Sussex. By investigating the structure and function of the alternative oxidase enzyme in plants, fungi, and parasites, Professor Moore's work is confirming the potential of the alternative oxidase enzyme to provide therapeutic support for a number of health conditions.

We also meet Professor Regina Fluhrer and her team at the University of Ludwig Maximilians. This team studies the function and mechanisms of the specialist enzymes known as intramembrane proteases to better understand their role in health as well as disease, including Alzheimer's disease, malaria, hepatitis C infections and B (immune) cell deficiency. We learn how Professor Fluhrer is laying the foundation for the development of future drug targets applicable to a wide range of diseases.

We then turn to the research by Professor Lloyd Kasper (conducted while at the Geisel School of Medicine, Dartmouth College), Javier Ochoa-Repáraz at Eastern Washington University, and Dr Javier Ochoa-Repáraz at Symbiotix Biotherapies. Their work has demonstrated that the microbes living within the gut contain and release compounds such as a specific bacterial sugar that can regulate the development and function of our immune system. Critically, their findings may lead to developments in the treatment of multiple sclerosis and other immune-related diseases such as inflammatory bowel disease.

Also working on understanding the importance of the gut for our health are Professor Phillip Hylemon and Professor Huiping Zhou from the Medical College of Virginia, Virginia Commonwealth University. Their work has demonstrated that bile acids do much more than simply aid digestion, as was the dominant view until recently. Rather, bile acids are now known to regulate complex biological pathways and we conclude this section by considering the important implications of this work for the treatment of various diseases, including cancer.

A NEW METHOD TO UNDERSTAND CELL COMMUNICATION

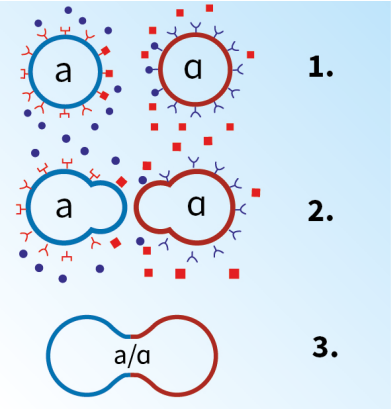
The question of how cells communicate with their environment has long fascinated scientists. Typically, cells receive information from the outside through a group of proteins known as membrane receptors. For many years, these receptors have been the focus of research for **Professor Jeffrey Becker**, a microbiologist based at the University of Tennessee. His aim is not only to understand the secrets behind this type of communication, but also to develop new and better methods to study the mechanisms involved in this process.

Being able to understand the outside world is one of the most fundamental requirements for every cell. The most common way they do this is through special proteins – called receptors – placed in the membrane, which receive the outside signal and pass the message on to the appropriate channels inside the cell. Through these interactions between receptor and signal, the cell can respond and adapt to different triggers, including light, hormones, and many others; but crucially without the need to let foreign compounds enter inside.

The fact that there are almost 1,000 different forms of the largest family of receptors (G protein-coupled receptors) in human cells is testament to importance of cell communication. Although they may perform very different functions they share a very similar structure: one end stuck out of the cell followed by seven loops in the membrane itself and finally the other end pointing inside the cell. They are present in a variety of tissues and organs, and regulate numerous physiological mechanisms, including vision, smell and taste, the heart, nervous system, and even reproduction.

Not surprisingly then, malfunction in human cell receptor activity is linked with many diseases, including obesity, blindness, cancer, schizophrenia, Alzheimer's disease, hypertension, and diabetes. They are already an attractive target for drug therapy, with an estimated 30–50% of current medications targeting these receptors, but a better understanding of their contributions to various diseases could go a long way to treat many other conditions.

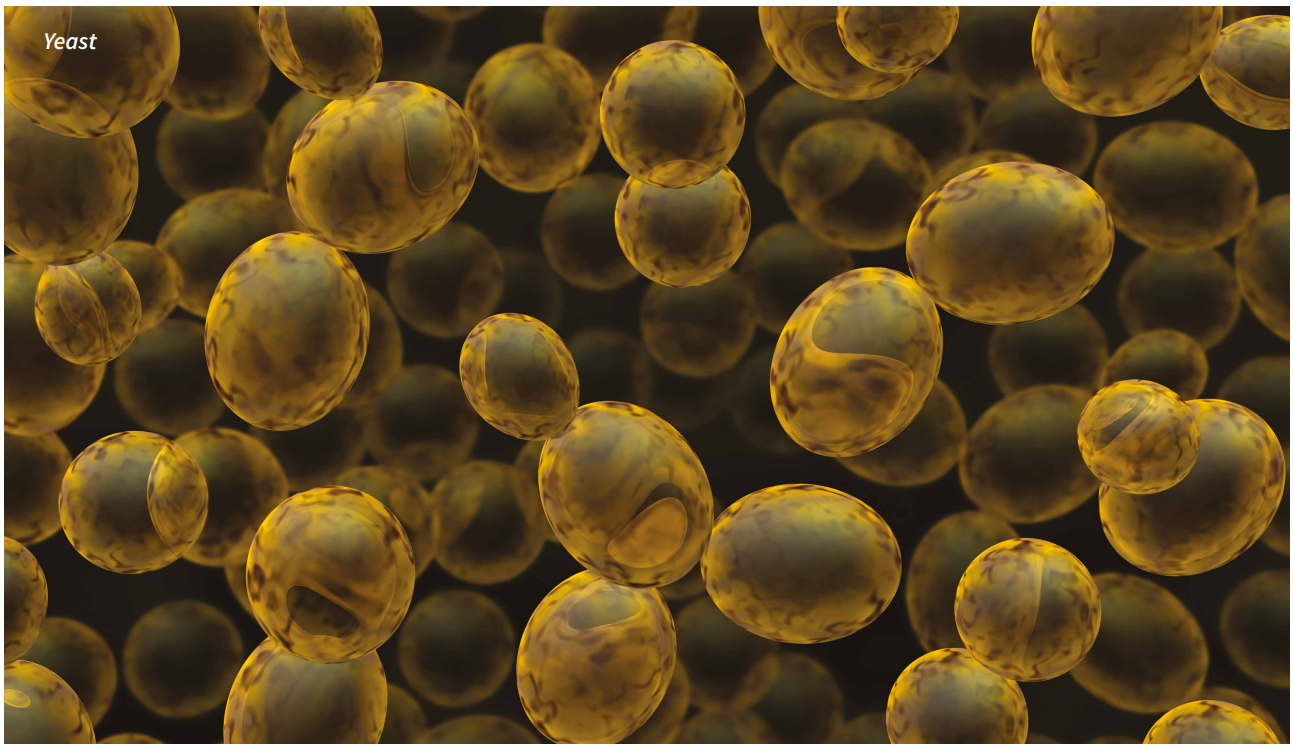
The problem is that the mechanisms regulating the activity of these receptors are still not fully understood, mostly due to limitations in the methods used. Studying fast interactions involving minute amounts of receptor and signal is an incredibly difficult task. It has been the prime objective of Professor Jeffrey Becker, at the University of Tennessee, to develop more accurate ways to measure and study these mechanisms, which will in turn allow for a deeper understanding of the interactions between receptors and signals.



Yeast Mating

Using Yeast as a Model

To really grasp how these receptors work during signalling, researchers need to track their interactions with incoming messages. There has been much progress in the past few decades about the structure of receptors, but methods to follow interactions in live cells as they happen in real-time are very tricky to develop.
Professor Becker and his team have used a receptor from yeast as a model in all their research. Given the well-characterised genome and availability of strong genetic tools, yeast provides an ideal system for this type of research. Although yeast and mammals are very



Yeast

different organisms, these receptors work in very much the same way. Researchers have been able to use mammalian receptors in yeast and vice-versa and, irrespective of combination, these compounds are able to maintain normal functions. This means that any research progress achieved with yeast will most likely also apply to mammalian cells.

Using a multitude of approaches over the course of several years, Professor Becker and his team have made many important discoveries regarding the mechanisms behind these receptors. Examples include the identification of spots that interact with incoming signals, areas involved in transmitting the signal to inside the cell, and a region curbing excessive receptor activity. The researchers also identified several changes in the shape of the receptor as it receives a signal, including how an interaction spot becomes available to an incoming message. These findings clearly suggest that receptors are heavily involved in different functions, from receiving external messages to fine tuning the signalling process.

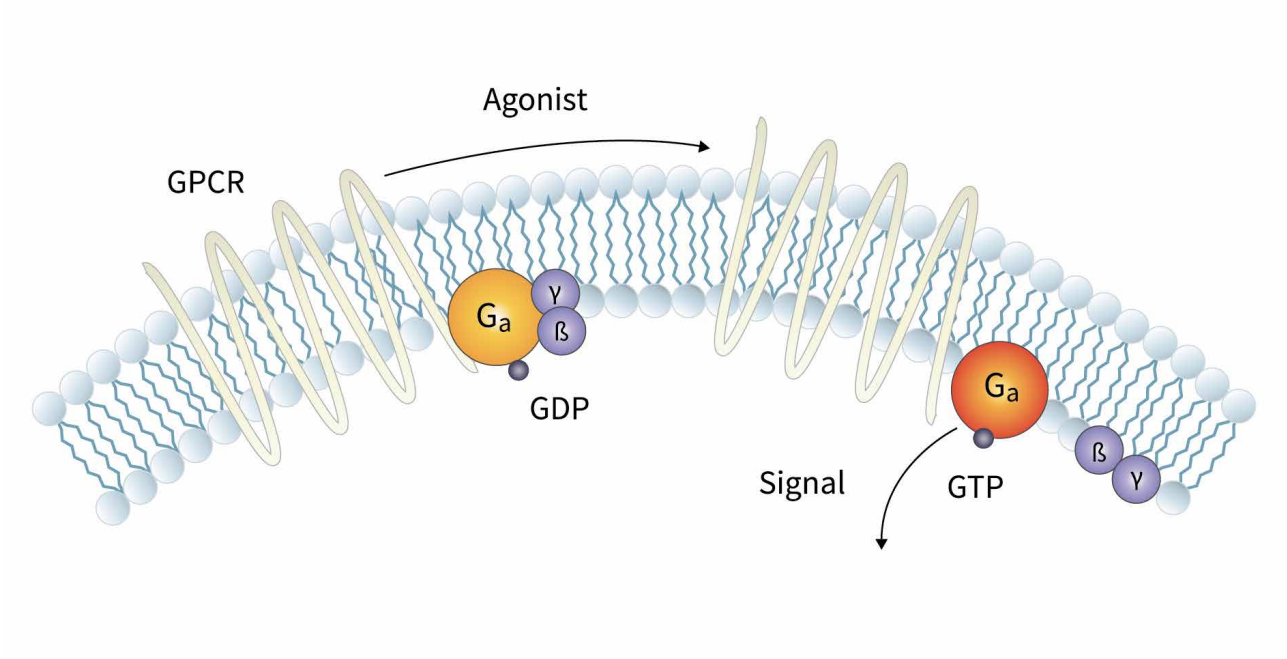
Unnatural Amino Acids

While the techniques used so far to study receptor signalling have allowed considerable progress, the methods typically used have some disadvantages. A major problem is that proteins interacting with the receptor may be misidentified according to the major technique of antibody co-precipitation used. Another problematic technique to identify interacting proteins involving the use of chemical cross-linking may also lead to artefacts resulting from the inability to control cross-linking during the signalling process.

In an attempt to solve these issues, Professor Becker and his team are now developing a faster and potentially more reliable way to measure such receptor/signal interactions. This has been an ongoing endeavour – their work on a rapid laser-activated approach to measure signalling in live cells started back in 2015.

At that stage, the team had just started using a method pioneered elsewhere for yeast which relies on the ability of cells to use all amino acids available to them, including amino acids not found naturally. Crucially, these unnatural amino acids can be synthesised to contain a variety of tags to facilitate detection, such as fluorescent areas or highly reactive groups.

After a few adjustments to the original method developed by Professor Peter Schulz at the Scripps Institute, Professor Becker and his team were the first to demonstrate that unnatural amino acids can be incorporated into these receptors in the yeast cell and still retain function. ‘To our knowledge, this is the first report of the expression in the native host cell of a receptor containing [an unnatural] amino acid’ wrote Professor Becker in 2008. ‘It shows that receptors containing unnatural amino acids can be used to capture ligand and study changes in domain-domain interactions during receptor activation’. Ten years on, Professor Becker and his team are still continuing to work on ways to extend this method. Their current mission is to upgrade the old technique by using UV-laser short pulses to detect fast interactions. To test this approach, Professor Becker and his team used an unnatural amino acid and a protein derived from blood as experimental guinea-pigs in place of real signals and receptors. The researchers



Activation of the Receptor Pathway by the Alpha-Factor Agonist

saw that a ligand containing the unnatural amino acid working as a signal was hydrophobic (meaning it doesn't like water) and so could only bind to similarly hydrophobic areas in the blood-derived protein. At this stage there is no reason to suggest that interactions between real signals and receptors will be any different, which means that Professor Becker's new method will be equally valid in testing cell signalling mechanisms.

Professor Becker further explained in 2017, 'We believe the methodology can be extended to capture protein interactions in a live cell using unnatural amino acid incorporation into proteins in the native state. The ability to capture such interactions in the living cell in a very short time using a laser should allow real-time analysis of protein interactions of intracellular pathways and processes.'

Looking to the Future

Given the encouraging results of this preliminary work, Professor Becker's next goal is to apply this technique to their yeast model. The team hopes this method will provide more accurate snapshots of the interactions between different signals and the receptor at specific times before, during, and after the signalling mechanism.

Crucially, this method offers what others cannot: cross-linking reactions on a time scale of seconds rather than minutes. This is essential to find transient protein interactions in living cells, and it is hoped that the short time period for the laser pulses will limit any artefactual trapping of proteins which are not relevant.

The Importance of Innovation and Collaboration

Professor Becker's overarching aim is to develop a methodology to measure signals associated with the receptor both during the inactive state (that is, while not receiving a message) and the active state (receiving a message), which can be applied not only to study yeast receptors, but also more generally to all species – including humans.

This innovative work will, for the first time, make it possible to see if and how a receptor changes during the few seconds of the signalling process and detect where and when each signal interacts with the receptor. This will greatly improve our understanding of how signalling is regulated. It is not unreasonable to suggest that this technique could be successfully applied to important receptors in mammalian cells, and that the knowledge of where signals interact could provide new targets for drug design leading to novel and effective therapies. Critically, the long-term plan is to adapt the method to human cells and conduct vital studies to better understand how these receptors work.

This impressive body of research underscores the undoubtable power of collaboration in science. With this in mind, our article concludes with a note of thanks from Professor Becker to his long-term collaborator, Professor Fred Naider, at City University in New York: 'It has been my extraordinary privilege to have had a perfect collaborator, Professor Fred Naider for most of my research career. Professor Naider and I have a beautiful synergy merging our interests in chemistry (Fred) and biology (me).'

Meet the researcher

Professor Jeffrey Becker
Department of Microbiology
University of Tennessee
Knoxville, TN
USA

After obtaining his doctorate at the University of Cincinnati in 1970 and then completing postdoctoral research for two years, Professor Jeffrey Becker moved to the Department of Microbiology at the University of Tennessee. Currently, he holds the position of Chancellor's Professor Emeritus at the same university and his main area of research is the structure and function of peptides and their receptors, membrane transport, and medical mycology. Throughout his career, Professor Becker has received multiple awards and academic honours, been a highly-sought consultant and panellist, and has been a fellow of the American Association for the Advancement of Sciences since 2008. Professor Becker has received continuous funding from National Institutes of Health for over 40 years and has published over 250 peer-reviewed papers. In addition to teaching, Professor Becker has supervised 200 undergraduate students, 35 doctoral students, and 8 postdoctoral fellows to date, highlighting his commitment to research and the development of scientists in this field.

CONTACT

E: jbecker@utk.edu
W: http://web.bio.utk.edu/becker/

KEY COLLABORATORS

Fred Naider, College of Staten Island, City University of New York
Melinda Hauser, University of Tennessee

FUNDING

National Institutes of Health
American Cancer Society
National Science Foundation
U.S.-Israel Binational Science Foundation

FURTHER READING

M Hauser, C Qian, ST King, S Kauffman, F Naider, RL Hettich, JM Becker, Identification of peptide-binding sites within BSA using rapid, laser-induced covalent cross-linking combined with high-performance mass spectrometry, Journal of Molecular Recognition, 2017, 31, e2680.

MS Uddin, F Naider, JM Becker, Dynamic roles for the N-terminus of the yeast G protein-coupled receptor Ste2p, Biomembranes, 2017, 1859, 2058–2067.

MS Uddin, M Hauser, F Naider, J Becker, The N-terminus of the yeast G protein-coupled receptor Ste2p plays critical roles in surface expression, signalling and negative regulation, Biochimica et Biophysica Acta, 2016, 1858, 715–724.

GK Umanah, LY Huang, JM Maccarone, F Naider, JM Becker, Changes in conformation at the cytoplasmic ends of the fifth and sixth transmembrane helices of a yeast G protein-coupled receptor in response to ligand binding, Biochemistry, 2011, 50, 6841–6854.

GK Umanah, L Huang, FX Ding, B Arshava, AR Farley, AJ Link, F Naider, JM Becker, Identification of residue-to-residue contact between a peptide ligand and its G protein-coupled receptor using periodate-mediated dihydroxyphanylalanine cross-linking and mass spectrometry, Journal of Biological Chemistry, 2010, 285, 39425–39436.

LY Huang, G Umanah, M Hauser, Son C, B Arshava, F Naider, JM Becker, Unnatural amino acid replacement in a yeast G protein-coupled receptor in its native environment, Biochemistry, 2008, 47, 5638–5648.

THE UNIVERSITY OF TENNESSEE KNOXVILLE

THE METAORGANISM: THE MICROBIOME AND ITS HOST

A human body is host to a multitude of microorganisms, without which we would not be able to survive. As such, humans, and all other organisms are truly metaorganisms composed of a host and a complex microbiome. **Professor Thomas Bosch** at the Zoological Institute at the Christian-Albrechts-University of Kiel, Germany, studies the complex interactions which take place within metaorganisms between host cells and microbes.

For most of the history of medicine, the human body has been regarded as a solitary entity. While it has been known for centuries that we harbour many microorganisms in and on our bodies, for the majority of this time these microbes have been regarded as pathogens at worst and insignificant hitchhikers at best.

However, over the past decade researchers have begun to realise that we, along with all plants and animals, share a long co-evolutionary history with our microbes. Far from parasites, they play a vital role in our health and wellbeing – they are written into our genome and we could not experience a normal life without them.

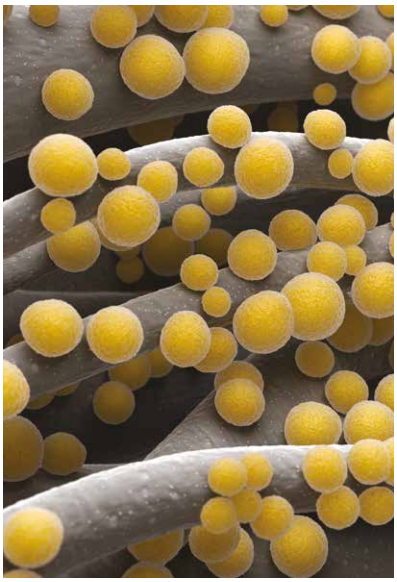
There are no solitary organisms, instead, every plant and animal on Earth is a metaorganism. A metaorganism is a host and its complete microbial community is commonly referred to as the microbiome. Over half the cells in a healthy human body are not human at all but belong to the multitude of species that compose our microbiome.

We now know that our bodies have evolved to utilise these microbes in critical aspects of our biology, from digestion to immune function, to mental health. We are just beginning to scratch the surface of understanding what it means to be a metaorganism and how microbial relationships shape biology as a whole. Professor Thomas Bosch at the Christian-Albrechts-University of Kiel, in Germany, is an evolutionary developmental biologist and pioneer of metaorganism research seeking to shine a light on these relationships.

Exploring a Model Metaorganism

While scientists are beginning to appreciate the powerful contributions of the microbiome to human health and biology and are beginning to form an understanding of the species of microbes that are commonly associated with good health, it is difficult to study microbial relationships in detail in humans.

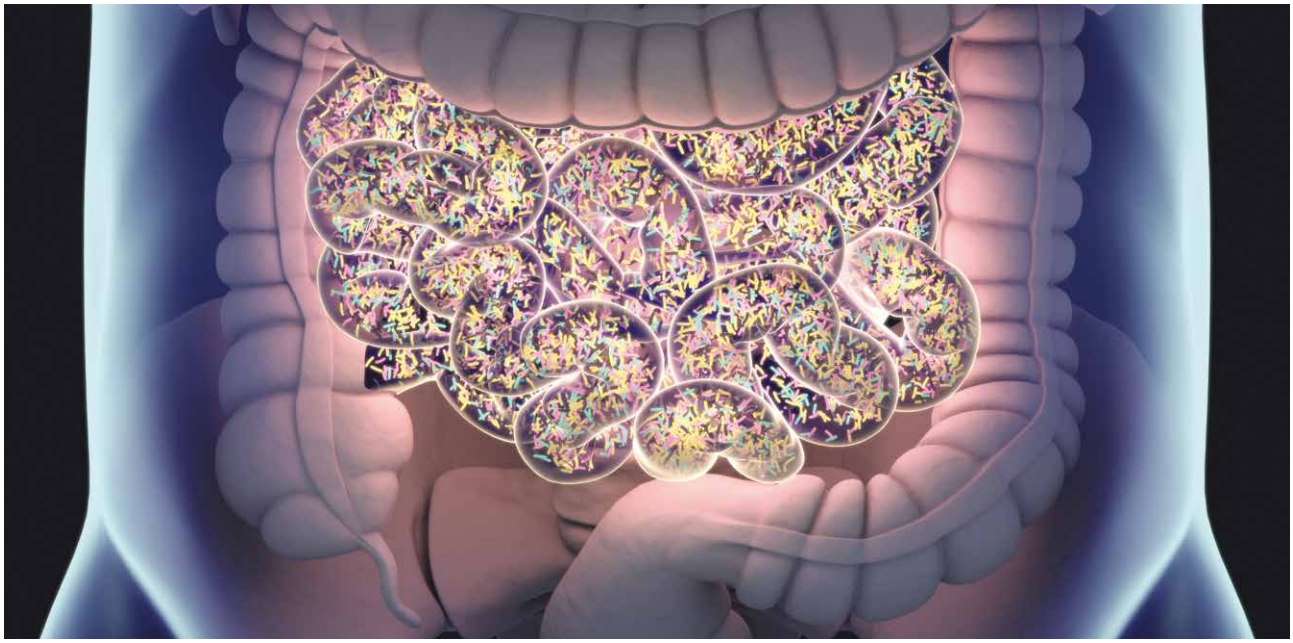
The vast array of human diets, lifestyles, and living conditions, combined with our long lifespan and the inability to monitor a person 24 hours a day, all



make it near impossible to perform experiments with human subjects that can accurately tease out the effects of a specific microbe, let alone a complex community of microbes.

However, getting a handle on these interactions is critical for the progression of modern medicine. Professor Bosch notes that, ‘disturbing the microbial interactions within metaorganisms is one of the major causes of many “modern” and chronic

‘My research is focused on understanding the multi-organismic nature of life. All organisms are metaorganisms. Disturbing the interactions within metaorganisms is one of the major causes of many “modern” and chronic diseases.’



diseases.’ In order to develop a high-resolution understanding of the evolutionary history of metaorganisms and the health implications of our microbial relationships, it helps to study them in smaller, more simple creatures.

The phylum *Cnidaria*, whose members include jellyfish, sea anemones, and coral, also contains a tiny freshwater organism called Hydra. These little creatures, less than 15 millimetres in length, resemble tiny sea anemones and are easy to raise in large numbers in a laboratory. Hydra are a perfect model system for exploring fascinating questions about interactions between hosts and microbes.

Their biology is simple enough to control for a multitude of environmental factors like diet and living conditions and complex enough to display highly evolved connections with their microbes. Further, they have an unlimited lifespan, showing an ability for self-renewal that is unparalleled in the animal kingdom. Interestingly, the gene that regulates this process is also deeply tied to the Hydra’s relationship with its microbiome. Professor Bosch and his team utilise these small aquatic animals in their research to illuminate how evolution has shaped host-microbe relationships to form metaorganisms.

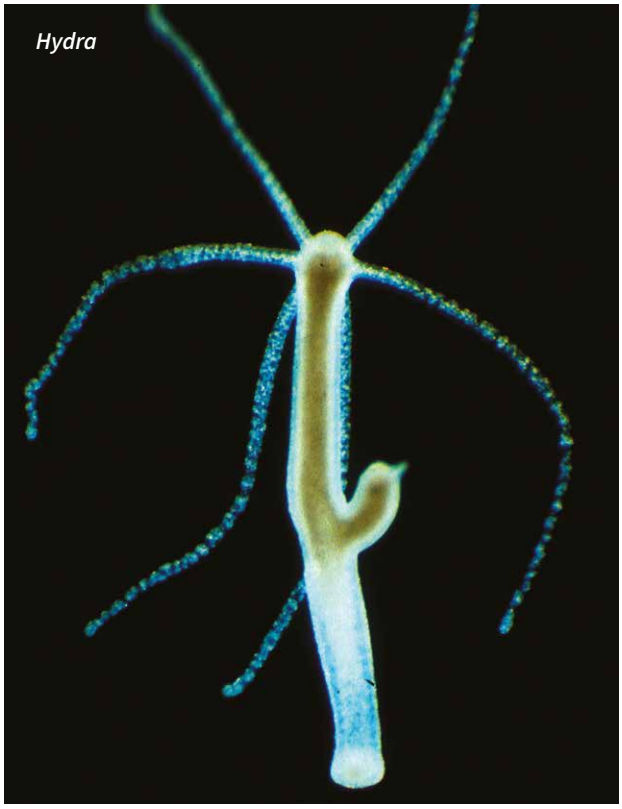
The Immortal Hydra

Hydra initially drew scientists’ attention as a target for research efforts due to a unique characteristic – immortality. These tiny organisms do not age and can live for an unlimited amount of time, thanks to special cells called stem cells with the ability to proliferate indefinitely. Naturally, researchers have been curious to determine the mechanisms underlying this regenerative ability and understand how they relate to mechanisms of ageing in more complex animals, like humans.

Genes contain the instructions for building proteins – when a gene is expressed, the sequence of that gene is copied from DNA to an RNA template that will be used in a protein’s construction. All of the cells in your body have the same DNA, but most of their genes have been locked down so that only the genes relevant to the function of a cell are expressed. Stem cells are cells where gene expression is not locked down yet, such that they still have the ability to become multiple other cell types.

Professor Bosch and his team set out to determine which gene (or genes) in Hydra conferred immortality. Hydra have three types of stem cells that are each able to continuously regenerate into different tissue types but otherwise, have unique roles and gene expression profiles. Since all three share immortal properties, Professor Bosch suspected that the gene of interest would be one that was expressed in all three lines. To test this, the team collected the RNA templates that were present in each of the stem cell types and sequenced them to determine which genes were being expressed. Once they ruled out genes that were not expressed in all the stem cell lines, a single candidate emerged: *FoxO*.

To test the finding further, the team manipulated *FoxO* expression and confirmed that when its expression was high, stem cells proliferated and when its expression was low, cells lost the ability to regenerate. This was an


Hydra

exciting finding as *FoxO* variants in insects alter their lifespan and mutations in the human version of *FoxO* have been found in humans that live past 100.

This manipulation also revealed another critical finding – the *FoxO* gene is tightly associated with the Hydra's immune system and appeared to regulate how the Hydra interacts with its microbes. It has been shown that altering animals' microbiomes can extend life and it may be that the Hydra's microbiome also plays a role in its immortality.

The Hydra Shapes its Microbiome

How the microbiome is regulated is a still largely a biological mystery. We know that organisms depend on their microbes to complete a large number of functions and that there must be constant communication between the host and its microbial communities to maintain this relationship. However, the mechanisms of this communication are not yet known. Professor Bosch became interested in understanding how Hydra manage their relationship with their microbes beyond *FoxO*.

Organisms like animals and humans are constantly coming in contact with new microbes through both their skin and their gut, yet healthy organisms maintain a fairly consistent composition of microbial species on these surfaces. It is not known how this balance is maintained. Numerous studies have demonstrated that microbes interact with the nervous system through the exchange of neuropeptides, compounds that communicate with nerve cells or neurons. Professor Bosch observed that loss of neurons led to changes in bacterial composition and predicted that Hydra's neuropeptides may play a role in shaping which microbes live on its skin and in its gut.

They observed young Hydra as they grew and discovered that as the nervous system developed, the bacteria on the hatchling's body changed. Further, some species of bacteria were relocated to specific parts of the young Hydra's body. They found that the growing neurons were releasing neuropeptides with antimicrobial properties that were specific to certain species of bacteria.
Different types of neurons released different neuropeptides, so that microbe species were relegated to different areas of the body. For example, a bacterium that was prevented from growing on the Hydra's trunk was allowed to grow abundantly on its tentacles. Amazingly, the Hydra's nervous system communicates with its skin microbiome to determine how microbes are distributed.

The Microbiome Shapes its Hydra

In the human body, many of our organs must contract regularly to maintain normal function. Though we have specialised pacemaker cells that keep things moving, it has been found that people with disorders that affect the contraction of their gut also frequently have abnormal gut microbiota. Though the Hydra's gut is much less complex than a human gut, it does contract spontaneously at regular intervals.

Professor Bosch and colleagues sought to understand how altering the Hydra's gut microbiome might impact these contractions. To do this, they treated a group of Hydra with antibiotics that cleared the animal's gut bacteria. The antibiotic-treated Hydra showed a striking reduction in gut contractions, despite no apparent changes in the ability of the gut cells to contract.

When the same animals were re-exposed to their normal gut bacteria and their gut microbiome was re-established, normal contractions resumed. Interestingly, recolonising the Hydra with only one species of gut bacteria alone did not improve contractions and recolonising them with a mix of bacteria that was different from their original microbiome only partially restored gut movement. Hydra gut activity appears to depend on exactly the right mix of microbes for healthy function.

Understanding the Metaorganism

Professor Bosch's research in Hydra is illuminating the nuances of metaorganism biology. Even in a creature as simple as the Hydra, a complex interplay of genetics and physiology mediates and is mediated by, the microbiome. The relationships between a host and its microbial communities are deeply entrenched in the evolutionary history of both organisms, and the two are truly inseparable.

Meet the researcher

Professor Thomas C.G. Bosch
Director, Zoological Institute
Head, Interdisciplinary Research Centre Kiel Life Science (KLS)
Christian-Albrechts-University Kiel
Germany

Professor Thomas Bosch completed his undergraduate and graduate studies in Biology at the University of Munich, earning his doctorate in 1986. He continued on to a postdoctoral position at the University of California, Irvine, USA and a professorship for Zoology at the Friedrich Schiller University of Jena, before joining Kiel University in 2000. Understanding the evolution and fundamental principles governing life processes in multicellular animals is a major goal of his research. He has published during his career over 150 research papers and articles and two books. From 2010 to 2013 he served as Vice-President of the university and now heads the Kiel Life Science (KLS) interdisciplinary research centre. He is the spokesperson for the DFG funded Collaborative Research Centre (CRC/SFB 1182) 'Origin and Function of Metaorganisms', which studies the role of multi-organismic interactions in health and disease. He is also the Editor-in-Chief of *Zoology* and former President of the German Society of Developmental Biology (GfE). His research work has been recognised by a number of awards including the Dr honoris causa degree from St Petersburg State University, Russia (2004). Professor Bosch is a Senior Fellow of the Canadian Institute of Advanced Research (CIFAR) and Fellow of the Wissenschaftskolleg (Institute of Advanced Studies) in Berlin.

CONTACT

E: tbosch@zoologie.uni-kiel.de
W: www.bosch.zoologie.uni-kiel.de
www.metaorganism-research.com

FUNDING

German Science Foundation (DFG)

FURTHER READING

T Rees, T Bosch and AE Douglas, How the microbiome challenges our concept of self, PLoS Biology, 2018, 16, e2005358.

R Augustin, K Schröder, APM Rincón, S Fraune, F Anton-Erxleben, E-M Herbst, J Wittlieb, M Schwentner, J Grötzinger, TM Wassenaar and TCG Bosch, A secreted antibacterial neuropeptide shapes the microbiome of Hydra, Nature Communications, 2017, 8, 698.

AP Murillo-Rincon, A Klimovich, E Pemöller, J Taubenheim, B Mortzfeld, R Augustin and TCG Bosch, Spontaneous body contractions are modulated by the microbiome of Hydra, Scientific Reports, 2017, 7, Article number: 15937.

A-M Boehm, K Khalturin, F Anton-Erxleben, G Hemmrich, UC Klostermeier, JA Lopez-Quintero, H-H Oberg, M Puchert, P Rosenstiel, J Wittlieb and TCG Bosch, *FoxO* is a critical regulator of stem cell maintenance in immortal Hydra, Proceedings of the National Academy of Sciences, USA, 2012, 109, 19697–19702.

C | A | U
Kiel University
Christian-Albrechts-Universität zu Kiel

AN ENZYME FOR THE FUTURE

Professor Tony Moore and his team at the University of Sussex are investigating the alternative oxidase. This enzyme provides an alternative route for molecular oxygen in the pathways of energy production that are often considered to be wasteful of energy but hold the key to the development of many different novel therapeutic treatments.

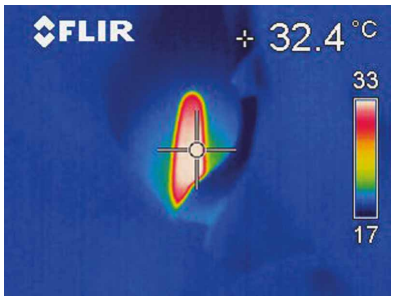
Energy is vital to every aspect of life. We as humans require it to perform everyday bodily functions, from breathing to keeping our hearts pumping, relying on incredibly complex mechanisms to furnish our energy demands. One of these mechanisms, called the respiratory chain is utilised by all eukaryotic cells in multicellular organisms such as plants and animals to produce energy.

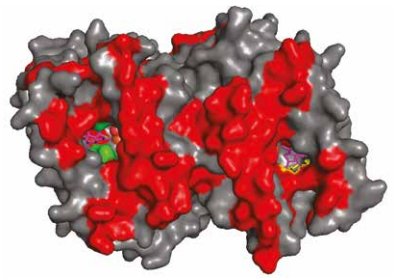
In order to use this mechanism effectively, eukaryotic cells such as those within our own bodies contain mitochondria – tiny double-membrane-bound compartments called organelles that function as the powerhouse of the cell. Within the mitochondria is a convoluted and complicated folded inner membrane that houses the electron transport chain – this is the site of respiration and energy production. It is through this electron transport chain that mitochondria harness energy in the form of a simple molecule known as adenosine triphosphate (ATP).

However, the mechanism through which this occurs is highly complex and relies on several proteins within the mitochondrion's inner membrane to function correctly. In essence, the production of energy in all eukaryotic cells relies on a series of oxidation-reduction reactions that eventually result in the addition of hydrogen to oxygen, thereby reducing it to water.

In doing so, the energy released during these oxidation-reduction reactions is used to 'pump' protons, positively charged hydrogen ions separated from their associated electrons, across the membrane thereby generating a proton electrochemical gradient – with a high concentration of protons on the outer side of the membrane and a low concentration on the inner side of the mitochondrial membrane.

Like water flowing downstream the protons are driven to follow the gradient and as the protons move back from the outer side (with a high concentration of protons) to the inner side (with a low concentration of protons), the energy released during this process is used to drive a molecular motor called the ATP synthase, resulting in the release of ATP for cellular activities. Multi-enzyme complexes located in the inner mitochondrial membrane mediate this reaction to release the energy that all complex cellular organisms then utilise to power their cellular activities and hence survive.

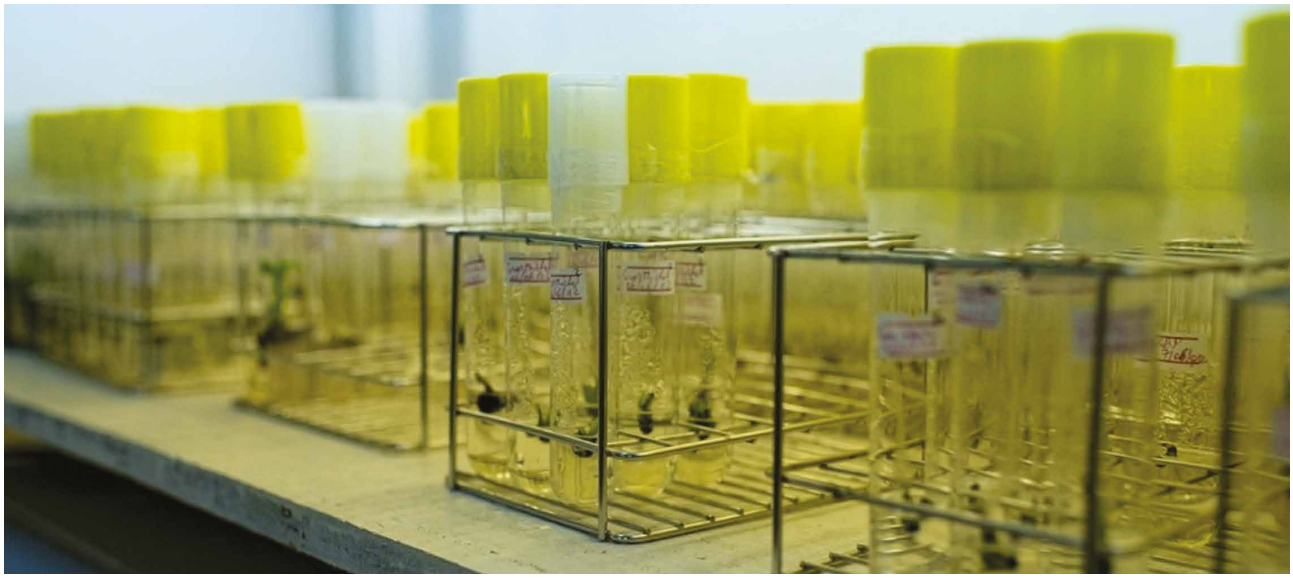




Respiration

The process of respiration or energy production through the electron transport chain starts when the multi-enzyme complexes, NADH and succinate dehydrogenases (called complexes I and II respectively) transfer electrons into a pool of ubiquinone molecules and on to a multi-enzyme complex (the cytochrome bc1 complex) that then oxidises the pool and transfers the electrons to the final enzyme, known as cytochrome c oxidase. It is this protein which then reduces oxygen into water.

'A key challenge in understanding how to control the alternative oxidases in plants, fungi and parasites rests upon the identification of the substrate and inhibitor-binding site(s) in this protein.'



During each of the steps in the process, as electrons flow 'downhill' along the chain, free energy is released. It is this free energy that is used to form the proton gradient which drives the production of ATP via the ATP synthase – this is the source of energy that can be used to fuel vital biological processes required for cellular activity.

Looking at the Alternatives

While this electron pathway may be the most well-established route for the production of energy, further research by Professor Moore's laboratory has found that alternative respiratory pathways also exist in plants, some fungi and parasites. This is due to the expression and presence of a single protein, known as the alternative oxidase (AOX). This enzyme, which is also located in mitochondria, enables plants, some fungi and parasites to by-pass the cytochrome bc1 complex and cytochrome oxidase activities thereby releasing the energy as heat rather than forming a proton gradient.

This is a particular concern in terms of fungicide resistance, as many commercial fungicides target the respiratory chain at the level of the cytochrome bc1 complex thereby blocking the fungi's ability to respire. If the alternative oxidase is present, however, it by-passes the site of fungicide action and hence enables the fungus to continue to respire and grow. Understanding the structure of AOX and how it works is, therefore, an important area of scientific research as it may provide information on an alternative target for the development of novel fungicides.

Professor Anthony Moore at the University of Sussex has dedicated over 40 years of his career to investigating the structure and function of the AOX enzyme in both plants and parasites. He describes how, 'the Moore Lab' is particularly interested in how the structure of this important but enigmatic protein influences its function in plants, fungi and parasites. Although this protein has been known about for over 100 years, relatively little is known about its mechanism of action or physiological significance.'

One of the consequences of alternative oxidase activity as indicated above is to facilitate the dissipation of energy as heat which some plants use to volatilise 'smelly compounds' to support pollination or to enable the plant to 'burst through snow' in order to flower during the winter months.

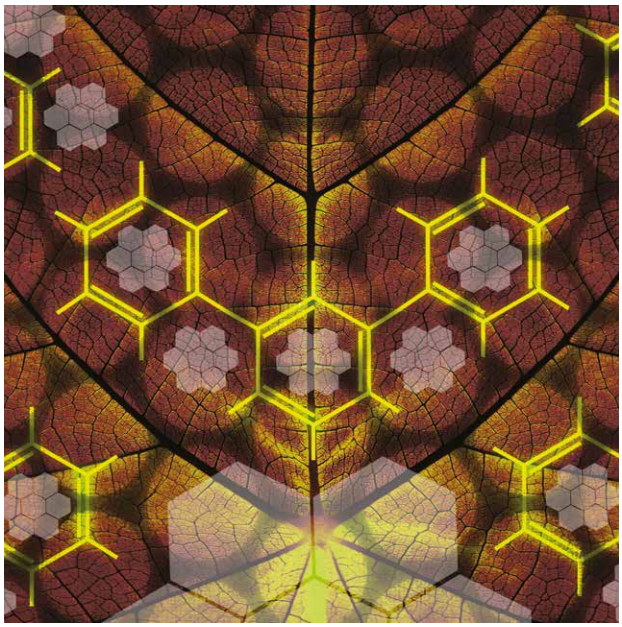
The AOX pathway can also be called into action in response to stresses such as drought, cold, harmful reactive oxygen species (ROS) and infection. Understanding the mechanism by which the protein is expressed and how it responds to these stresses have been particularly important to Professor Moore and his team, who have also recently identified AOX in human fungal pathogens, in addition to plants.

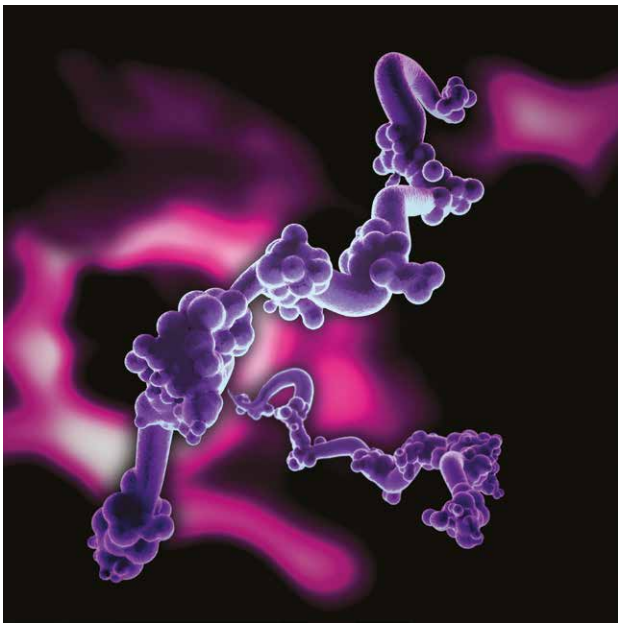
The Structure of Drug Development

Professor Moore and his team, in collaboration with Professor Kiyoshi Kita at the University of Tokyo and Nagasaki, have also been investigating the enzymatic structure of AOX within the protozoan parasite *Trypanosoma brucei*, known to cause African sleeping sickness.

The reason this parasite has been of such interest to Professor Moore and his collaborators in Japan is because of its total dependence, in the blood-stream form, on the alternative oxidase pathway to respire. Because this protein is absent from humans, understanding the nature of this alternative oxidase offers an attractive target for the design of new medicines to kill the parasite by disrupting its ability to survive.

Recently, the Japanese and UK team determined the structure of the AOX at atomic level resolution both in the





presence and absence of a number of specific AOX inhibitors. This has enabled them to identify the substrate and inhibitor binding site of the *trypanosomal* AOX. Such information is proving critical in the synthesis of novel inhibitors and effective drugs to hopefully treat trypanosomiasis or sleeping sickness.

Professor Moore states that, 'a key challenge in understanding how to control the alternative oxidases in plants, fungi and parasites rests upon the identification of the substrate and inhibitor-binding site(s) in this protein.' The substrate-binding site is the site at which the enzyme binds ubiquinone and possibly inhibitor molecules and so knowing more about the structure of this site and how AOX might bind to inhibitors will enable targeted drugs to be produced that can control its action.

Current and Future Research

In other research, Professor Moore and his team, have analysed the structures of AOXs from different species and in a review released last year, explained how differences in the response and sensitivity to specific AOX inhibitors could be related to structural differences in the channel within the protein which leads to the substrate binding site. They suggest that such structural differences indicate that all AOX proteins are not the same and hence such knowledge is critical for future structure-based drug design.

One particular area that such information has proven critical has been in the development of novel drugs to treat candidiasis. In addition to parasites such as trypanosomes, individuals with a compromised immune system are particularly susceptible to opportunistic diseases such as cryptosporidiosis, candidiasis and those caused by the microsporidia parasites. Examples of human fungal organisms that produce candidiasis include *Candida albicans* and *Candida auris*.

Candida auris is an emerging multi-drug resistant human fungal pathogen, which is resulting in a world-wide candidaemia epidemic of global concern. Its genome has been sequenced and Professor Moore's group have confirmed that it does indeed contain a gene encoding the alternative oxidase. Independent testing of their current family of inhibitors has provided some spectacular results since some of Professor Moore's inhibitors are proving effective at specifically inhibiting the growth and development of *Candida auris*. This is a major breakthrough as *Candida auris* is currently resistant to the usual drugs used to treat candidiasis.

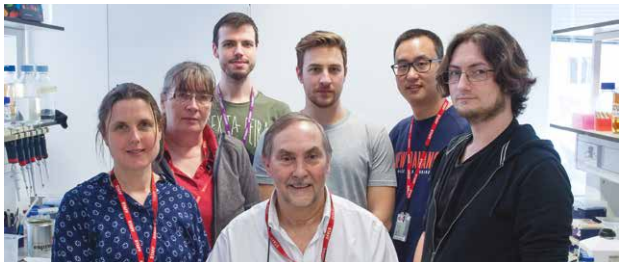
Professor Moore is also collaborating with Dr Marten Szibor and Professor Howy Jacobs in Finland on the possible role the AOX protein could have in alleviating sepsis – a condition caused by an inflammatory response to infection that can lead to multi-organ dysfunction. The condition causes over 44,000 deaths per year, but his collaborator's early results show a lot of promise.

Through the administration of AOX protein, a major symptom of sepsis known as septic shock, could possibly be protected against. This is thought to be due to the acceleration of the recovery process as a direct result of stimulated mitochondrial activity due to the presence of AOX. The promising and innovative nature of this work also shows potential in the effective design and development of novel AOX proteins to treat Alzheimer's and Parkinson's disease where changes in mitochondrial activity have been associated with the disease.

Professor Moore's team has proved the importance of investigating these fundamental metabolic mechanisms. AOXs are now showing substantial promise in providing therapeutic support for a number of health conditions – an outcome spearheaded by Professor Moore's ongoing dedication to understanding this enigmatic enzyme.

Meet the researcher

Professor Tony Moore FRSB, FLS
Biochemistry & Biomedicine
School of Life Sciences
University of Sussex
Brighton
UK



Professor Anthony (Tony) Moore is Professor of Biochemistry at the University of Sussex. He first developed an interest in mitochondria whilst at Shell Research Ltd, prior to undertaking a PhD in the same area at the University of Aberdeen. He later went on to study mitochondria at institutes such as the Johnson Research Foundation at the University of Pennsylvania, before joining the University of Sussex in 1979. Throughout his career, he has made many significant landmark discoveries into the understanding of alternative pathways of electron transport. He and his team at the Moore Lab are currently looking into the function, mechanisms and structure of alternative oxidases (AOX), enzymes which provide an alternative respiration pathway to plants, fungi, parasites and prokaryotes. They are working in collaboration with industrial and clinical partners to develop novel fungicides and therapeutics based on their research. In 2018, Professor Moore was selected as a finalist for the BBSRC Innovator of the Year Award and is currently in the process of spinning out a company called 'AOX Technologies Ltd' to commercialise his fungicides.

CONTACT

E: A.L.Moore@sussex.ac.uk
W: http://www.sussex.ac.uk/lifesci/moorelab/

KEY COLLABORATORS

Professor Kiyoshi Kita, Nagasaki University
Dr Marten Szibor, University of Tampere
Professor Howy Jacobs, University of Tampere
Dr John Misselbrook, MD Agform Ltd, UK

FUNDING

Biotechnology and Biological Sciences Research Council (BBSRC)
Wellcome Trust

FURTHER READING

B May, L Young and A Moore, Structural insights into the alternative oxidases: are all oxidases made equal? Biochemical Society Transactions, 2017, 45, 731–740.

A Moore, T Shiba, L Young, S Harada, K Kita and K Ito, Unraveling the heater: new insights into the structure of the alternative oxidase, The Annual Review of Plant Biology, 2013, 64, 637–663.

UNIVERSITY OF SUSSEX

A UNIQUE FAMILY OF INTRAMEMBRANE PROTEASES

Intramembrane proteases are proteins located in the core of the cell membranes of mammals and other organisms. Discovered at the end of last century, they are still poorly understood and implicated in a number of diseases such as Alzheimer's Disease, malaria and hepatitis C. **Professor Regina Fluhrer** and her team at the University of Ludwig Maximilians, Germany, study the function and mechanisms of intramembrane proteases, in order to better understand their role in health and disease.

Intramembrane Proteases – a Recent Discovery

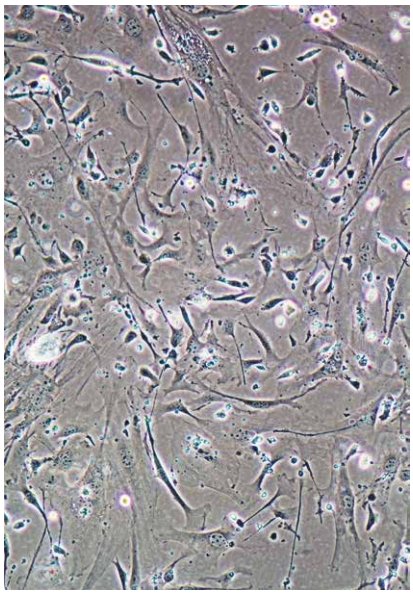
Intramembrane proteases are specialised enzymes that are situated in the core of cellular membranes. They can be present in the plasma membrane that surrounds cells, but also in membranes that surround specialised compartments inside the cell. These specialised compartments include, for instance, the endoplasmic reticulum, where proteins and lipids including steroid hormones are made, and the Golgi apparatus, where proteins are processed and sorted.

Also known as transmembrane proteases, they cut off portions of other proteins located in the membrane, causing fragments to be released and secreted into the environment outside of the organ or cell and at the same time fragments are released into the interior solution of the cell. Once released, these protein fragments can act as signalling molecules or stimulate other biochemical reactions.

Evolutionary distinct from other proteases, intramembrane proteases are still not completely understood, but have been shown to be involved in pivotal physiological and pathophysiological processes, like cell differentiation, regulation of metabolic processes and the development of Alzheimer's disease.

Professor Regina Fluhrer is a researcher at the Ludwig Maximilians University (LMU) and the German Centre for Neurodegenerative Diseases (DZNE) in Munich who has been studying this specialised group of proteins for the past 15 years. 'Intramembrane proteases are implicated in pathologies like Alzheimer's disease, Malaria, Hepatitis C infections and B (immune) cell deficiency,' explains Professor Fluhrer. 'Since these proteases may be attractive drug targets we also intend to understand their cleavage mechanism in detail.'

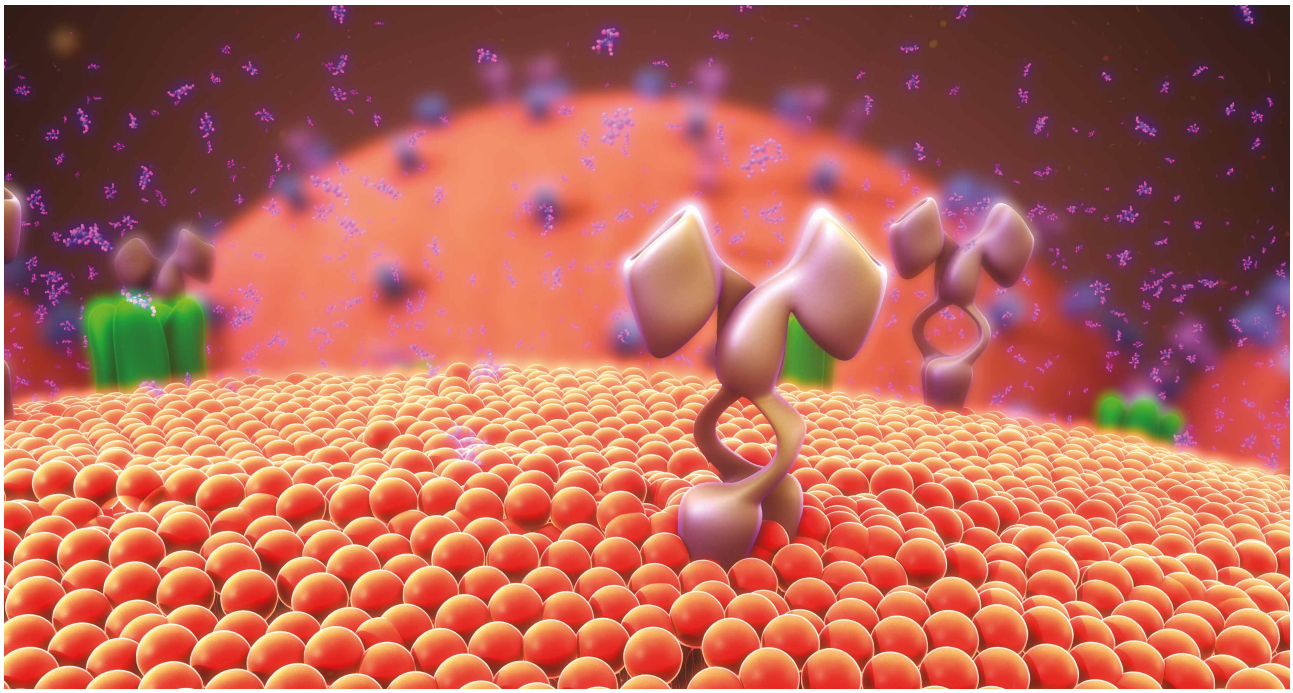
Intramembrane proteases are classified based on their cutting or 'cleavage' mechanisms, which depend upon the molecular groups present in their active sites. The active site of an enzyme is the location where a substrate (in this case another protein) binds in order to initiate a chemical reaction. Professor Fluhrer's research currently focuses on the signal peptide peptidase (SPP) and four SPP-like (SPPLs) proteases, SPPL2a,



SPPL2b, SPPL2c and SPPL3, which are part of a family called GxGD-type intramembrane proteases.

Proteins are formed from strings of amino acids. These intramembrane enzymes contain an aspartate amino acid residue in their active site and are found in a variety of compartments in the cells of mammals. SPP and SPPL2c are found in the endoplasmic reticulum, SPPL3 in the Golgi apparatus, SPPL2b in the plasma membrane, and SPPL2a in lysosomes

‘Intramembrane Proteases are implicated in pathologies like Alzheimer, Malaria, Hepatitis C infections and B cell deficiency.’



– compartments used primarily for breaking down cell waste.

Although all SPP/SPPL family members contain a similar active site, the substrates and cleavage mechanisms of different intramembrane aspartyl proteases seem to be more diverse than initially thought. Professor Fluhrer’s work focuses on unravelling the protein targets and cleavage mechanisms of this group of proteases.

Ectodomain Shedding and Protein Secretion

When intramembrane proteases cleave their membrane-anchored substrates, the portions of the proteins they cleave that extend into the extracellular space (the area outside of the cell) are released from the membrane surface (secreted). Typically, for GxGD intramembrane proteases the extracellular portions of their substrates are required to be shorter than 60 amino acids for efficient cleaving to occur. Substrates with longer extracellular portions get shortened through a mechanism called ectodomain shedding before they are processed by GxGD intramembrane proteases.

Professor Fluhrer’s lab recently demonstrated that human SPPL3 is an exception to this rule since it directly cleaves a protein called foamy virus envelope protein without first shortening its extracellular domain. Based on this finding, the researchers set out to identify and characterise new SPPL3 protein targets, to understand the cleavage mechanism of SPPL3 and elucidate its function in cells.

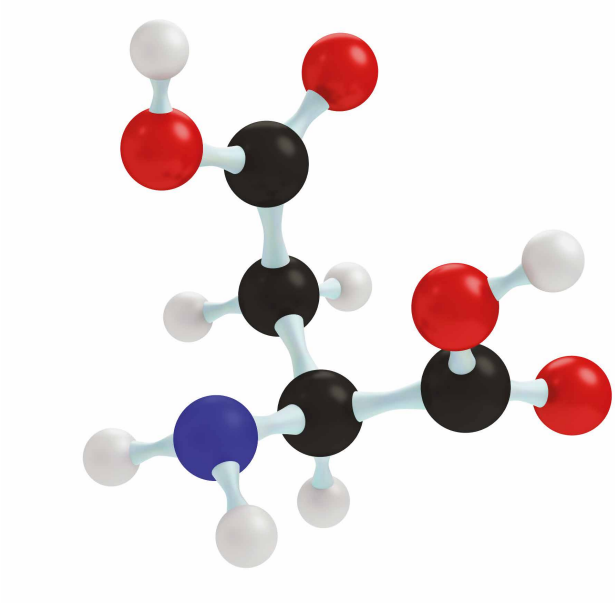
Protein Glycosylation

Glycosylation is a biochemical reaction that takes place in the endoplasmic reticulum and Golgi apparatus of the cells of eukaryotic (multicellular) organisms. It occurs when one or more sugar molecules are attached to a protein to create a glycoprotein. Approximately half of all proteins in a cell undergo glycosylation, an important and highly regulated pathway of modifying proteins. Glycosylation is important for protein, function and stability, and is known to affect the three-dimensional configuration of some proteins.

N-glycosylation is the attachment of a sugar, known as a glycan, to a nitrogen atom on an asparagine amino acid residue within a protein, resulting in a molecular group known as N-glycan. N-glycan synthesis is initiated in the endoplasmic reticulum, with subsequent processing and modification of the N-glycan taking place in the Golgi apparatus. Within the Golgi, numerous enzymes called glycosyltransferases, which add sugars to the protein, and glycosidases, which remove sugars, convert these glycans into even more complex N-glycans. N-glycosylation is complete when the N-linked glycoproteins are either secreted or become embedded in the plasma membrane.

SPPL3 Controls Protein N-glycosylation

Recently, SPPL3 was shown by Professor Fluhrer and her colleagues to control protein N-glycosylation, when key Golgi glycosyltransferases were identified as SPPL3 protein substrates. Since then, most newly discovered SPPL3 substrates have been found to modify N-linked glycans. Other SPPL3 substrates



contribute to the biosynthesis of other sugar molecules called glycosaminoglycans, as well as O-glycan synthesis, where a sugar is attached to an oxygen containing residue on the protein.

Professor Fluhrer's team found that the majority of these new SPPL3 substrates are located in the Golgi apparatus and are implicated in multiple glycosylation pathways in the cell, confirming that SPPL3 plays a crucial role in cellular N-glycosylation, but also a more general role in regulating cellular glycosylation pathways.

Using cells grown in the laboratory as well as tissues from mice that lack SPPL3, the researchers confirmed that SPPL3 is one of the major proteases responsible for releasing the extracellular portions of various glycosyltransferases and glycosidases into the extracellular space, the area outside of the cell. This leads to a reduction in their cellular activity and the researchers demonstrated that N-glycosylation in the cell is reduced when the extracellular portions of glycosyltransferases are cleaved by SPPL3.

The team found that reduced levels of SPPL3 resulted in increased glycosylation, whereas elevated amounts of SPPL3 caused decreased glycosylation of many secretory and membrane glycoproteins. Mice deficient in SPPL3 also had a build-up of glycosylation products in the Golgi. This is because the active site of glycosidases and glycosyltransferases is located within their extracellular portions so when these enzymes are cleaved by SPPL3, their activity in the Golgi is reduced.

As a consequence of altered glycosylation, Professor Fluhrer's group found that when levels of SPPL3 were increased in cells in the laboratory, the sizes of glycoproteins in those cells were reduced. In contrast, when SPPL3 levels were decreased, this resulted in increased molecular weight of the glycoproteins.

In related studies, a comparison of substances secreted by cells showed that a glycosyltransferase called GnT-V is the SPPL3 substrate that affects glycosylation in these cells most significantly, whereas other substrates such as the N-glycan-modifying enzymes called ß3GnT1 and ß4GalT1 have a less severe effect. When glycans were separated from proteins and analysed, the branched glycan chains normally generated by GnT-V were found to be strongly diminished in cells producing SPPL3 in large amounts.

Although cleavage of the glycosyltransferases by SPPL3 remains to be shown directly, these results set the stage for further investigations into SPPL3 activity. Professor Fluhrer and her group provide evidence that by using one protease – that of SPPL3 – to control the process of N-glycosylation, cells are able to rapidly and efficiently change the glycosylation state of many glycoproteins within the cell.

Future Directions: Many Questions Remain

Currently SPPL2c is the only member of the SPP/SPPL family for which no substrates have been identified. According to Professor Fluhrer's preliminary results, SPPL2c is mainly found in the testes in mice and humans. Her team also found that cells that express SPPL2c show reduced SPPL3 protein levels. In future studies, Professor Fluhrer's group hopes to confirm the location of SPPL2c, to identify proteins modulated by SPPL2c and to find the mechanism that causes SPPL3 levels to decrease in cells that express SPPL2c.

In addition, questions about SPPL3 still remain. Preliminary data from Professor Fluhrer's lab indicate that SPPL3 protein levels depend on nutrient concentrations in the cell and that low SPPL3 levels are observed under high glucose conditions. Going forward, the researchers will investigate whether increased SPPL3 levels induced by low glucose are a result of changes in protein production, processing or degradation.

They will also determine if SPPL3 protein levels change during cell differentiation (specialisation), and whether SPPL3 protein levels change in models of Alzheimer's disease in mice. They will measure SPPL3 expression in brain tissue from human Alzheimer's disease patients and compare this to patients with no disease, and in tumour tissue at various stages of cancer.

Intramembrane proteases are implicated in numerous disease processes in humans. Various drugs targeting the better characterised soluble proteases have been approved for treatment of human disease but currently no drugs have been approved against intramembrane proteases, as basic research on these proteins is still in its early stages. In her continued research on the physiological functions and mechanisms of these proteins, Professor Fluhrer is laying the groundwork for future drug targets against intramembrane proteases to be identified, along with new treatment options that can potentially be used against a diverse array of diseases.

Meet the researcher

Professor Regina Fluhrer
Biomedical Center, Ludwig Maximilian University (LMU) &
German Center for Neurodegenerative Diseases (DZNE)
Munich
Germany

Professor Regina Fluhrer is a professor of Biochemistry at the Biomedical Center of the Ludwig Maximilians University (LMU) and at the German Center for Neurodegenerative Diseases (DZNE) in Munich, Germany, where she is also a Coordinator and Deputy Speaker and a member of the Tenure Track Committee. From 2015 to 2017 she was member of the LMU Board of University Representatives. She received her PhD in 2003 in Biochemistry at the Ludwig Maximilian University of Munich, where she also completed her postdoctoral work. It was during this time that she became interested in the SPP/SPPL-family of intramembrane aspartyl proteases. Professor Fluhrer has also been a recipient of the Boehringer Ingelheim APOPIS Award for Young Researchers and in 2012 she was recognised for teaching excellence by the Bavarian Ministry of Science.

CONTACT

E: Regina.Fluhrer@med.uni-muenchen.de
W: http://www.biochemie.abi.med.uni-muenchen.de/fluhrer_lab/index.html
https://www.dzne.de/en/research/research-areas/fundamental-research/research-groups/fluhrer/research-areasfocus/

KEY COLLABORATORS

Professor Bernd Schröder, Christian-Albrechts-University
Professor Stefan Lichtenthaler, DZNE Munich
Professor Christian Haass, LMU Munich
Professor Axel Imhof, LMU Munich
Dr Regina Feederle, Helmholtz Centre

FUNDING

The Ludwig Maximilians University of Munich (LMU)
German Center for Neurodegenerative Diseases (DZNE)
German Research Foundation (DFG)

FURTHER READING

T Mentrup, R Fluhrer, B Schröder, Latest emerging functions of SPP/SPPL intramembrane proteases, European Journal of Cell Biology, 2017, 96, 372–382.

P-H Kuhn, M Voss, M Haug-Kröper, B Schröder, U Scheperse, S Bräse, C Haass, SF Lichtenthaler, R Fluhrer, Secretome analysis identifies novel signal peptide peptidase-like 3 (Sppl3) substrates and reveals a role of Sppl3 in multiple golgi glycosylation pathways, Molecular and Cellular Proteomics, 2014, 14, 1584–1598.

M Voss, U Künzel, F Higel, P-H Kuhn, A Colombo, A Fukumori, M Haug-Kröper, B Klier, G Grammer, A Seidl, B Schröder, R Obst, H Steiner, SF Lichtenthaler, C Haass, R Fluhrer, Shedding of glycan-modifying enzymes by signal peptide peptidase-like 3 (SPPL3) regulates cellular N-glycosylation, EMBO Journal, 2013, 33, 2890–2905.

M Voss, B Schröder, R Fluhrer, Mechanism, specificity, and physiology of signal peptide peptidase (SPP) and SPP-like proteases, Biochimica et Biophysica Acta (BBA) – Biomembranes, 2013, 1828, 2828–2839.

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

DZNE Deutsches Zentrum für Neurodegenerative Erkrankungen in der Helmholtz-Gemeinschaft

UNLOCKING THE SWEET SECRETS OF THE MICROBIOTA

The pioneering research of **Professor Lloyd Kasper** while at Geisel School of Medicine, Dartmouth College, and **Javier Ochoa-Repáraz** at Eastern Washington University, has revealed that microbes living within our gut contain and release compounds such as a specific bacterial sugar known as a polysaccharide that can regulate the development and function of our immune system, and excitingly may represent a new class of treatment for multiple sclerosis.

Multiple sclerosis (MS) is an inflammatory disease that leads to severe damage to the human central nervous system (CNS) and progressive disability. The overall incidence is increasing and current estimates suggest that it affects as many as 1,000,000 individuals in the United States and over three and a half million patients worldwide. MS is a chronic autoimmune disease, where the body's defence system, the immune system, starts to attack the body's own tissues, causing inflammation and damage.

Although MS can be considered a total brain condition, the specific disease process in MS is the immune system attack of the coating that protects nerves, called myelin, resulting in damage that disrupts the messages travelling between the brain and the rest of the body. This attack is to both white and grey matter brain tissue causing a wide range of neurological difficulties including limb numbness, loss of balance, tremors, vision, memory problems, and dysfunction of speech or swallowing.

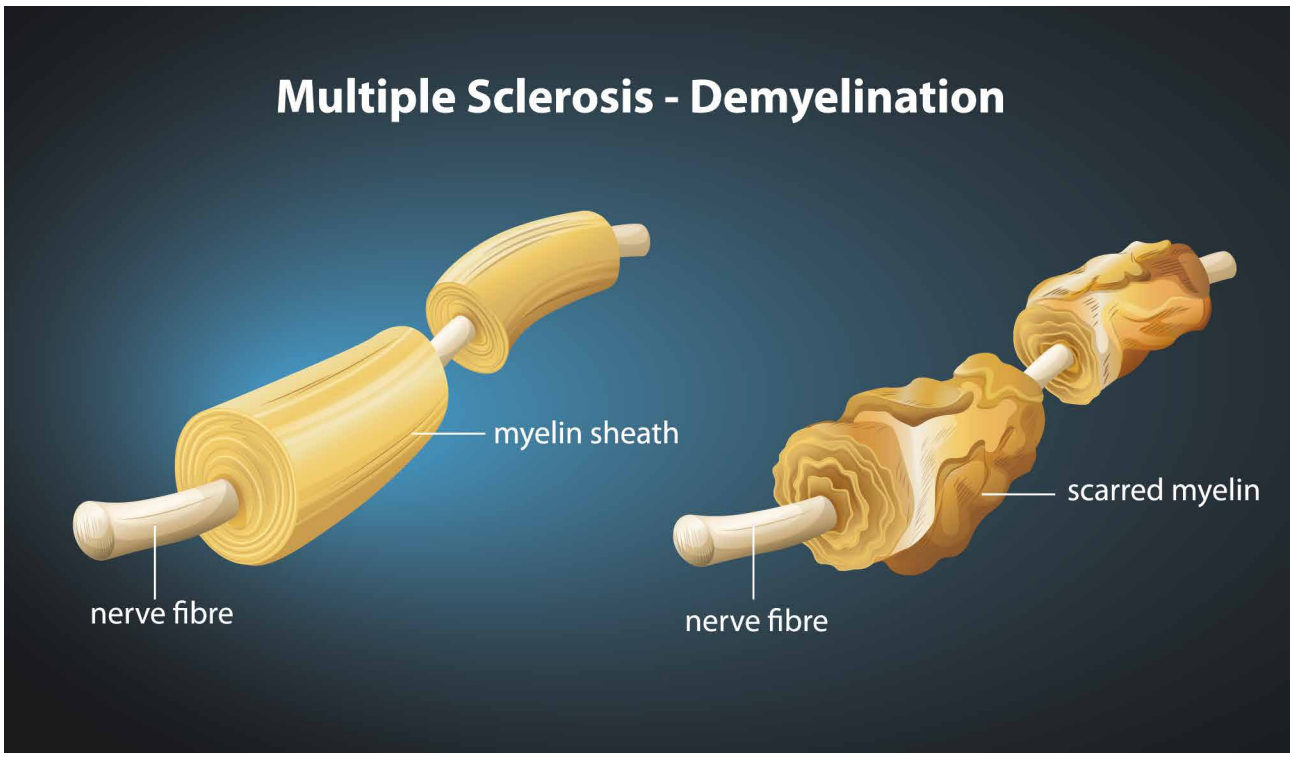
Relapsing-remitting multiple sclerosis (RRMS) is an inflammatory condition that is the most common initiating pattern of the disease, characterised by periods where symptoms worsen, called relapses, followed by unpredictable periods of stability or remission.

There is also a degenerative component in which the nerve damage is cumulative and the disease can eventually evolve into secondary-progressive multiple sclerosis (SPMS), where the immune inflammatory relapse attacks become less frequent but the degenerative disability continues without periods of remission. Those individuals develop secondary progressive disease six to ten years after the onset of disease. Current therapies are principally directed at the inflammatory relapsing phase of the disease.

There is no cure for MS and currently approved treatments that modulate the immune system aim to reduce the frequency and duration of relapses thereby improving symptoms. Unfortunately, many of these treatments that strongly suppress the immune system can have serious deleterious side effects including cardiac problems, infection and long-term lymphopenia – a reduced number of white blood cells that play a vital role in the immune

system. There is, therefore, an urgent unmet clinical need for an effective, easily administered treatment for MS, that prevents this autoimmune damage but does not compromise the immune system itself.

Tackling this head on are Professors Lloyd Kasper, Professor of Medicine, Microbiology and Immunology while at Geisel School of Medicine at Dartmouth College and Javier Ochoa-Repáraz, Assistant Professor at Eastern Washington University. Professor Kasper is a co-Founder of the biopharmaceutical company Symbiotix Biotherapies Inc. Along with the other co-founders and the CEO of Symbiotix, Nader Yaghoubi, MD, PhD, he is developing an exciting new class of therapeutics, that takes advantage of the protective role played by the bacteria that live within us.



The Microbiome

The human body is colonised by a huge number of microscopic organisms or microbes, collectively referred to as the human microbiota. This ecosystem is made up of trillions of cells, including bacteria, archaea, viruses and fungi. Far from being neutral bystanders, these microbes live in a close relationship with us that is vital for normal health.

The biggest populations of microbes reside in our gut where they aid digestion, prevent infection by disease-causing organisms, and educate our immune system on what to fight. It's well recognised that the gut microbiota contributes to the normal function of the immune system, and the link between these microbes and human health is an exciting focus of future scientific research.

Landmark research by Professors Kasper and Ochoa-Repáraz and colleagues has unveiled key insights into how these microbes interact with the human immune system and has changed our understanding of this special relationship that has evolved over thousands of years. In a recent study, the researchers demonstrated that special two-way interactions exist between the host and gut microbiome that offers benefits to both the host and the microbiome.

In experiments using a mouse model of experimental autoimmune encephalomyelitis (EAE) in a specific mouse species (Non-Obese Diabetic – NOD mice) that resembles features of severe stages of secondary progressive MS, the mice were found to have an imbalance in their microbiome, compared to healthy controls.

Interestingly, it was demonstrated that the disease affects the microbiota and vice versa, and that antibiotic treatment could minimise progression of disease and reduce mortality, providing a foundation for the idea that therapeutic interventions that target the gut microbiome at early stages of CNS inflammation can potentially limit the progression of this devastating disease.

Bacterial Protectors

Excitingly, the researchers have also shown that powerful bacterial molecules can have far-reaching effects on the host and that they can actively suppress the human immune system.

Exploring the link between microbial communities and autoimmune disease, Professors Kasper and Ochoa-Repáraz with their team have closely studied the gut bacteria *Bacteroides fragilis* (*B. fragilis*) for many years.

In an elegant series of experiments, the team demonstrated that *B. fragilis* has a beneficial effect on the mouse model of MS. In this mouse EAE model, treatment with the *B. fragilis* bacteria slows the development of disease and induces immune tolerance.

Taking a closer look at how *B. fragilis* bestows such protective effects, crucial to maintaining a healthy immune system, previous studies by the other two Symbiotix co-founders (Professors Sarkis Mazmanian from Caltech and Dennis Kasper from Harvard Medical School) found that eight different sugars (polysaccharides) are produced on the surface of the bacteria that envelope the bug and form its protective coat. Their published research found that one sugar, in particular, called Polysaccharide A (PSA), is responsible for the protective effect.

Polysaccharide A has an interesting and unique structure, known as a zwitterion,

in that it is both positively and negatively charged. In the absence of this zwitterionic property, the molecules lose their immunological potency for inducing activation of a population of protective immune cells known as regulatory T cells. The purified Polysaccharide A is protective both prophylactically (before the disease is induced) and therapeutically (after clinical disease is established) when orally administered to EAE mice, the experimental model of human MS, used in both academia and industry to study the disease.

The purified Polysaccharide A showed a protective effect, delaying the onset and reducing the severity of the disease. The sugar significantly suppressed inflammation and prevented central nervous system attack of the myelin sheath protecting the nerves, thereby reducing the severity of disease in all the animals model of the disease. This ground-breaking work offers the exciting potential of giving rise to an entirely new class of therapy for MS.

Regulatory T Cells

The team then went on to show that the Polysaccharide A produced by *B. fragilis* plays a powerful protective role in the immune system and is capable of shaping particular immune responses that involve Regulatory T cells.

Regulatory T cells are white blood cells that play an important role in regulating or suppressing other cells in the immune system, thereby modulating the immune response. One of the primary inadequacies of the immune system in those with MS is that regulatory T cells are present in sufficient numbers in the circulation but appear to be dysfunctional in that they cannot effectively reduce the expression or production of the potent inflammatory molecule, interleukin-17.

Regulatory T cells control the immune response and therefore have the potential to help prevent autoimmune diseases, like MS, and limit the extent of inflammatory disorders. Circulating throughout the body to sites of inflammation, regulatory T cells dampen and suppress the inflammatory process by releasing a specific anti-inflammatory molecule, interleukin-10, that acts in opposition to interleukin-17.

Professor Kasper and his team took white blood cells from healthy human donors and demonstrated that the protective Polysaccharide A sugar stimulates dendritic cells – a type of messenger in the immune system that presents 'foreign' material to the T cells. Once stimulated, the dendritic cells activated the immunosuppressive regulatory T cells. Conversely, the team also demonstrated that T cells isolated from patients with multiple sclerosis have the capacity to acquire regulatory characteristics when stimulated in experimental conditions with the protective Polysaccharide A sugar.

Excitingly, their work clearly demonstrates that a gut-derived bacterial sugar has the ability to mount a regulatory T cell response using peripheral blood cells obtained from those with multiple sclerosis. As there are very few substances that can increase regulatory T cell number and activity, Symbiotix Biotherapies Inc. is developing these sugars as an entirely new approach to treating diseases in which regulatory T cell activity is essential.

Exploring the response to the protective Polysaccharide A sugar more closely, the team went on to identify the principal anti-inflammatory substance released by the T cells that can dampen the inflammation process in MS. This substance, interleukin-10, was then shown to be increased by regulatory T cells isolated from patients with MS following exposure to polysaccharide A in the laboratory.

Similarly, enhanced levels of interleukin-10 production were observed with oral administration of Polysaccharide A in mice. Confirming that the Polysaccharide A response was interleukin-10 dependent, mice that could not produce interleukin-10 showed no beneficial response following treatment with the sugar.

Working out the mechanisms by which the polysaccharide A elicits its immune dampening response, Professor Kasper and the Symbiotix team have forged an innovative new approach to the development of new treatments for MS. Symbiotix Biotherapies Inc. now aims to translate these groundbreaking research studies that have resulted in the first therapeutic molecule to emerge from the human microbiome, which they have named Reglemers™, into novel therapeutics for MS.

Next Steps

Professor Kasper and his team have moved this work forward with the support from the National Institutes of Health's Small Business Technology Transfer (STTR) programme and a Phase II Small Business Innovation Research grant (SBIR), as well as investor capital. Their near-term goal is to advance this revolutionary treatment option for MS into human clinical trials. The aim is to provide an orally administered, safe and effective treatment for MS without the potentially deleterious side effects associated with some of the more recently Food and Drug Administration (FDA) approved therapies for treating relapsing MS.

The company is now engaged in manufacturing and scale-up in preparation for the production of larger amounts of material for toxicology studies and human clinical testing. Striving to improve outcomes for patients with MS, Professor Lloyd Kasper and Javier Ochoa-Repáraz's research could pave the way for reducing the damaging effects of the disease, improving chances for patients to enjoy a much better quality of life. Beyond MS, this bacterial-derived sugar has potential applications in other serious immune-mediated diseases, including inflammatory bowel disease.

Meet the researchers

Professor Lloyd Kasper
Dartmouth Medical School
Lebanon, NH
USA

Dr Nader Yaghoubi
President and CEO of
Symbiotix Biotherapies
Boston, MA
USA

Dr Javier Ochoa-Repáraz
Eastern Washington
University
Cheney, WA
USA

Lloyd Kasper is a Professor Emeritus of Immunology/Microbiology and Medicine at the Geisel School of Medicine at Dartmouth College where he has been actively involved in basic and applied immunology research supported by grants from the National Institutes of Health, Immune Tolerance Network, March of Dimes, the National Multiple Sclerosis Society and industry. His basic research interests have been in immunoparasitology and in the pathogenesis of multiple sclerosis and the role of the gut microbiome in the regulation of neuronal demyelination. When not doing science at Dartmouth, Kasper can be found painting and skiing in Taos, New Mexico.

CONTACT

E: lloyddartmouth@gmail.com
W: http://symbiotix-bio.com

Nader Yaghoubi, MD, PhD, is President and CEO of Symbiotix Biotherapies, a pioneering microbiome company developing novel molecular therapeutics based on the microbiome. He has over 20 years of experience in the creation, operations and financing of life science companies. Dr Yaghoubi received an MD and PhD in Molecular Pharmacology from the combined degree program at Boston University School of Medicine. He is extensively involved in mentoring young companies and has served/serves as a mentor, advisor and reviewer with the Massachusett's Biotechnology Council's MassCONNECT program, Mass Life Sciences Center programs, Springboard Venture Capital Forum and numerous international and university business plan competitions.

Javier Ochoa-Repáraz, PhD, is Assistant Professor at Eastern Washington University (EWU). He received his PhD in Cellular and Molecular Biology from the University of Navarra in Spain. His postdoctoral training was at Montana State University and Dartmouth College exploring the impact of gut mucosal immune responses to microbes on inflammatory neuronal demyelination. With Dr Lloyd Kasper at Dartmouth College, he explored the mechanisms of immunomodulation induced by gut symbionts in the context of multiple sclerosis using animal models of the disease. At EWU, he continues working on the reciprocal interaction between the gut microbiome and disease.

BILE ACIDS ARE NOT JUST FOR DIGESTION

Over the last two decades, bile acids have gone from being thought of as mere helpers during digestion of fats and fat-soluble vitamins, to crucial players in the signalling pathways operating in the liver. **Professor Phillip Hylemon** and **Professor Huiping Zhou**, from the Medical College of Virginia, Virginia Commonwealth University, USA, lead two of the main research groups worldwide trying to unveil these pathways and the repercussions of these in terms of disease.

Researchers have known that bile acids play a vital role during digestion since 1870. It is now a well-accepted fact that these acids are needed for the metabolism of lipids, cholesterol, and fat-soluble vitamins (namely, vitamins A, D, E, and K). Under normal circumstances, bile acids are synthesised in the liver, then stored in the gallbladder until they are released into the gut after a meal. After digestion, these acids travel to the liver along with digested nutrients and finally return to the gallbladder for use at the next meal.

For a long time, this was considered to be the only function of bile acids. However, over the past 20 years, this idea of a passive role is slowly being replaced with a more dynamic one as a signalling molecule. It all started in 1999 when the first signalling pathway involving nuclear receptor and bile acids was identified. This mechanism turned out to be a key regulator in the metabolism of bile acids, glucose, and lipids in both the liver and intestines.

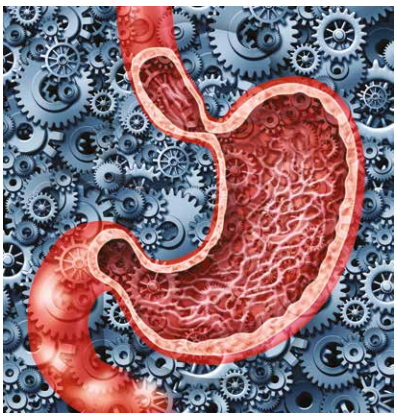
A few years later, a second pathway was unveiled that again linked bile acids to the regulation of glycogen, sugar, and lipid metabolism via a cell surface receptor (Called TGR5). Interestingly, this pathway is present in the bile duct leading to the liver, but not in the liver itself.

This notable absence of the signalling pathway in the liver triggered the curiosity of Professor Phillip Hylemon and Professor Huiping Zhou. They believed that the likelihood of having a specific mechanism in liver cells was strong enough to warrant investigation. As Professor Hylemon explains, their overarching aim is to 'investigate the role of bile acid-activated cell signalling pathways in hepatic metabolism using both *in vitro* and *in vivo* model systems.'

Consequences of a High-fat Diet

In 2012, after testing more than a dozen different possibilities, the duo saw their suspicions confirmed when they identified a new connection between bile acids and the liver. This new pathway involves a way for bile acids to communicate with liver cells without actually entering the cell. The system cleverly uses an intermediary located in the cell membrane (called spingosine-1 phosphate receptor 2, S1PR2), that in turn triggers a multitude of responses from inside the cell. In other words, it works as a messenger between bile acids and liver cells.

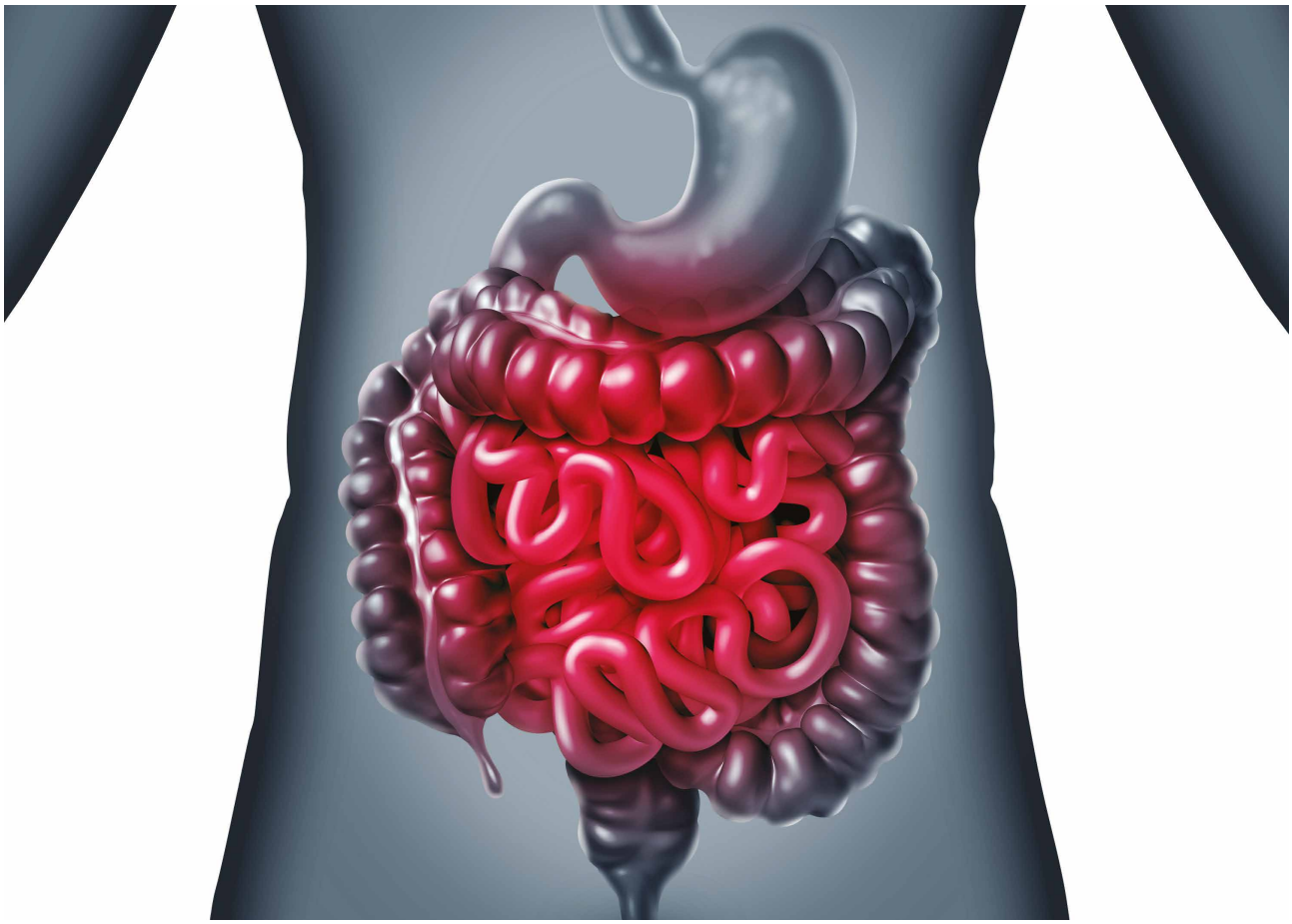
The team confirmed the importance of this new mechanism in lipid metabolism when researchers spotted how it can affect key genes involved in digestion and lipid transport. These included,



for example, genes responsible for transporting lipids in the blood and synthesis/degradation of cholesterol.

Given these results were obtained from experiments in a petri dish, the next step for Professor Hylemon and Professor Zhou was to explore the physiological effects on live animals. Interestingly, when fed a high-fat diet, mice were able to step-up on the activity of this pathway to avoid any long-term injuries. However, when this protective effect was blocked, then the mice quickly developed enlarged and fatty livers with an accumulation of cholesterol and triglycerides. The researchers speculated this happened because important genes involved in the transport and metabolism of lipids had failed, resulting in lipid accumulation in the liver.

‘Identification of bile acid-induced [mechanisms] in liver cells not only establish a novel theory in bile acid biology but also provide new mechanistic insights into the pathophysiology of metabolic diseases including NAFLD.’



This is particularly relevant in today’s society as a Western-type diet is typically high in saturated fats, which over time can overwhelm the ability of the liver to metabolise fats. As the researchers stated in 2015, ‘dysregulation of this cell signalling system may have significance in the development of fatty liver and related diseases especially in individuals on a high-fat diet.’

Of even greater concern is that a high-fat diet is often associated with obesity and non-alcoholic fatty liver disease (NAFLD), which can, in turn, be a precursor of cirrhosis and cancer of the liver. This is currently one of the most common chronic liver diseases in adults but ‘effective therapeutic strategies are still limited,’ explains Professor Zhou. Indeed, ‘identification of bile acid-induced [mechanisms] in liver cells not only establish a novel theory in bile acid biology but also provide new mechanistic insights into the pathophysiology of metabolic diseases including NAFLD,’ continues the researcher.

Bile Acids and Bile Duct Cancer

Bile acids cannot be thought of solely as protectors. While the beneficial effects include stimulation of bile formation and effective lipid metabolism, there is a dark side to bile acids. Effects such as cellular toxicity when in excess and impaired bile synthesis are just two examples. To complicate things further, there is some evidence that bile acids activate the same signalling pathways to produce these contradictory effects, making it a challenge for researchers to uncover the differences.

One of these examples comes in the form of bile duct cancer. For the last two decades, researchers have been aware that bile acids can promote the growth and development of this type of cancer. Albeit rare, these cancers are usually very aggressive and associated with a poor prognosis for the patients, especially if diagnosed late.

There is some evidence linking abnormal bile acid production and cancer development, with patients exhibiting elevated bile acid concentration in the bile, apparently caused by bile duct obstructions. However, the specific mechanisms by which bile acids can promote cancer development and biliary tumour growth are still a mystery.

Between 2014 and 2015, Professor Hylemon and Professor Zhou reported that excess bile acids are able to trigger an inflammatory response – a key stimulator of cancer development – and this way promote cancerous cell growth in the bile duct. Unsurprisingly, the researchers believe that this could be a promising novel therapeutic target for this type of cancer. For Professor Hylemon, this discovery may turn out to be their most important contribution to science. 'We now hypothesise that this is key to regulation of cell proliferation, inflammation and fibrosis in the liver,' notes the researcher.

With this in mind, the team successfully reduced liver injuries in mice with bile duct obstruction (a common precursor for bile duct cancer) by blocking the activity of bile acids, which in turn eliminated the source of inflammation. Mice with normal bile acid activity were not so lucky, and the signs of inflammation were quickly visible. These data seem to point towards an inflammatory response as the main mechanism by which bile acids induce damage to the liver but further research is needed.

Bile Acids and Oesophagal Cancer

Bile duct cancer is not the only type of cancer with bile acid involvement. There is also some evidence to suggest that bile acids can promote oesophageal cancer development but specific details are still to be elucidated.

This type of cancer ranks as the seventh most frequent and the sixth leading cause of death caused by cancer worldwide. One in particular – oesophageal adenocarcinoma – represents more than half of all oesophageal cancers and its incidence has increased dramatically over the past few years. Risk factors include Barret oesophagus, gastroesophageal reflux disease, being male, smoking, poor diet, and obesity. Although treatment by removing all or part of the oesophagus (known as oesophagostomy) combined with chemotherapy can significantly improve long-term survival rate, options for treatment are limited when diagnosis is made late in the disease.

Similar to observations made by Professor Hylemon and Professor Zhou in relation to bile duct cancer, bile acids were also found to promote invasive growth of cancer cells in oesophageal adenocarcinoma. This is likely achieved by inducing inflammation and triggering multiple pathways to boost cell division and migration, as well as the capability for causing tumours and spread of disease. 'The study not only provides new insights into the pathogenesis of oesophageal adenocarcinoma and the role of bile acids in disease progression,' the researchers stated, 'but strongly suggests that targeting signalling pathways represents a novel therapeutic strategy for the treatment of oesophageal adenocarcinoma.'

This idea applies beyond bile duct cancer. Professor Hylemon's and Professor Zhou's teams are beginning to build a very strong case in favour of targeting bile acids and associated mechanisms as a strategy to develop new treatments for cancers where these substances are involved.

Future Directions

It is now well accepted that bile acids are not just for digestion, but also regulate complex biological pathways. Professor Hylemon and Professor Zhou's findings extensively support the idea of an interplay between bile acids and nutrient metabolism in the liver/bile duct.

Despite the progress over the past 20 years, future studies to discover different pathways and mechanisms are needed. With this in mind – and because the cause of cancer cannot be attributed to a single factor – Professor Hylemon and Professor Zhou are now looking at how bile acids fit into the network of signals that regulate tumour proliferation and inflammation. Ultimately, says Professor Hylemon, 'it may allow the scientific community to target this novel pathway for treatment of various diseases.'

Meet the researchers

Professor Phillip Hylemon
Professor of Microbiology and Immunology and Medicine
Virginia Commonwealth University
School of medicine
Richmond, VA
USA

Professor Huiping Zhou
Professor of Microbiology and Immunology and Medicine
Virginia Commonwealth University
School of medicine
Richmond, VA
USA

Professor Phillip Hylemon obtained his PhD in microbiology from Virginia Polytechnic Institute and State University in 1971. He is now Professor of Microbiology and Immunology and Medicine at Virginia Commonwealth University. His research investigates the role of bile acids in lipid metabolism in the liver using both in vitro and in vivo model systems. Professor Hylemon has a long track record in this field, being continuously funded by the National Institutes of Health since 1975. His current aim is to understand how bile acids can develop a network of cell signalling pathways that regulate tumour cell proliferation, inflammation, and fibrosis.

CONTACT

E: phillip.hylemon@vcuhealth.org
W: https://medschool.vcu.edu/expertise/detail.html?ID=697

Professor Huiping Zhou obtained her PhD in molecular biology from the University of Kentucky in 1998. She joined Virginia Commonwealth University in 1999 as a post-doctoral researcher. After a few years as an associate professor at the Department of Immunology and Microbiology, she gained full professor status in 2016. Professor Zhou has received multiple awards, including the Women in science, Dentistry & Medicine Professional Achievement Award in 2018 and Research Career Scientist Award from Department of Veteran Affairs. Professor Zhou has been continuously funded by the National Institutes of Health and Department of veteran Affairs since 2005. Her long-term collaboration with Professor Hylemon has resulted in over thirty joint publications in the last ten years.

CONTACT

E: huiping.zhou@vcuhealth.org
W: https://medschool.vcu.edu/expertise/detail.html?id=hzhou

KEY COLLABORATORS

William M Pandak, MD, Virginia Commonwealth University
Jasmohan S Bajaj, MD, Virginia Commonwealth University

FUNDING

NIH/NIDDK 1R01DK115377, 1R01DK104893, 1RO1DK057543 (Professor Zhou and Professor Hylemon)
VA Merit Review Award 1I01BX004033, Research Career Scientist Award IK6BX004477 (Professor Zhou)
1I01BX001328 (Professor Hylemon)

TECHNOLOGICAL AND METHODOLOGICAL ADVANCES

CELEBRATING TECHNOLOGICAL AND METHODOLOGICAL INNOVATION IN HEALTHCARE

From improved imaging techniques to surgical innovation, in this section, we explore a range of important technological and methodological advances that are revolutionising the future of healthcare. In the current age in which precision medicine is coming to the fore, these advances are playing a key role in driving forward more timely and accurate diagnosis of disease, as well as significantly improving patient outcomes.

We begin this section by meeting Professor Jeffrey C. Hoch from the University of Connecticut, USA, who leads the NMRbox platform resource. This invaluable initiative provides a freely available, not-for-profit and centralised resource allowing researchers across the world to maximise the potential offered by nuclear magnetic resonance, a biomolecular approach with applications as far ranging as structural biology, metabolic studies, disease diagnosis, and drug discovery.

We then turn to Professor Ulrich Flögel and Dr Sebastian Temme at the University of Düsseldorf, who together are pioneering an innovative magnetic resonance imaging approach that allows the visualisation of tissue damage associated with conditions such as inflammatory bowel disease and neurogenerative brain disorders. We read how better understanding the extent of inflammation in the tissues of individuals allows much more focussed and patient-centred treatment.

Remaining on the topic of medical imaging, we meet Dr Björn Schönnagel and his team at the University Medical Centre Hamburg-Eppendorf, Germany. The foetal cardiovascular system is notoriously difficult to image due to the movement of the beating heart. We read how Dr Schönnagel has developed a Doppler ultrasound device that is compatible with existing imaging techniques, allowing much improved diagnosis of congenital heart disease in infants still in the womb.

For adults, cardiovascular diseases such as coronary heart disease (CHD) present a leading cause of death worldwide, and those fortunate enough to survive can be left with serious long-term complications. Dr York Hsiang and his team at the University of British Columbia are using microengineering techniques to create 'smart stents,' specifically designed to overcome the problems associated with existing surgical approaches to the treatment of CHD. We read how they now plan to take their innovative approach from the laboratory into the real world of the clinic.

The transition from laboratory to clinic is also the current focus of the innovative company Cylerus. Based in the USA, Cylerus is dedicated to developing new methods to improve blood vessel (vascular) access for patients requiring haemodialysis as the result of end-stage renal disease (ESRD). We read about their impressive progress thus far aiming to improve healthcare and quality of life for patients with ESRD, and their plans to bring their novel Drug-Eluting Cuff delivery device even closer to clinical practice.

Our final researchers featured in this section are Professor Alexander-Friedrich and Dr Avci-Adali at the University Hospital Tübingen, Germany. Patients may require facial reconstructive surgery for a range of reasons, including oral cancer and tooth loss, and the surgery is often fraught with difficulties. We read how Professor Alexander-Friedrich and Dr Avci-Adali are pioneering a new type of maxillofacial implant developed using bone tissue engineering, taking advantage of the power of stem cells to maximise patient outcomes.

We conclude this section with an exclusive interview with Aisling Burnand, Chief Executive Officer of the Association of Medical Research Charities (AMRC), the national membership organisation for health and medical research charities in the UK. We read how the ARMC plays a vital role in supporting healthcare research, and how rapid advances in healthcare technologies and methodologies are leading to an unprecedented transformation in health and medicine.

A BOX IN THE CLOUDS

Nuclear magnetic resonance (NMR) is without doubt one of the most exciting analytic methods available in biomolecular medicine. Applications include structural biology, metabolic studies, disease diagnosis, and drug discovery. However, the use of NMR can be daunting and complicated, with a multitude of diverse computer programs for analysing the data to choose from. **Professor Jeffrey C. Hoch** from the University of Connecticut, USA, leads the development of the NMRbox platform, an extensive, freely available, not-for-profit resource aiming to help bring order to this chaos.

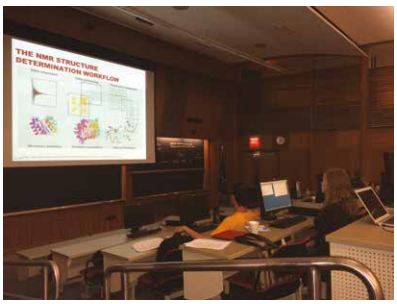
'DNA is the blueprint of life', goes the oft-repeated phrase. Yet few who hear it ever delve deeper to ask what that blueprint is actually for, to hear about proteins, the fascinating machinery, scaffolding, and sensors which are encoded by that string of information. Present in every cell, essential for life itself, with a dizzying range of structures, shapes and functions, proteins are the difference between the dead blueprint and the living factory.

Yet for all the ubiquity of proteins, there remain many questions that remain difficult to answer. One of the most basic questions is, quite simply, 'what does a protein look like?' Proteins are, of course, too small to see by eye, even with the most powerful of light microscopes. Thus, scientists have been working hard to develop other methods of determining the structure and shape of proteins. Nuclear magnetic resonance, or NMR, is one of these methods, and it can be thought of as the science of hitting atoms with a changing magnetic field and listening to the radio waves that they produce. Two of the unique capabilities of NMR (compared to X-ray crystallography, for example), are the ability to investigate disordered systems and to characterise dynamics.

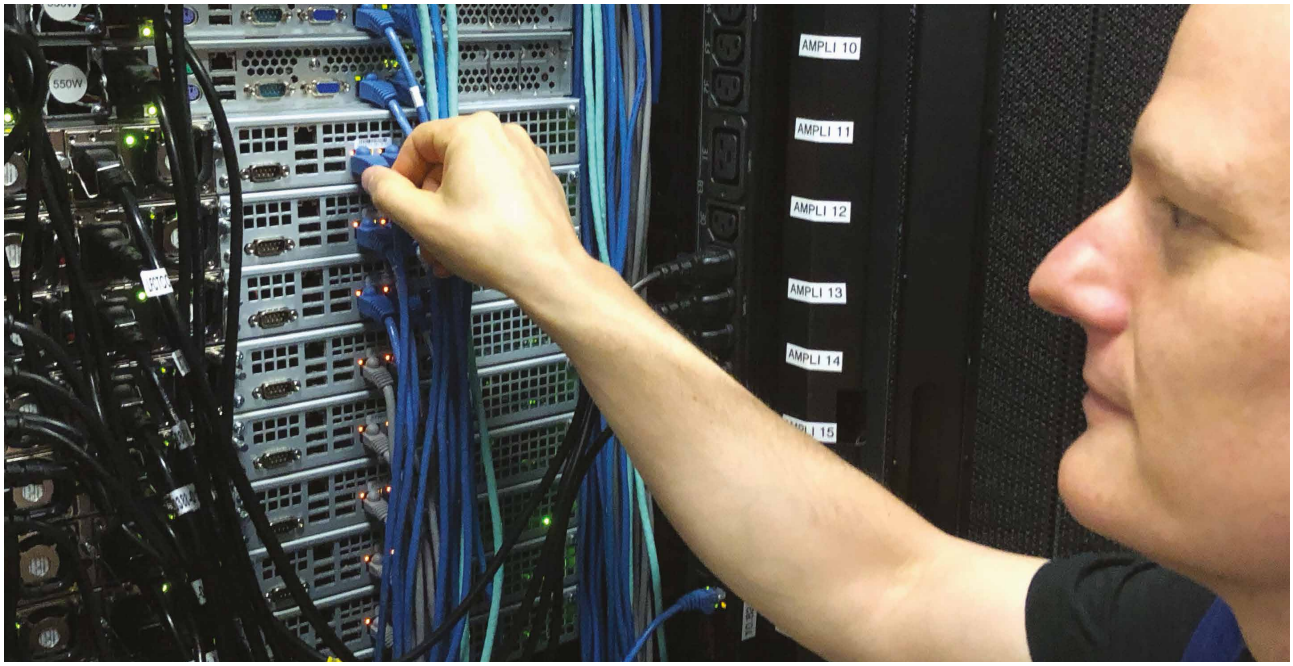
Deconvoluted

This is, of course, an oversimplification. Atomic nuclei surrounded by a strong magnetic field will tend to take up an aligned magnetic direction, something which occurs in all atoms with odd numbers of protons and neutrons. Introducing a second, weaker magnetic field will disrupt that alignment, and doing so using an oscillating field will lead to transient pulses of disruption. The nuclei respond by emitting an electromagnetic signal, which can then be detected by the NMR machine. Most importantly, the frequency emitted is dependent on the atomic environment – a carbon nucleus bound to four hydrogens will emit a different signal to one bound to three hydrogens and a hydroxyl group. This is the attribute which allows NMR researchers determine chemical structures.

Proteins are complex molecules and thus the NMR signal from a protein will consist of a number of different signals mixed together. Pulling these signals apart to determine which belongs to which nuclei is a difficult process, requiring separation by mathematical techniques such as Fourier transforms (which convert a mixture of waveforms into discrete peaks) or maximum entropy reconstruction, which is often combined with sparse sampling (deliberately taking fewer readings than would normally be required). Nor is this enough to determine a structure. Thus, a researcher will need to use several different NMR methods (with delightful names such as COSY, NOESY and TOCSY) in order to fully determine a protein structure. This cross-checking and signal assignment will often take up the majority of a researcher's time.



‘To set up a new NMR lab from scratch, you need months to find, assemble, and install the software, and technical expertise to maintain it.’



As may already be apparent, NMR is a complicated and heavily computer-dependent field of research, generating vast reams of data which require specialised software to interpret, visualise, and understand. This has led to a proliferation of software variants, most of which have been developed by academic groups in response to their own particular needs. Keeping an overview of this multitude of software options is made even more complex by development cycles, forking of projects to develop new variants, or the eventual discontinuation of development when the researchers graduate, retire, or simply run out of funding.

While this complexity is rarely a problem for those working on their own software, it does challenge those who follow after. Simply finding the ideal program is an ordeal in itself; scientific journals alone contain citations of hundreds of different software packages. To further complicate matters, the value of scientific discoveries increasingly rests on their ability to be reproduced, which in turn requires that the software used to make those discoveries is available for other researchers. Yet the range of software versions and oft-lacking archiving processes in academic laboratories means that software, particularly that used in older publications, is difficult to find.

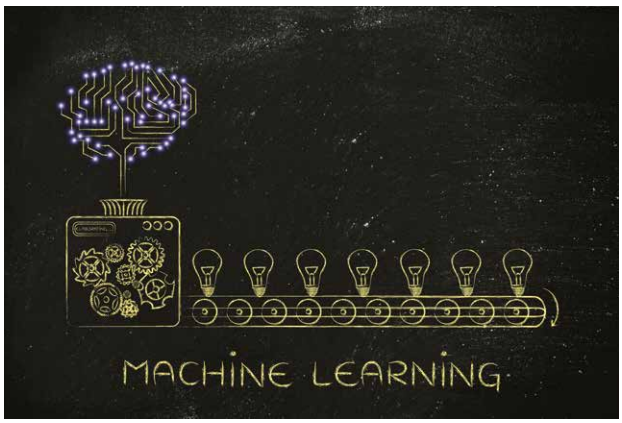
Bringing Order to the Mix

This difficulty caught the attention of Professor Jeffrey C. Hoch of the University of Connecticut. Having been part of the NMR research field for over four decades, he had noticed the increasing difficulty which new entrants had with developing or identifying ideal software for their needs. ‘To set up a new NMR lab from scratch, you need months to find, assemble, and install the software, and technical expertise to maintain it’ comments Dr Hoch.

To help offset this steadily-increasing difficulty, Dr Hoch and his collaborators used a grant from the National Institutes of Health to establish the NMRbox, a set of virtual machines dedicated to the needs of NMR researchers. Hosted on a central server, it provides access to over one hundred NMR software packages which run the gamut from visualisation to validation. A detailed registry of all software currently available is provided, thus allowing newcomers to the NMR field to quickly find the ideal program. It also automatically links to protein structures and publications which have been generated using those programs to allow the comparison of different packages.

NMR work uses large data sets and requires computationally challenging interpretation to determine protein structures. This is often beyond the capabilities of academic laboratories, or requires long calculation times even if it is possible. NMRbox solves this problem by providing a ‘virtual machine’ for each researcher who logs in. This acts as a personal computer environment within the server in which they have access to all of the required software, yet the software itself runs on the high-performance equipment located in the server station. This essentially offloads the difficult work onto the dedicated (and expensive) equipment in the central location, while scientists simply need to download the results when they are available.

This centralisation of software also provides efficiency gains. The dedicated IT support frees scientists from the struggles of understanding why each program is refusing to work, while central upgrading processes ensure that the current version is always in



play. This allows time-pressed researchers to focus directly on their work. Moreover, the NMRbox platform explicitly stores older versions of each package, allowing older papers to be accurately reproduced.

Server-based approaches are rapidly growing in popularity in all areas of computing – we simply consider the many things are now proudly advertised as being 'in the cloud'. This has also proven true for NMRbox, which is going from strength to strength. The user-base now covers researchers from over 33 countries, with more expected in the coming year. This rapid rise in popularity has prompted the developers to double their computing capacity in the coming year, turning NMRbox into an essential part of any NMR research program.

The Magnet Gap

Although systems such as NMRbox allow researchers from across the world to examine their NMR data, it does not help with the act of gathering the data itself. For this, researchers need access to an NMR machine, a large and expensive piece of equipment which requires liquid nitrogen and helium to maintain powerful magnets and where the strength of the magnetic field puts a hard limit on the resolution which can be achieved. As Dr Hoch notes, 'Sensitivity and resolution are key limiting factors in the application of NMR to challenging biomolecular systems, with higher magnetic fields improving both sensitivity and resolution.'

The next generation of NMR machines are already in development, working at 1.2 GHz, the equivalent of a magnetic field strength of 28 tesla (for comparison, the strength of a typical fridge magnet is around five milli-tesla). This exceptional magnetic strength means that the next generation will be able to detect far more interactions than ever before, particularly necessary for research into unstructured proteins or large complex biomolecules.

The world of research is a highly competitive one, with fierce rivalries not only between scientists but between nations. The level of funding and the equipment available to researchers within a particular region can spur the creation of a centre of excellence or force frustrated scientists to move abroad.

These decisions connect the political and the scientific arenas, and are the source of much discussion. One of these factors is known as the 'magnet gap', the difference in regional availability for the upcoming generation of NMR machines. The instruments are extremely sensitive but also extremely expensive, and efforts to fund the next generation of machine within the United States have lagged those in other countries. At the same time, around ten instruments have been ordered by various locations within Europe.

Expert scientists within the USA are already beginning to worry about this. 'In addition to the competitive disadvantage that investigators in the US will face when European scientists gains access to 1.2 GHz instruments,' notes Dr Hoch, 'students and trainees seeking access to state-of-the-art instrumentation will be forced to leave the US for training, resulting in a brain drain.' This process has long been occurring from less-funded countries to research titans such as the USA and European Union – however the prospect of researchers fleeing the USA is an uncomfortable turn-around for scientists and politicians alike.

Implications for Machine Learning

Professor Hoch predicts that there is 'an explosive growth of machine learning on the horizon.' The study of how systems can automatically learn from data without being explicitly programmed to do so has huge and far-reaching implications for medical science. One potential barrier, however, is the lack of abundant training data, and here NMR lags behind other fields. There are public repositories of NMR data, one of which Professor Hoch is co-head (Biological Magnetic Resonance Data Bank – BMRB – based at the University of Wisconsin), but the amount of data deposited is minuscule compared to the amount of NMR data collected. As of yet, it is nowhere near the level of 'big data'. One hope is that NMRbox will help make it easier for investigators to deposit their data in BMRB, thus taking forward this emergent field of research.

The Box of the Future

The NMRbox platform is already proving to be highly popular with researchers in the protein structure field. Yet Dr Hoch and his collaborators are not standing still, they intend to include yet more features into the system to assist with collaborative work and experimental reproducibility. With workflow metadata, processes for Bayesian inference and overarching systems to combine multiple software platforms, there is no lack of things to do.

Meanwhile Dr Hoch is wholly encouraging for all those who wish to get starting in NMR research via his platform system. Registration is free, he notes, so 'users can kick the tires and figure out which method is best.'

Meet the researcher

Professor Jeffrey C. Hoch
Department of Molecular Biology and Biophysics
UConn Health
Farmington, CT
USA

Part of the NMR research scene for over four decades, Professor Jeffrey C. Hoch of the University of Connecticut is a well-established expert in this field. Starting with a PhD in physical chemistry from Harvard University, he has risen through the academic ranks to his current role in the Department for Molecular Biology and Biophysics. During this time, Professor Hoch has managed to appear on over 100 publications, pen several books, and accumulate a long list of invited talks and awards, while also fitting in the role of Director at the National Center for Biomolecular NMR Data Processing and Analysis. He is head of the NMRbox initiative.

CONTACT

T: +1 860-679-3566
E: hoch@uchc.edu

KEY COLLABORATORS

The success of NMRbox is the result of close collaboration among teams based at UConn Health and the University of Wisconsin, and an active group of external collaborators.

UConn Health
Mark Maciejewski
Adam Schuyler
Michael Gryk
Ion Moraru
Yulia Pustovalova
Irina Bezsonova
Dmitry Korzhnev
Gerard Weatherby

University of Wisconsin
Pedro Romero
Hamid Eghbalnia
Eldon Ulrich
Miron Livny

External Collaborators
Frank Delaglio (US National Institute of Standards and University of Maryland)
Tatyana Polenova (University of Delaware)
David Rovnyak (Bucknell University)
Hari Arthanari (Harvard Medical School)
Robert Dempski (Worcester Polytechnic Institute)
Elizabeth Bafaro (Worcester Polytechnic Institute)

FUNDING

National Institutes of Health:
P41GM111135 (in support of NMRbox)
R01GM123249 (in support of signal processing research using NMRbox)

FURTHER READING

K Bourzac, How to Track Metabolites in Tissues Using NMR; The Scientist Magazine, 2018, 184.

MW Maciejewski, et al, NMRbox: A Resource for Biomolecular NMR Computation, Biophysical Journal, 2017, 112, 1529–1534.

JC Hoch, Beyond Fourier, Journal of Magnetic Resonance, 2017, 283, 117–123.

M Mobli, MW Maciejewski, AD Schuyler, AS Stern, JC Hoch, Sparse sampling methods in multidimensional NMR, Physical Chemistry Chemical Physics, 2012, 14, 10835–10843

M Mobli, JC Hoch, Nonuniform sampling and non-Fourier signal processing methods in multidimensional NMR, Progress in Nuclear Magnetic Resonance Spectroscopy, 2014, 83, 21–41.

UCONN HEALTH

SHEDDING LIGHT ON BIOMEDICAL HOT SPOTS WITH CUTTING EDGE IMAGING

Professor Ulrich Flögel and **Dr Sebastian Temme** at the University of Düsseldorf are pushing the boundaries of a sophisticated imaging technique. This non-invasive tool can accurately visualise damaged tissue and different cell types in real time. The technology they are developing has huge implications for early and sensitive detection of many diseases including cardiovascular disease, arthritis, and neurodegenerative brain diseases.

Magnetic resonance imaging, or MRI, is a powerful tool that uses strong magnetic fields and radio waves to produce detailed images of the inside of the body. MRI offers many benefits over other imaging techniques – it is patient-friendly as it is non-invasive, and also gives extremely clear, detailed images of soft-tissue that other imaging techniques cannot achieve. MRI scans can be used to examine almost any part of the body, such as the brain and spinal cord, heart and blood vessels, bones, joints and internal organs.

The most commonly used MRI scanning technique typically works by harnessing the magnetic properties of hydrogen atoms to build these images of the body. Certain atoms, including hydrogen atoms, can absorb and emit energy in the form of radio waves when they are in a strong magnetic field. Hydrogen atoms are the most commonly used in MRI as they are present in water and fat and are abundant within the body.

In recent years, fluorine MRI (^{19}F MRI) has also generated a lot of scientific interest. This type of MRI scan takes advantage of the magnetic properties of fluorine atoms in molecules instead of hydrogen to build images of the body.

Fluorine is naturally occurring and present in vanishingly small amounts in our bodies, which means that any detected signals from fluorine are highly specific – because there is virtually no background signal.

The signal emitted from fluorine is very similar to hydrogen. This not only allows similar slightly modified hardware to be used for both, but by merging fluorine-based ^{19}F MR images with the matching MR images using hydrogen this enables the ^{19}F signal to be precisely identified anatomically. Building on this imaging system, Professor Flögel and Dr Sebastian Temme at the University of Dusseldorf are pioneering the development of a sophisticated ^{19}F MRI tool which holds promising potential applications for diagnosis and treatment of a wide variety of diseases.

Creating Contrast Between Inflamed and Healthy Tissue

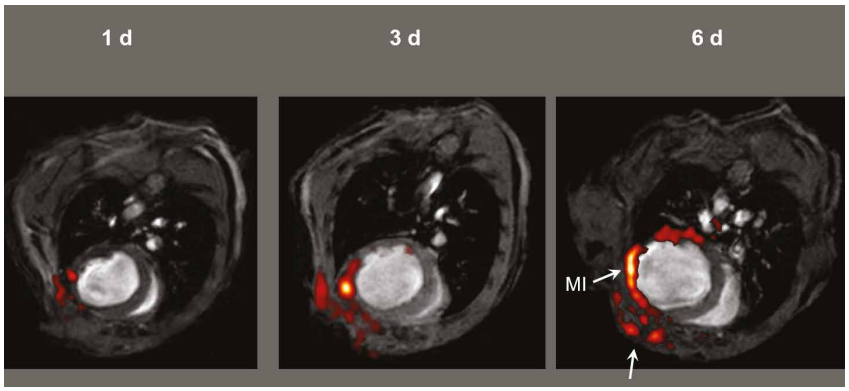
Inflammation occurs when the body defends itself against invading microbes or to repair damaged tissue. Inflammation is associated with many diseases including atherosclerosis, inflammatory bowel disease, neurodegenerative brain diseases and ischemic heart disease. The exact

3D ^{1}H/^{19}F MRI imaging of an arthritic knee joint. CREDIT: Supplemental Movie S1: Science Translational Medicine, 2012, 4, 146ra108.

diagnosis of these diseases, especially in their early stages, currently poses a challenge: inflamed areas of the body are notoriously difficult to distinguish from healthy tissue.

Changing this is Professor Ulrich Flögel, Head of Magnetic Resonance Imaging and Spectroscopy, Molecular Cardiology, and Professor for Experimental Cardiovascular Imaging at the Heinrich-Heine University of Düsseldorf, Germany. Excitingly, Professor Flögel has developed MRI technology that uses a fluorine-containing tracer, or contrast agent, that specifically targets inflamed areas of the body, and thus can accurately visualise 'hot spots' of tissue damage by inflammation.

‘Since ligands and targets can be easily adapted to a variety of problems, this approach provides a general and versatile platform for molecular imaging which strongly extends the frontiers of MRI.’



$^{1}H/^{19}F$ MRI imaging after myocardial infarction (MI) – a heart attack. CREDIT: Circulation, 2008, 118, 140–48.

In a 2008 study, the team used a tracer synthesised from perfluorocarbons – a family of unreactive chemicals. Professor Flögel’s tracer of choice was perfluoro-15-crown-5 ether, whose chemical properties make it ideally suited to ^{19}F MRI. Insoluble in water, the compound first needed to be emulsified so that it could be injected into the bloodstream. The researchers optimised the size of the emulsion particle with the aim that it would be selectively taken up by the cells of the immune system.

The team injected the emulsion particle containing the contrast agent into the bloodstream of two different groups of mice that suffer from inflammation. By overlaying the MRI images using their perfluorocarbon tracer with complementary high-resolution hydrogen-based MRI images, the team precisely revealed time-dependent accumulation of the tracer in the injured tissue.

As Professor Flögel explains, these ^{19}F signals, ‘can be unequivocally identified as background-free hot spots’ of damage by inflammation. The team convincingly showed that the contrast agent was taken up efficiently by monocytes and macrophages – two white blood cells of the immune system that play a key role in inflammation. The tracer was preferentially taken up by these white blood cells circulating in the bloodstream and accumulated in the lymph nodes – the body’s white cell-producing organs.

Visualising the Immune Response

Professor Flögel joined forces with immunobiology specialist Dr Sebastian Temme and the pair set out to develop the tool further. Using the same ^{19}F-perfluorocarbon tracer particles, they showed that the ^{19}F MRI signal was directly proportional to the amount of contrast agent taken up by the white blood cells.

The team’s finding suggests that not only is it possible to clearly detect specific areas of inflammation, but it is also possible to assess the severity of inflammation damage. This powerful tool holds promise for the early detection of inflammation-related disease, enabling timely treatment and the capability to monitor treatment regimes.

Potential for Graves’ Disease

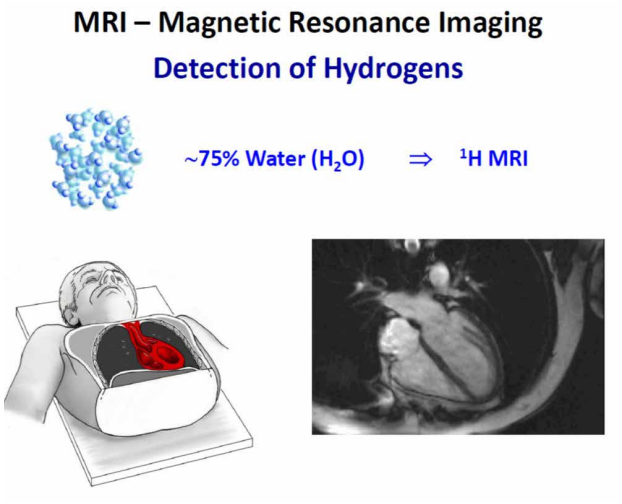
Their effective imaging tool has a wide range of therapeutic potential. Indeed, in a recent study, Professor Flögel demonstrated its benefit for Graves’ eye disease. An autoimmune condition, Graves’ disease is characterised by the excessive production of thyroid hormone. A common complication is Graves orbitopathy, or Graves’ eye disease, where inflammation of the eye can cause discomfort and distorted vision.

A better understanding of the degree of inflammation in the condition would hugely benefit individuals, providing an opportunity to offer patient-specific treatment. Professor Flögel set out to develop an MRI-based approach to track the inflammation using mice with a form of Graves’ eye disease.

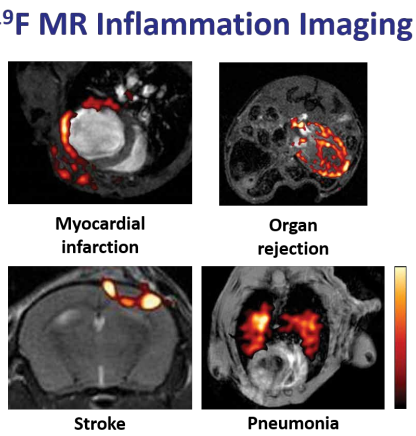
Employing his ^{19}F-perfluorocarbon tracer MRI technology, Professor Flögel detected structural changes in the eye – increased swelling and increased fatty deposits – as well as accurately visualising immune cell infiltration to inflamed areas within affected eyes. Professor Flögel argues that this approach of monitoring immune cell infiltration and structural changes to soft tissue can be translated to other autoimmune conditions, and thus offers a broad range of applications.

Visualising Blood Clots

Forging the way ahead, Professor Flögel and Dr Temme have gone on to show that specific, active targeting of ^{19}F-perfluorocarbons can be exploited to visualise other clinically-relevant



CREDIT: Christine Opfermann-Rüngeler.



CREDIT: Circulation, 2008, 118, 140–48, The American Journal of Transplantation, 2011, 11, 235–44 and Circulation: Cardiovascular Imaging, 2010, 3, 202–10.

structures including blood clots and different cell types.

A thrombosis is a blood clot that forms with a blood vessel, stopping the proper flow of blood around the bloodstream. This can have serious health consequences, including death, but can be prevented by early identification and treatment. Identification of blood clots in their early stages is challenging: small developing blood clots only have a minor impact on blood flow so do not give a clear signal using conventional MRI, and in its early stages is hard to distinguish from healthy tissue.

Professor Flögel and Dr Temme cleverly designed an MRI tracer to specifically detect blood clots in the body. Building on Professor Flögel's successful ^{19}F-perfluorocarbon tracer, they added a small part of a biologically relevant clotting factor – called α2-antiplasmin – a key protein involved in the clotting process. The α2-antiplasmin fragment was bound to the tracer under very mild conditions, ensuring that it remained biologically viable.

Excitingly, the team showed that they could effectively actively target developing blood clots with a diameter of less than 0.8 mm with a very high signal to noise ratio in two groups of mice with thrombosis. Their newly developed method of gently binding a clinically-relevant compound to ^{19}F-perfluorocarbon tracers represented a leap forward. By using the same platform for attaching biologically-active compounds to ^{19}F-perfluorocarbon tracers, the team has also successfully visualised activated platelets and fibrin – both relevant for clot formation. Amazingly, they have also successfully combined the detection of all three simultaneously.

Multicolour ^{19}F MRI

Pushing the frontiers of MRI technology, the team is now focussing their efforts on developing a multicolour MRI tool. To achieve this, they use different ^{19}F-perfluorocarbon particles that each have their own distinct MRI 'signal', where each tracer particle is viewed as a different colour.

In preliminary experiments on human blood clots outside of the body, Professor Flögel and Dr Temme accurately visualised the clot using three colours. Three different ^{19}F-perfluorocarbon particles were targeted to the α2-antiplasmin, the activated platelet tracer and also to the fibrin target described above. This powerful multicoloured MRI approach enables simultaneous detection of multiple targets and has the potential to truly transform MRI.

Cutting-edge Imaging

Professor Flögel and Dr Temme are working to further expand their 'colour palette' to allow the simultaneous detection of multiple clinically-relevant targets. Their work explores new strategies to increase the sensitivity and specificity of the tool. As Professor Flögel explains: 'Since ligands and targets can be easily adapted to a variety of problems, this approach provides a general and versatile platform for molecular imaging which strongly extends the frontiers of MRI.' In particular, the research team is focussed on targeting cardiovascular disease.

They are currently working on a novel combined imaging/intervention approach to cardiovascular precision medicine by tracking inflammation during the development of coronary artery disease. In the near future, this exciting emerging field of ^{19}F MRI has the potential to revolutionise imaging-based cell tracking, imaging of disease and drug development.

Meet the researchers

Professor Ulrich Flögel
Head of Magnetic Resonance Imaging and Spectroscopy, Molecular Cardiology
Heinrich-Heine University of Düsseldorf
Düsseldorf
Germany

Dr Sebastian Temme
Department of Molecular Cardiology, Experimental Cardiovascular Imaging
University Hospital Düsseldorf
Heinrich-Heine University of Düsseldorf
Düsseldorf
Germany

Professor Ulrich Flögel completed a PhD at the Institute of Organic Chemistry, University of Bremen, Germany in 1994. In 1997 he became Research Group Leader in Magnetic Resonance Imaging and Spectroscopy, Molecular Cardiology at Heinrich-Heine University of Düsseldorf, Germany, and in 2014 was appointed full Professor at the same institute. He received the Scientific Award of the Höchst in 1995 and the Edens Award, Heinrich-Heine University Düsseldorf in 2007. Professor Flögel's research focuses on the interplay of function, energetics, metabolism, and inflammation and its role in the development of cardiovascular diseases using innovative multinuclear MRI/MRS techniques.

CONTACT

E: floegel@uni-duesseldorf.de
W: http://www.nmr.hhu.de

Dr Sebastian Temme undertook his PhD in Immunobiology in 2009 at Rheinische Friedrich Wilhelms University before completing postdoctoral studies first at Department of Genetics, Immunobiology, Rheinische Friedrich-Wilhelms-University, Bonn and then at the Department of Molecular Cardiology, Heinrich-Heine University of Düsseldorf. Dr Temme was awarded the Edens Award of Heinrich-Heine University Düsseldorf in 2015. He is currently principal investigator on the DFG-funded grant 'Imaging of Thrombo-inflammatory Processes' until 2020.

CONTACT

E: sebastian.temme@uni-duesseldorf.de
W: http://www.nmr.hhu.de

KEY COLLABORATORS

Professor Rolf Schubert, Freiburg, Germany
Professor Jürgen Schrader, Düsseldorf, Germany
Professor Bodo Levkau, Essen, Germany
Professor Malte Kelm, Düsseldorf, Germany
Professor Gustav Strijkers, Amsterdam, Netherlands
Professor Juerg Schwitter, Lausanne, Switzerland
Professor René Botnar, London, UK
Professor Karlheinz Peters, Melbourne, Australia
Professor Eric Ahrens, San Diego, USA

FUNDING

Sonderforschungsbereich / Collaborative Research Center
Deutsche Forschungsgemeinschaft (DFG), German Research Foundation
Graduiertenkolleg / Research Training Group 1089
National Institutes of Health
Research commission Heinrich-Heine University (HHU) Düsseldorf

FURTHER READING

U Flögel, Z Ding, H Hardung, S Jander, G Reichmann, C Jacoby, R Schubert and J Schrader, In vivo monitoring of inflammation after cardiac and cerebral ischemia by fluorine magnetic resonance imaging, Circulation, 2008, 118, 140–148.

B Ebner, P Behm, C Jacoby, S Burghoff, BA French, J Schrader and U Flögel, Early assessment of pulmonary inflammation by ^{19}F MRI in vivo, Circulation: Cardiovascular Imaging, 2010, 3, 202–10.

U Flögel, S Su, I Kreideweiß, Z Ding, L Galbarz, J Fu, C Jacoby, O Witzke and J Schrader, Noninvasive detection of graft rejection by in vivo ^{19}F MRI in the early stage, American Journal of Transplantation, 2011, 11, 235–44.

U Flögel, S Burghoff, PL van Lent, S Temme, L Galbarz, Z Ding, A El-Tayeb, S Huels, F Bönner, N Borg, C Jacoby, CE Müller, WB van den Berg and J Schrader, Selective activation of adenosine A2A receptors on immune cells by a CD73-dependent prodrug suppresses joint inflammation in experimental rheumatoid arthritis, Science Translational Medicine, 2012, 4, 146ra108.

FOETAL MAGNETIC RESONANCE IMAGING AND THE DIAGNOSIS OF CONGENITAL HEART DEFECTS: A NEW APPROACH

Foetal magnetic resonance imaging (MRI) is increasingly used for the imaging of many types of medical conditions and research continues into its use. However, MRI of the foetal cardiovascular system has encountered significant difficulties due to the constant movement caused by the beating of the heart which results in blurring of images. **Dr Björn Schönnagel** and his team of the University Medical Centre Hamburg-Eppendorf, Germany, have recently introduced a newly developed Doppler ultrasound (DUS) device that is compatible with MRI and allows registration of the foetal heartbeat in utero. This information is mandatory to synchronise the cardiac movement with MR image acquisition, and therefore allows successful high-quality imaging of the human foetal heart.

The foetal MR imaging team: Björn Schönnagel (left), Dr Manuela Tavares de Sousa (middle), Professor Jin Yamamura (right). Credit Björn Schönnagel.

Congenital heart disease (CHD) represents one of the most common birth defects in foetuses with no chromosomal abnormalities and is a leading cause of infant mortality and sickness. Currently, the incidence of CHD is 6–9 per 1,000 live births.

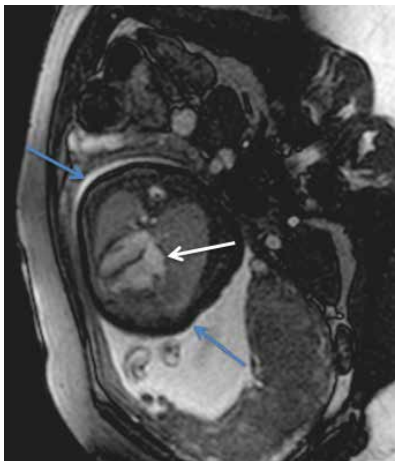
Monumental breakthroughs in surgery and diagnostics have been achieved in the past century, leading to an increased survival rate for newborns with severe CHD. As such, over 85% of babies born with a CHD now live into adulthood. However, the accurate diagnosis of CHD in the prenatal stage is often crucial for successful short- as well as long-term treatment – and ultimately survival. Critically, the development of diagnostic imaging modalities is likely to be invaluable for medical teams in supporting parents and informing the planning of postnatal care.

Existing Approaches in Foetal Heart Imaging

Currently, echocardiography (that is, ultrasound of the heart) is used to create images of the foetal heart during pregnancy. During this procedure, a small probe is placed on the mother's abdomen, which sends out ultrasonic sound waves at a high frequency. When the probe is placed in certain locations and at certain angles, the ultrasonic sound waves are reflected by the mother's and baby's tissues (also the baby's heart), depending on their

‘We believe that this novel technique of direct foetal cardiac gating is a promising approach in the prenatal evaluation of the foetal cardiovascular system, especially in cases where echocardiography is limited or inconsistent.’

Positioning of the MR-compatible Doppler ultrasound device before foetal MRI examination. Credit Björn Schönnagel.



MR images with sharp delineation of the foetal heart (white arrow) using the MR-compatible Doppler ultrasound device. The foetal thorax (blue arrows) is surrounded by maternal pelvic organs.

reflection properties. The reflected waves are recorded and displayed as an image, thus providing images of the foetal heart which are used for diagnostic purposes.

However, not all cases of CHD can easily be detected by echocardiography. Especially in the later stages of pregnancy echocardiography can be limited as a technique due to the position of the foetus, calcification or hardening of the foetal bones, maternal obesity, and the reduction of the volume of amniotic fluid. This means that new techniques could be helpful in improving the accurate detection and classification of heart defects during this later phase of pregnancy.

Magnetic resonance imaging (MRI) is a medical imaging technique that uses a strong magnetic field and radio waves to generate ultrafast images of the organs of the body. To date, MRI has been used for the imaging of many different foetal organs, especially the central nervous system. In many ways it can be superior to ultrasound, e.g., imaging planes can be easily acquired in any anatomical orientation, not being dependent on the acoustic window like ultrasound, and image quality is not affected by issues such as foetal positioning, interference of bone and only in a lesser extent to maternal obesity. The performance, accuracy, and quality of MRI improve with gestational age, in contrast to foetal ultrasound, which becomes progressively more difficult. In addition, MRI can also provide accurate functional information, such as blood flow characteristics. However, MRI of the foetal cardiovascular system has been hampered due to a number of technical difficulties. The greatest concern is that due to the constant movement caused by the beating of the foetal heart, blurring of images occurs if the image acquisition and the cardiac cycle are not precisely synchronised. In the adult patient, synchronisation of the heartbeat and magnetic resonance image acquisition is achieved by triggering the heartbeat (known as cardiac gating) using conventional electrocardiogram (ECG; with surface electrodes on the patients’ chest wall) or pulse oximetry. However, due to the inaccessible position of the foetus in utero, assessment of ECG or pulse oximetry information to directly trigger the foetal heartbeat is impossible.

With the help of Dr Fabian Kording (physical engineer), the foetal imaging team of Dr Björn Schönnagel, Professor Jin Yamamura and Dr Manuela Tavares de Sousa of the University Medical Centre Hamburg-Eppendorf, Germany, have recently developed an MR-compatible Doppler ultrasound (DUS) device that allows successful foetal cardiac gating for high-quality MRI of the human foetal heart. The developed DUS device is based on the principles of a cardiotocogram, which is used in pregnancy to monitor foetal heart rate and uterine contractions. The probe of a cardiotocogram is placed on the abdomen of the pregnant woman and uses Doppler ultrasonic sound waves for detecting both the foetal heartbeat and uterine contractions. The scientists modified this conventional probe by replacing several parts of the probe to make it compatible for use in high magnetic field environments such as MRI. In a series of experiments, they have been able to confirm the effectiveness of this technique for foetal cardiac gating.

Early Developmental Work

A foetal sheep animal model was the first important step in the development of the MR-compatible DUS device. Together with the MR-compatible DUS probe dedicated software components were developed for optimisation of foetal heartbeat recognition. To

Planning an acquisition of MR images using the MR-compatible Doppler ultrasound device. Credit Björn Schönnagel.

demonstrate the effectiveness of the MR-compatible DUS device, the researcher team performed foetal heart triggering in five foetal sheep as part of their early developmental work. The scientists placed the DUS probe on the abdomen of the pregnant sheep. When a constant DUS signal (that is, a foetal heartbeat) was received, the probe was fixed in place by a belt. In this way, DUS signals were translated into trigger signals by the dedicated software and trigger pulses were conducted to the MR system to allow synchronisation of MR image acquisition with the foetal heartbeat.

Efficacy in Human Foetal Assessment

In an initial study, fifteen human foetuses at late gestational age were examined using the new MR-compatible DUS device for foetal cardiac gating. The DUS probe was fixed on the maternal abdomen to generate trigger signals from foetal cardiac motion during the MR examination. The triggering signals and image quality were analysed, demonstrating reliable trigger signals and high image quality of the dynamic images of the foetal hearts.

Dr Schönnagel and colleagues also looked at the feasibility of foetal phase-contrast-MR angiography, which is a functional MRI method for characterisation of blood flow. The study investigated blood flow haemodynamics of the descending aorta, the largest artery in the human body, in third-trimester human foetuses. Using their DUS device for foetal cardiac gating, they compared foetal blood flow rate assessed by MRI with the reference standard of Doppler ultrasound measurements of blood flow. They found that their MR-compatible DUS device for foetal cardiac gating allows for foetal MR angiography in the foetal descending aorta revealing high correlation with Doppler ultrasound measurements.

The group also used their DUS device for foetal cardiac triggering with MRI to see if they could successfully differentiate normal foetal hearts from foetuses with CHD in comparison to foetal echocardiography as the reference standard. The researchers looked at eight foetuses with a normal heart and four with a CHD. All foetuses were in the third trimester of pregnancy. A variety of anatomical landmarks and quantitative measurements of the foetal heart were assessed for comparison. Direct cardiac gating using the DUS device allowed continuous triggering of the foetal heart. Foetal cardiac MRI and echocardiography revealed an overall consistency of both quantitative and qualitative measures for differentiation of foetuses with normal hearts and foetuses with CHD.

For the first time, Dr Schönnagel and his group were able to show that dynamic foetal cardiac MRI using external cardiac triggering allows diagnosis of CHD and that furthermore, this was in similar in effectiveness to the reference standard of foetal echocardiography. The researchers note the importance of this finding, commenting 'We believe that this novel technique of direct foetal cardiac gating is a promising approach in the prenatal evaluation of the foetal cardiovascular system, especially in cases where echocardiography is limited or inconsistent.'

The Future of Direct Foetal Cardiac Gating

Drawbacks still remain in the use of MRI for foetal cardiovascular imaging. More specifically, the imaging plane needs to be defined before imaging begins and as such, is vulnerable to the effects of foetal movement. Additionally, the foetal cardiac trigger signal may be lost due to foetal movement, necessitating replacement of the DUS probe on the maternal abdomen. The start-up company 'northh medical' is currently on the way to advance and manufacture the DUS device for widespread usage.

However, despite these difficulties, MRI has the exciting potential to aid in the evaluation of foetal CHDs, especially in later-stage pregnancies where foetuses may not be adequately evaluated by Doppler ultrasound alone. The ability to trigger the foetal heart could potentially be a significant technological advance in foetal cardiovascular MRI, aiding in the diagnosis of abnormal foetal heart structure and blood flow in pregnancies where ultrasound is not possible or where the findings of which are inconclusive. The use of MRI paired with the novel device developed by Dr Schönnagel and his team may become a viable alternative to foetal ultrasound in the future, offering better imaging of the foetal cardiovascular system and consequently, diagnosis of CHD worldwide.

Meet the researcher

Dr Björn Schönnagel
University Medical Center Hamburg-Eppendorf
Hamburg
Germany

Dr Björn Schönnagel completed his medical degree in 2008 at Georg August University Göttingen and is now a senior physician in the Department of Diagnostic and Interventional Radiology and Nuclear Medicine at the University Medical Centre in Hamburg, Germany, where he has worked since 2009. Dr Schönnagel is an active researcher as well as practitioner, and a co-founder of 'northh medical GmbH,' the company responsible for the development of the MR-compatible Doppler ultrasound device.

CONTACT

W: https://www.uke.de/english/physicians-and-scientists/arztprofilseite_bj%C3%B6rn_sch%C3%B6nnagel.html and http://northh-medical.com/
P: +49 (0) 40 7410 - 54010
E: b.schoennagel@uke.de

KEY COLLABORATORS

Manuela Tavares de Sousa, MD, Department of Obstetrics and Fetal Medicine
Jin Yamamura, MD, Department of Radiology
Jochen Herrmann, MD, Department of Pediatric Radiology
Carsten Rickers, MD, Department of Pediatric Cardiology
Fabian Kording, PhD, Kai Fehrs, PhD, and Christian Ruprecht PhD, northh medical GmbH

FUNDING

German Research Foundation

FURTHER READING

F Kording, et al, Doppler ultrasound triggering for cardiovascular MRI at 3T in a healthy volunteer study, Magnetic Resonance in Medical Sciences, 2017, 16, 98–108.

M Tavares de Sousa, et al, Dynamic fetal cardiac magnetic resonance four chamber view imaging using Doppler ultrasound gating in the normal fetal heart and in congenital heart disease: comparison to fetal echocardiography, 2018, Ultrasound in Obstetrics and Gynecology, doi: 10.1002/uog.20167.

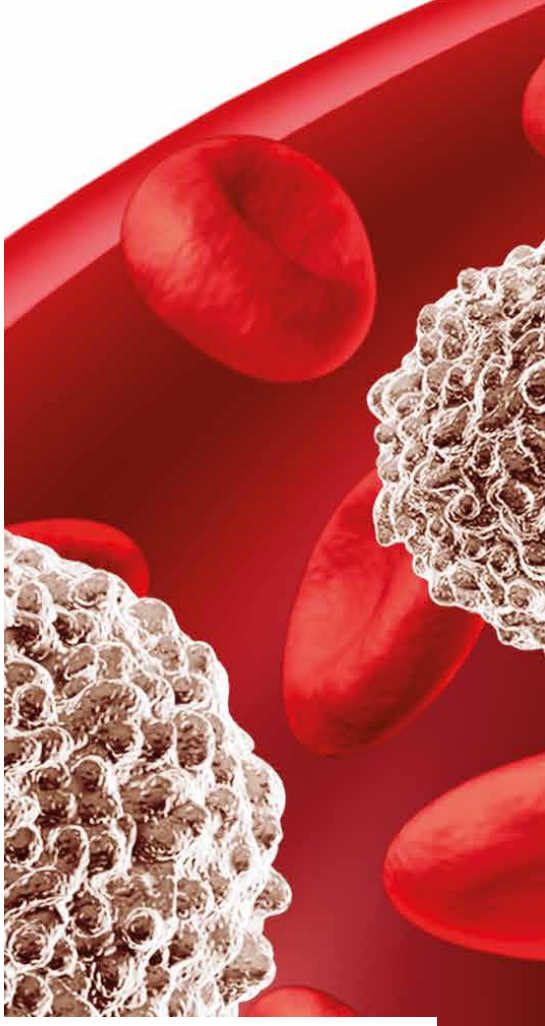
F Kording, et al, Dynamic fetal cardiovascular magnetic resonance imaging using Doppler ultrasound gating, 2018, Journal of Cardiovascular Magnetic Resonance, doi: 10.1186/s12968-018-0440-4.

BP Schoennagel, et al, Fetal blood flow velocimetry by phase-contrast MRI using a new triggering method and comparison with Doppler ultrasound in a sheep model: a pilot study, Magnetic Resonance Materials in Physics, Biology and Medicine, 2014, 27, 237–244.

BP Schoennagel, et al, Fetal dynamic phase-contrast MR angiography using ultrasound gating and comparison with Doppler ultrasound measurements, 2019, European Radiology, doi: 10.1007/s00330-018-5940-y.

GOING WITH THE FLOW: NEW METHODS FOR TREATING CARDIOVASCULAR DISEASE

When an artery becomes blocked or damaged, a mechanical scaffold called a stent is often implanted into the vessel to improve blood flow. However, metallic stents can cause re-narrowing at the sites where they are implanted. This process is known as restenosis, which can lead to lethal complications. **Dr York Hsiang**, Professor of Surgery at the University of British Columbia, and his team use microengineering techniques to develop novel stents that can better detect restenosis, and treat it earlier when it occurs.



Cardiovascular Disease: A Growing Problem

Cardiovascular disease is a leading cause of death worldwide, and includes a number of conditions including heart disease, strokes, and diseases of the peripheral circulation. According to the British Heart Foundation, heart and circulatory diseases cause more than a quarter of all deaths in the UK. An average of 460 people each day die from these diseases, and survivors can live with serious long-term complications.

A major cause of cardiovascular disease is a narrowing of the arteries caused by the build-up of fatty plaques on the artery walls, a process known as atherosclerosis. These plaques harden over time, narrowing the arteries and limiting the flow of blood. Atherosclerosis can occur anywhere in the body, and different conditions can develop based on where it occurs – the most common being coronary heart disease (CHD), in the case of atherosclerosis of the coronary arteries.

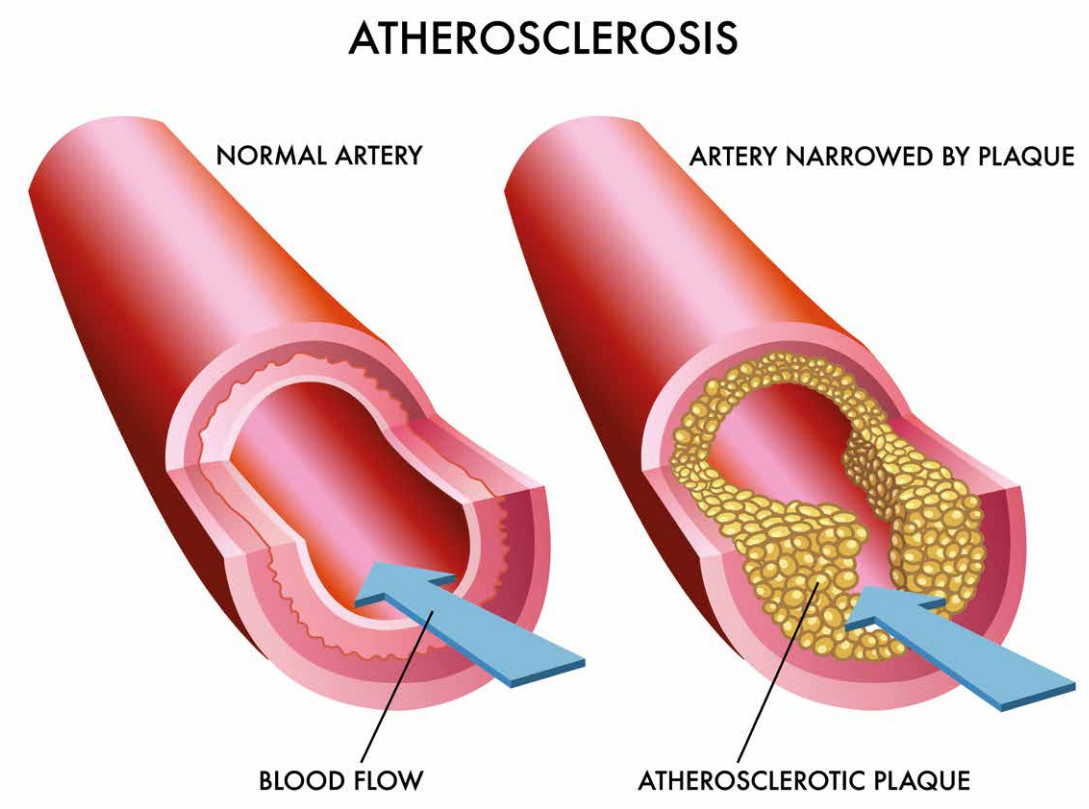
Stents as Arterial Scaffolds

A procedure known as balloon angioplasty is effective in treating atherosclerotic plaques. This procedure involves inserting a small deflated balloon attached to a catheter into the atherosclerotic area, then inflating the balloon which breaks up the plaque, to increase the diameter of the affected blood vessel and improve blood flow. Following the procedure, the blood vessel may re-constrict. If this happens, a metal scaffold – a stent - can be inserted into the site of the angioplastied area to hold the blood vessel open.

Although widely used and effective, stents are foreign metal objects. When placed within the blood vessel, the body's natural response is to reject the foreign substance by inflammation. This inflammatory response from the blood vessel wall occurs due to the immune system responding to damage caused by implantation of the stent. Over time, the build-up of inflammatory tissue within the stent, leads to re-narrowing of the stented blood vessel. This event,

called restenosis, occurs in 20–30% of patients and can lead to serious complications; in some cases, restenosis can be severe enough to completely block blood flow and cause heart attacks or strokes.

Current approaches to combat restenosis are effective but still have limitations. Stents can be coated with drugs that suppress the immune system to help prevent restenosis. However, the use of drug-eluting stents has been linked to an increased long-term risk of death due to sudden clotting of the stent. With 3 million stents being implanted worldwide, new, more effective methods to address restenosis are needed.



Diagnosing Restenosis

Dr York Hsiang, Professor of Surgery at the University of British Columbia, and his team have been working on new stent devices for the early diagnosis of restenosis. Dr Hsiang and his team's recent projects include the development of a stent that can monitor early signs of restenosis in the patient.

Current methods for diagnosing restenosis are limited in their effectiveness and can be extremely costly. Primary methods used to detect restenosis include imaging techniques such as magnetic resonance imaging and X-ray angiography. These methods are expensive, cannot give real-time information regarding the condition, and in some cases, do not give information about where restenosis has occurred. They also require the patient to be exposed to high doses of ionising radiation. The most effective current diagnostic techniques use catheters to specifically image the stented area in real-time but these are costly and cause patient discomfort.

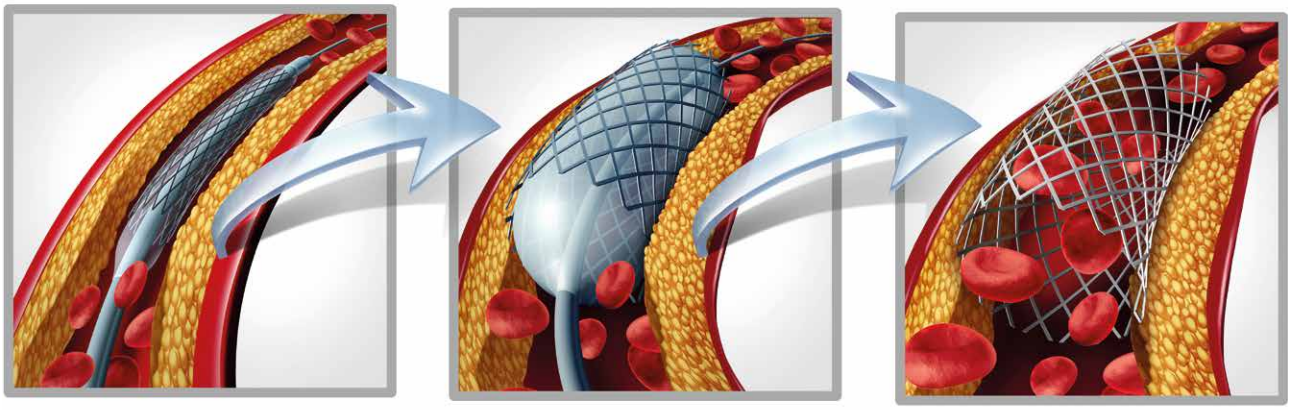
Smart Stents

Dr Hsiang and his team recognised the need for a non-invasive and rapid method to diagnose restenosis following stent implantation. In order to address this issue, they looked to microengineering approaches, aiming to produce a stent that uses integrated microelectronics to monitor the progression of the disease.

In a 2018 study, Dr Hsiang and his colleagues designed and tested a stent able to monitor the development of restenosis in real time but in a non-invasive way. This 'smart stent' is manufactured like a normal stent and can be implanted using standard balloon catheters, and is composed of pressure sensors joined by a conductive metal frame.

The metal part of the stent, as well as keeping the blood vessel open like a traditional stent, acts as a miniature antenna that can relay information about the blood pressure inside the stent. Readings can be taken by placing an external antenna against the skin that emits radio waves, powering the sensors and providing data to the external antenna regarding blood pressure. A drop in blood pressure indicates that the stent is becoming blocked and treatment may be needed.

The team tested the device in the lab, using salt water flowing through plastic tubing as a simulation of the cardiovascular system. They found that they could measure changes in the properties of the stent based on the pressure inside the graft. They then tested the device using an animal model, by sewing a graft containing the sensor-integrated device into a pig artery. The team found that when they mechanically restricted blood flow through the artery, they could detect these changes using the antenna.



Example of a stent used to open a blocked artery

The Road to the Clinic

The advantages of this device are that real time information about restenosis can be taken without the need for invasive or high-energy diagnostic procedures. The device may also be used to detect restenosis at an earlier stage than other diagnostic techniques, with less danger to the patient.

The next steps for the group are optimising the device for use in the clinic. The team aims to improve the sensitivity of the device to detect more subtle changes in pressure that may be seen during early restenosis. Their overall aim is to be able to diagnose restenosis before symptoms occur. They then hope to integrate the antennae device with internet functionality, allowing in-stent pressure to be monitored at home.

Treating Restenosis

Although the smart stent was capable of monitoring blood flow without using invasive methods, the current treatments for restenosis remain invasive and potentially dangerous. In cases where stents are partially blocked, a second balloon angioplasty may be required. In cases of full blockage, more complicated surgeries are required, or a new stent may need to be inserted. All these surgeries require catheterisation, carrying with it a risk to the patient and significant discomfort. The group therefore investigated the use of a smart stent for treating restenosis.

Recent studies have shown that hyperthermia, or the application of mild heat, could be effective for the suppression of restenosis. Studies have heated an implanted stent using various means to prevent restenosis. One method involves inserting a catheter into the stent and inducing heat in the stent using an electromagnetic field. However, this is invasive, and the high power used poses a health risk to the patient. Other methods such as external beam radiation to heat the stent have been tried, but again pose a risk to the patient due to the use of ionising radiation.

Through their work with the smart stent, Dr Hsiang and his team found that stainless-steel antenna stents can produce heat when excited with radiofrequency waves. This finding led to a very recent study in which the team tested a smart stent capable of heating without the need for invasive catheter procedures. The stent, like the previous smart stent, used a radiofrequency inductor, which heats up when exposed to radio waves of a specific frequency.

To test their device, Dr Hsiang and his team implanted an artificial graft containing the stent into the femoral artery of pigs and monitored the temperature of the stent using both fibre optic sensors attached to the artificial graft, and infrared imaging. They found they could increase the temperature by increasing the power of the radiofrequency waves supplied to the implant. This showed that use of an actively heating stent was clinically possible, and sets the stage for future studies to improve and optimise the system for clinical use.

Where Next?

Based on the promising results from their studies, future work by the team will focus on bringing these inventions into the clinic to provide practical and safe diagnostic and treatment tools for patients with stents. Future studies will also aim to integrate these devices with anti-thrombotic coatings to combine the benefits of these coatings with the benefits given by the smart stents.

By using microengineering techniques, Dr Hsiang and his team have provided new diagnostic and treatment techniques for restenosis. With 3 million stents being implanted worldwide each year, new methods dealing with the consequences of stent implantation will no doubt improve patient quality of life through more reliable and less invasive methods for their management.

Meet the researcher

Dr York Hsiang
Professor of Surgery
Department of Surgery
University of British Columbia
Vancouver, BC
Canada

Dr York N Hsiang completed his MB ChB at the University of Otago, before embarking on General and Vascular Surgery training at the University of British Columbia in Vancouver. Following this, he undertook research training with an MHSc degree in Clinical Epidemiology and Biostatistics and a research fellowship at Harbor-UCLA Medical Center in California. He was promoted to Professor of Surgery in 2000. His research focuses on the development of novel stents, devices that are implanted into damaged or blocked blood vessels to keep them open. Using microengineering approaches, Dr Hsiang's group designs new stents that can treat or detect issues associated with stent implantation using remote methods. Dr Hsiang is the recipient of numerous awards and holds a number of grants for his work. He has published a wealth of literature over the past 30 years concerning the treatment and diagnosis of cardiovascular diseases.

CONTACT

E: york.hsiang@vch.ca

KEY COLLABORATORS

Kenichi Takahata, Department of Electrical and Computer Engineering, University of British Columbia

FUNDING

The Canadian Institutes of Health Research
The Natural Sciences and Engineering Research Council of Canada
The Canada Foundation for Innovation
The British Columbia Knowledge Development Fund

FURTHER READING

GK Yang, YN Hsiang, Primary popliteal vein aneurysm, Clinics in Surgery, 2018, 3, 2076.

X Chen, B Assadasangabi, YN Hsiang, K Takahata, Enabling angioplasty-ready "smart" stents to detect in-stent restenosis and occlusion, Advanced Science, 2018, 10.1002/advs.201700560.

JD Misskey, C Yang, S MacDonald, et al, A Comparison of RUDI and DRIL for the management of severe access-related hand ischemia, Journal of Vascular Surgery, 2015, 62, 535–536.

SM Grenon, J Gagnon, YN Hsiang, Ankle-Brachial Index for Assessment of Peripheral Arterial Disease, *New England Journal of Medicine*, 2009, 361, e40.

CYLERUS: AN INNOVATIVE APPROACH TO VASCULAR DRUG DELIVERY

Prosthetic vascular grafts for dialysis access have a limited lifespan and usefulness due to inflammation, infection and especially blood vessel narrowing at the site of graft implantation. Consequently, patients need repeated surgeries to revise or replace the vascular conduits, which is an expensive and difficult procedure. **Cylerus** is an innovative company dedicated to developing new methods of drug delivery to prosthetic vascular grafts that has developed a novel medical device called the Drug-Eluting Cuff (DEC). The DEC significantly increases the lifetime of these critical access sites while decreasing costs and the harmful consequences to patients of repeated interventions to maintain graft function (patency) to support life-sustaining hemodialysis.

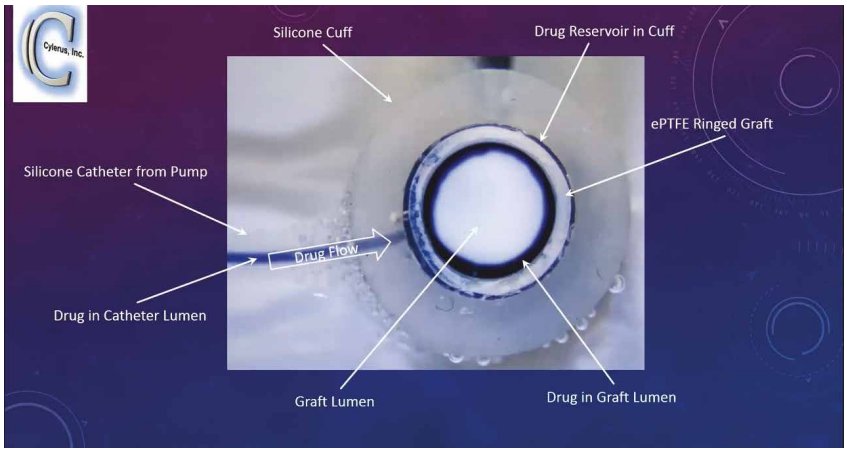
Patients with end-stage renal disease (ESRD) must undergo hemodialysis – filtering of the blood – to eliminate toxins and waste products from their circulation two to three times per week. Currently, there are over 400,000 patients requiring multi-weekly dialysis sessions in the United States and over one million worldwide. During dialysis, a dialyser and a special filter, called an artificial kidney, are used to clean the blood, taking over the function of diseased kidneys that no longer function or work effectively. To enable routine dialysis, the patient undergoes a minor surgical procedure in which vascular access is established that can be used to connect the patient's circulation to the dialyser.

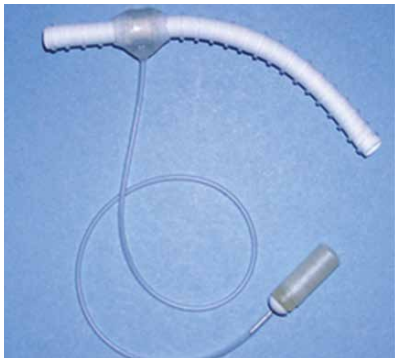
Choices for establishing chronic vascular (blood vessel) access are limited. Currently, the most common access utilised is a fistula – vascular access is made by joining an artery and a vein in the lower or upper arm. A fistula is considered the best choice by many healthcare professionals and patients; however, fistulas cannot be used unless the patient has healthy veins and it can take months for arteriovenous fistulas (AVFs) to mature. Furthermore, about half of AVFs fail to achieve adequate blood flow to sustain effective hemodialysis, requiring further intervention in the patient to establish vascular access.

Another option for ESRD patients to establish vascular access is implantation of a prosthetic (synthetic) graft. This is accomplished by using a soft piece of tubing-like material called ePTFE (expanded polytetrafluoroethylene), known as Teflon® in the unexpanded state, which provides a new blood-carrying conduit between an artery and a vein. The option of utilising a prosthetic graft is attractive as it can be used for dialysis much sooner than an AVF. However, the primary patency (or continued function) of prosthetic grafts is poor as three months after implantation, almost half of these grafts require re-intervention to maintain adequate blood flow to support hemodialysis.

Generally, an obstruction occurs at the outflow tract of the graft due to the abnormal accumulation of smooth muscle cells and connective tissue near the site where the graft is connected to the vein (the anastomosis site). Thus, to re-establish blood flow mechanical (thrombectomy) and pharmacological (thrombolysis) tools are employed to achieve patency and regain the grafts therapeutic function – this is defined by the practitioner as adequate blood flow to perform dialysis.

As vascular access sites fail, either AVF or prosthetic, ESRD patients need to undergo repeated surgical procedures in order to revise, fix or create a new access site. In doing so they face associated risks and complications such as blood clots (thrombosis), central vein stenosis (abnormal narrowing of the blood vessel due to trauma and inflammation) and infection. A number of mechanical approaches, such as the use of hooded ePTFE, and pharmacological, such as heparin-coated grafts, have been used in attempts to prevent or reduce the aggressive proliferation and migration





Porous ePTFE membrane under an electron microscope.

of cells that can occur at the graft site. However, these methods have been mostly unsuccessful. The costs of keeping grafts functioning in dialysis patients are staggering and now exceed $1 billion annually in the US alone.

The Drug-Eluting Cuff Synthetic Graft

The drug delivery Company, Cylerus, aims to improve the utility and functionality of prosthetic vascular grafts with their novel Drug-Eluting Cuff (DEC) delivery device. Cylerus, Inc. was formed in 2007 by Dr Stephen Hanson, a professor and previous chair of the Biomedical Engineering Department at Oregon Health & Science University in Portland Oregon. His goal was to develop new drug delivery methods for increasing the longevity and functionality of prosthetic vascular grafts.

Based in Melbourne, Florida, Cylerus specialises in vascular drug delivery innovations and has developed the DEC that can be combined with an implantable drug pump, and the FDA approved drug, sirolimus. This drug-device combination is intended to prevent cell proliferation at the site where a prosthetic graft is implanted in patients. The DEC is a product of many years of research that originated in Dr Hanson's laboratory at Emory University in Atlanta, GA and subsequently at Oregon Health & Science University (OHSU) in Portland, OR.

Cylerus is headed by President and CEO, Dr Ronald J. Shebuski, Sr, who leads the research and development effort to develop a formulation of sirolimus that is biocompatible with the DEC, catheter delivery tubing and drug pump. Dr Shebuski oversees the preclinical safety and efficacy studies of the DEC sirolimus combination that are the first steps to be completed before the device can be tested in ESRD patients. The DEC is being developed to prevent narrowing downstream of the prosthetic graft implantation site and may lead to more prosthetic graft usage if failure rates can be diminished significantly by the Cylerus approach. This could potentially swing the balance of vascular access from AVFs to greater use of DECs and prosthetic vascular grafts.

The DEC, manufactured for Cylerus by a contract research organisation, utilises standard wall six mm ringed or non-ringed ePTFE as the starting material and the other components are all medical grade silicone. A standard medical grade catheter connects the DEC to a drug-filled osmotic pump to deliver sirolimus to the interior of the graft in a continuous and circumferential manner. The drug pump delivers sirolimus – a cytostatic drug that interrupts the cell cycle and thus prevents excessive cellular proliferation – in a continuous manner. The drug pump can be refilled or replaced on a monthly or quarterly basis.

Sirolimus is delivered through the porous wall of the graft and to the immediate surrounding area, where it remains highly concentrated, preventing cellular and tissue build-up around the connection (i.e. anastomosis) between the graft and the vein. The specialised design of the DEC allows sirolimus to remain concentrated at the graft site while minimising its concentration in the systemic circulation. This is important as sirolimus is also FDA approved as an immunosuppressant drug (Rapamune®) for kidney transplant patients.

However, local DEC delivery of sirolimus provides pharmacologically effective concentrations at and downstream of the graft anastomotic site where it's needed, but systemic levels will be well below immunosuppressive levels of the drug (this has already been demonstrated preclinically). The total amount of sirolimus to be administered to the vascular access site by the Cylerus DEC is approximated at 1-2 mg/month. In comparison, Rapamune® is administered orally at a dose of 2-4 mg/day. The other FDA approved product that contained sirolimus (< 1 mg), to effectively prevent smooth muscle cell proliferation in drug-eluting oronary stents, was the Cypher™ stent.

The DEC and drug pump can be individually manipulated without the prosthetic graft material and

may potentially be used for medical applications aside from hemodialysis. For example, the DEC has the potential to deliver different types (drugs, cells or genes) and concentrations of therapeutic agents singly, in combination or sequentially. The delivery of the drug can also be adjusted or stopped, depending on the needs of the patient.

Promising Results

Dr Prabir Roy-Chaudhury, the Chief of Nephrology at the University of Arizona at Tucson, is a co-principal investigator for the project with Dr Shebuski and brings extensive experience in kidney failure, vascular access and hemodialysis to the Company. Dr Roy-Chaudhury has evaluated the DEC and demonstrated efficient drug delivery through the ePTFE graft wall using a blue dye as a drug surrogate (top figure on the previous page). The DEC provides the drug (sirolimus) to the inside wall of the vascular conduit in a continuous and circumferential manner.

Furthermore, the blood flow in a tube or cylinder is fastest in the middle of the bloodstream and slowest near the vascular wall, where sirolimus is entering the conduit. Thus, the flow dynamics are preferential for sirolimus to have intimate contact with the vascular wall where the smooth muscle cell accumulation occurs. Importantly, sirolimus concentrates at the graft anastomotic site and flows in a continuous and circumferential manner to the inner and downstream wall of the prosthetic graft site and so is an extremely effective means of drug delivery. A high local concentration of the drug is provided where you need it – continuously.

In earlier studies, researchers in the laboratory of Dr Hanson tested the DEC prosthetic graft device in a non-human primate model by measuring blood vessel thickening after 30 days. His research team implanted femoral arterial interpositional prosthetic ePTFE grafts on both sides of the groin. Sirolimus was then infused slowly (2 mg/month) to the graft on one side and vehicle saline on the other, and the results were compared. The researchers found an 80% reduction in smooth muscle cell proliferation at 30 days following graft implantation on the graft side that had been slowly infused with a very low dose of sirolimus compared to the graft side exposed to saline only.

To further test the sirolimus formulation, Dr Shebuski's team conducted a study in pigs over a period of 28 days where the drug was delivered continuously through osmotic mini-pumps at doses of 0.5 and 1.5 mg/month. The researchers found that the drug was stable for 28 days, maintaining a high concentration at the infusion site and a low concentration in the systemic circulation. The researchers now plan to carry out longer-term studies up to 90 days in length, in order to demonstrate the safety and effectiveness of the body temperature stable sirolimus formulation on smooth muscle cell accumulation in the gold standard porcine AV prosthetic graft model in Dr Roy-Chaudhury's laboratory at the University of Arizona with the assistance of Dr Diego Celdran, an expert vascular large animal veterinary surgeon and scientist.

Other drug delivery systems do exist – for example, drug-eluting balloons and drug-polymer reservoirs. However, these therapies can only deliver small doses of the drug at diminishing concentrations over short periods of time. In addition, they are generally delivered during the graft implantation surgery. Cylerus relies upon 1) the porosity of ePTFE, 2) the known action of sirolimus at very low concentrations to act as an anti-proliferative and 3) the ability to continuously deliver drug via a refillable and replaceable drug pump. This provides a system that will be more effective, safer and cheaper compared to other methods attempted thus far to maintain the utility of prosthetic vascular grafts.

The Cylerus DEC device has been shown to deliver sirolimus continuously at stable concentrations and is applicable to any porous prosthetic graft. Synthetic ePTFE grafts are also used in vascular reconstructions in diabetic patients and in those with peripheral arterial disease. Cylerus's DEC device could become the best choice for these patients, as well as for those with small vessels and diseased or defective blood vessels requiring prosthetic replacements.

The Future of Vascular Drug Delivery

Cylerus has developed a proprietary, long-acting, body temperature stable formulation of sirolimus and will soon complete preclinical safety and further efficacy testing in preparation for human clinical trials. The Company is currently Phase I/II Fast-Track SBIR funded by the National Institute on Aging (NIA), part of the National Institutes of Health (NIH), to complete preclinical studies with plans to file an IND (Investigational New Drug – IND) application with the FDA in 2020 to initiate Phase II clinical studies in the US.

The Company is now in the process of applying for further funding for continued pre-IND studies (Good Laboratory Practice – safety and biocompatibility study), regulatory document preparation and the start of human Phase II clinical trials. Cylerus is seeking a potential commercial partner to support further development activities and is applying for follow-on NIH grants to the currently funded Phase I/II Fast-Track SBIR (NIA) to support the clinical program to a large degree.

The novel Cylerus DEC is projected to reach 50% market share after five years, with roughly 75% of sales being vascular access and maintenance providers, and the remainder of sales to the peripheral vascular disease market. As Cylerus continues to progress in their efforts to bring this novel prosthetic graft saving technology to human trials, patients with ESRD will be one step closer to a better quality of life. The DEC will allow for improved sustainability of prosthetic vascular grafts leading to fewer re-intervention surgeries, markedly decreased healthcare costs, and an increased overall quality of life for ESRD patients.

Meet the Team

Dr Stephen R. Hanson

Dr Stephen R. Hanson is the Founder and Board Chairman of Cylerus and holds a PhD in Chemical Engineering from the University of Washington in Seattle, USA. From 2003 until 2012, he was a Professor of Surgery and a Professor in the Department of Biomedical Engineering at the Oregon Health Science University in Portland, Oregon. There, his research focused on preventing vascular graft failure. He produced the first generation of local drug delivery devices and completed numerous drug studies focusing on the prevention of blood vessel thickening following injury. In 2007, he founded Cylerus in order to bring his novel drug delivery technology and approach to market.

E: steverhanson33@gmail.com

Dr Diego Celdran

Dr Diego Celdran is a veterinary surgeon (DVM) and associate scientist (PhD) at the University of Arizona working in Dr Prabir Roy-Chaudhury's laboratory. He is an expert in large animal vascular surgery and is responsible for the placement of AV grafts in large animal models of prosthetic vascular graft safety and efficacy. Dr Celdran was trained at the College of Veterinary Medicine, Caceres, Estremadura Spain.

E: dceldran@deptofmed.arizona.edu

Dr Prabir Roy-Chaudhury

Dr Prabir Roy-Chaudhury is currently the Division Director for Nephrology and the Director of the Kidney and Vascular Centre at the University of Arizona, where his research focuses on uremic vascular biology and dialysis vascular access. Previously, he was a Medical Director at the University of Cincinnati Health Vascular Access Centre and a Professor in the Division of Nephrology at the University of Cincinnati College of Medicine. He holds a PhD in renal medicine from the University of Aberdeen in Scotland and an MD from the Royal College of Physicians.

E: proychaudhury@deptofmed.arizona.edu

Dr Ronald J. Shebuski, Sr

Dr Ronald J. Shebuski has been the President and CEO of Cylerus since 2010. He completed his PhD in Pharmacology at the University of Minnesota Medical School in Minneapolis in 1985. He has led successful drug development teams at several large US-based pharmaceutical companies and co-founded a number of start-up companies focusing on vascular biology. Dr Shebuski has extensive drug development experience in the commercial sector and also served as a Special Government Employee to the Center for Drug Evaluation and Research (CDER) at the FDA for two terms.

E: rshebuski@gmail.com

KEY COLLABORATORS

The University of Arizona

FUNDING

National Institute on Aging (NIA) – Phase I/II Fast Track SBIR Grant

WEBSITE

W: https://www.cylerus.com

STEM CELL-POWERED IMPLANTS TO REVOLUTIONISE MAXILLOFACIAL SURGERY

Bone tissue engineering expert **Professor Alexander-Friedrich** and cardiovascular tissue engineering expert **Dr Avci-Adali** at the University Hospital Tübingen, Germany, are working to harness the regenerative power of stem cells to improve maxillofacial surgery. Their collaborative research aims to develop a new type of maxillofacial implant that promises to be considerably less stressful for the patient and utilises the regenerative power of the patients' own body.

Demand for Bone Tissue and State of the Art Treatment

In Germany alone, more than 10,000 people are newly diagnosed with oral cell carcinoma each year resulting in tumours and in most cases removal of the jaw bone. Other causes of bone defects requiring surgery include tooth loss, bone cysts, and congenital defects such as cleft lips, jaws, and palates. Facial reconstructive surgery, however, is notoriously difficult. The current mainstay of treatment is bone transplantation involving a heavy and/or gruelling series of surgeries.

Surgeons take bone tissue from a patient's hip, leg, or shoulder blade, sculpt it into shape, and implant it into the bone defect within the jaw. Because the bone comes from the patient's own body, there is little risk of rejection by the immune system.

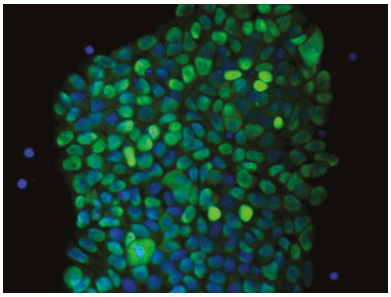
Usually, the removed bone biopsies will be moulded into titanium splints and fixed within the jaw to maintain face structure. However, it is difficult to precisely match the shape of the bone to the face, and subsequent swelling and other pressures can cause it to sag out of alignment, resulting in significant deformities. Moreover, further surgeries are often necessary, which significantly increases healthcare costs in addition to the patient's suffering.

The procedure of transplantation of the patient's own bone can also damage the harvest site and often causes chronic pain. To overcome these difficulties, a bone tissue engineering approach represents a suitable alternative.

Bone Tissue Engineering

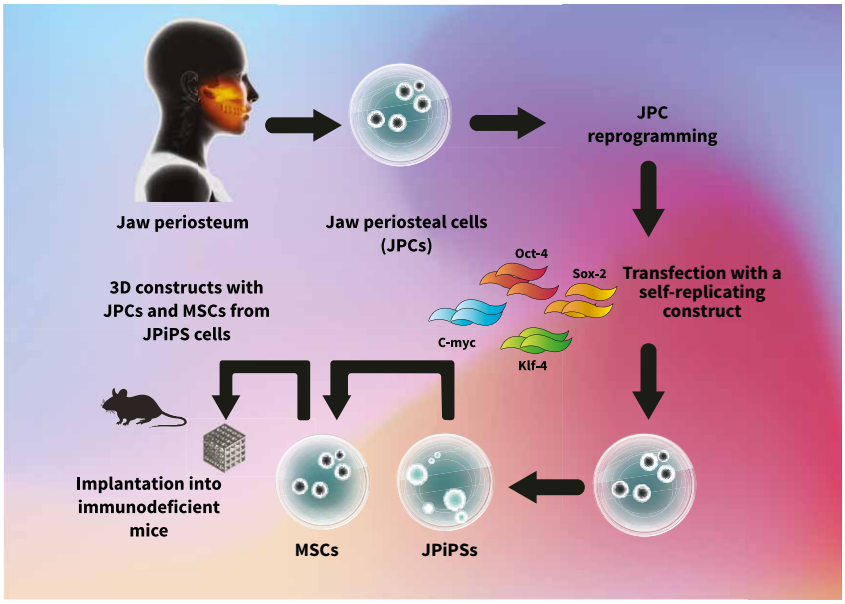
Tissue engineering takes advantage of specialised cells – called stem cells – that can be converted into different types of cells through a process called differentiation. As stem cells can self-renew and differentiate, they hold great promise for regenerating damaged or lost tissues.

All large bones are covered by a thin membrane called the periosteum, which contains bone-producing cells called osteoblasts. It is within the inner layer of the periosteum that the researcher's stem cells of interest are found. Some of these 'jaw periosteal cells', or JPCs, have high rates of bone formation, even in older patients, making them ideal candidates for jaw implant surgery.



iPSCs derived from human skin cells

Another significant advantage is that a periosteum biopsy, to extract the cells during surgery, is far easier and less painful than a bone marrow puncture, the conventional source of stem cells for bone regeneration. Professor Alexander-Friedrich explains: 'We are working with a relatively unusual stem cell type that has not been characterised to the same extent as bone marrow stem cells but is easily accessible.'



The experimental procedure: Human jaw periosteal cells (JPCs) will be isolated and grown under specific conditions. JPCs will be then reprogrammed into jaw periosteal induced pluripotent stem (JPiPS) cells. JPiPS cells will be differentiated into mesenchymal stem cells (MSCs) and then seeded into 3D constructs to generate bone constructs following implantation in immunodeficient mice for biocompatibility testing.

A fundamental prerequisite for the successful application of stem cells in tissue engineering therapies is a high yield of safe, reliable and good quality stem cells. The team of Professor Alexander-Friedrich isolates the JPC stem cells from tissue biopsies and they are then grown under specialised conditions in the laboratory to maximise cell yields with a high regenerative potential. Furthermore, Professor Alexander-Friedrich identified a marker (called MSCA-1) that could be used to reveal which JPCs have the most potent bone generation potential. By harvesting those cells containing MSCA-1, they could maximise the yield of cells with a high bone forming capacity.

Developing the Right Materials

In addition to the generation of suitable stem cells, which are sufficient for implantation, it is equally important to develop safe and clinically applicable biomaterials on which the stem cells are grown. Professor Alexander-Friedrich and Dr Avci-Adali are together exploring the most suitable implant materials that can mimic native tissue and direct stem cells to regenerate into functional tissue.

'A promising approach is the tissue engineering approach using stem cells and scaffold materials (biomaterials) for the generation of bone constructs to be transplanted into the occurring bone defects,' explains Professor Alexander-Friedrich. The two teams are exploring the suitability of different biomaterials for JPC growth and proliferation, as well as adhesion, differentiation, and effects on development potential.

Scaffold properties such as porosity and topography as well as mechanical stability are examined and adapted to mimic the natural structure of bone. Professor Alexander-Friedrich's team's aim is to combine different biomaterials that allow both elastic and mechanical stability that can withstand the mechanical force that occurs during mastication or chewing.

Additionally, in contrast to conventional metal implants, biomaterials in tissue engineering must degrade in a stepwise manner within the body and must be biologically compatible to prevent inflammation and damage to healthy tissue. The team has tested various commercially available materials including collagen, calcium phosphate and polylactide.

Dr Avci-Adali's working group focuses on the use of endothelial progenitor cells (EPCs), which are stem cells that are able to generate the endothelial cells lining all blood vessels in the human body. In her research group, these EPCs are applied for the creation of new blood vessels in tissue engineered constructs as well as in damaged tissues, such as in injured heart muscle after a heart attack, and also for the lining of artificial vascular implants with endothelial cells.

Moreover, the implant surfaces are covered with biomolecules in order to line the blood-contacting surfaces with endothelial cells or to induce homing of patient's EPCs to the implants and to improve vascularisation.

The biomaterial, which creates a three-dimensional structure for the incorporated cells to live on, as well as the supply of these cells with nutrients and oxygen and the removal of waste products, plays an important role in the successful regeneration of tissues. Thus, the formation of new blood vessels in the scaffolds can ensure the viability and functionality of the incorporated cells.

Furthermore, the cell and blood tolerance of biomaterials used for the formation of new tissues have to be analysed extensively prior to clinical applications. Dr Avci-Adali's group has extensive expertise in the analysis of cell and blood tolerance of different implants and biomaterials, such as hydrogels, vascular implants and prosthesis. Different tests in the laboratory, such as static and dynamic tests, are used to evaluate the blood tolerance of new biomaterials according to ISO-10993 standards.

Stem Cell Reprogramming

Using a reprogramming process, four factors can be introduced into the patient's fully differentiated cells, such as skin cells. These factors work together to alter specific cell activities, thereby switching the cellular identity

Dr Avci-Adali: 'The aim of the present project is to develop iPSCs from patients' own JPCs. These iPSCs show in comparison to those isolated from the bone membrane, a higher growing capacity with the ability to generate different cell types including bone cells.'

from the differentiated, fully developed, later stage cell, back into an early embryonic stem cell (ESC)-like state. These cells are called induced pluripotent stem cells (iPSCs) and have the potential to differentiate into different types of cells, such as nerve, bone, heart muscle, and liver cells.

The application of iPSCs offers two main advantages compared to ESCs. In comparison to the ESCs, the generation of iPSCs does not require the destruction of embryos. Thus, the major advantage of the use of iPSCs is the elimination of ethical issues, since the cells used for the reprogramming are harvested from a willing adult by a simple tissue biopsy, such as a skin biopsy. Furthermore, iPSCs are generated from a patient´s own fully differentiated cells, thus, no rejection reactions are expected.

Nucleic Acid-based Modification of Cells

Dr Avci-Adali's research team develops sophisticated methods for the modification and capture of cells on implants. For the modification of cells, the team uses synthetic nucleic acid-based molecules, such as self-replicating ribonucleic acids (RNAs) to produce the desired or missing proteins in cells.

The most important advantage of this technique is that it is a foot-print free method. The synthetic nucleic acid molecules are only transiently present in the cells and in comparison to other methods that use a viral-based modification of cells, there is no integration into the genome. Thereby, integration related mutation of cells and the occurrence of cancer is prevented. Thus, this method represents an interesting technology for clinical applications.

Furthermore, Dr Avci-Adali's research group uses nucleic acid-based small molecules, called aptamers, to capture specific cells on biomaterial surfaces. Therefore, biomaterials are created with aptamers designed to bind specific cells. This technology leads to the colonisation of biomaterials with the required cells promoting and improving tissue regeneration.

A New Generation of Implants

Professor Dorothea Alexander-Friedrich and Dr Meltem Avci-Adali, experts in tissue engineering at the University Hospital of Tübingen, are developing a new generation of biological implants. Their goal is to provide an implant that can seamlessly integrate into the patient's own bones and tissues, requiring only a short surgical procedure to insert, therefore preventing additional surgeries in other parts of the body.

The collaborators are pushing the boundaries for harvesting stem cells with maximum bone regeneration potential and they are exploring different biologically active coatings for implant materials that can promote the growth of cell populations, thus maximising the chances for successful treatment.

Professor Alexander-Friedrich explains: 'We have learnt a lot about the stem cells derived from bone membrane and suitable biomaterials. The biggest challenge that we are facing is the enrichment of sufficient cell numbers for large bone tissue engineering constructs.'

In their current German Research Foundation-funded study, the teams are exploring ways to convert the isolated JPS cells into an earlier, more embryonic-like state with increased potential for developing different cell types. These embryonic stage cells, or iPSCs, have the ability to develop into any cell type of the body. Dr Avci-Adali describes how the, 'aim of the present project is to develop a patient's own iPSCs. These stem cells show in comparison to those isolated from the bone membrane, a higher growing capacity with the ability to generate different cell types including bone cells.' Thereby, sufficient cell numbers for large tissue engineering implants can be obtained from the patient's iPSCs.

Having successfully generated iPSCs from bone membrane-derived cells, the teams' next steps are to fully assess these cells and better understand their bone-forming capacity. Using these cells, the researchers will generate three-dimensional tissue engineering constructs with high regenerative potential, to maximise the success of implantation.

When the optimisation of cells and biomaterials is complete, the newly developed implants will be first tested in animal models before moving on to human clinical studies. The team will test different three-dimensional tissue scaffold biomaterials seeded with mesenchymal stromal cells (MSCs), generated from JPCs derived from iPSCs, by implanting them under the skin of immune-compromised mice to assess how well the cells/biomaterials are tolerated in the body and can generate bone tissue.

Food for Thought

The restoration of individual tissues or organs through tissue engineering holds great potential for the future of medicine. The new generation of three-dimensional implants developed by Professor Alexander-Friedrich and Dr Avci-Adali will contain patients' own cells that have been coaxed to form the necessary cell types and tissues for restoring patients' normal body function without immune-rejection. This exciting approach will help the implant site to repair itself much more effectively than has ever been possible before.

Meet the researchers

Professor Alexander-Friedrich
Department of Oral and Maxillofacial Surgery
University Hospital of Tübingen
Tübingen
Germany

Dr Avci-Adali
Department of Thoracic and Cardiovascular Surgery
University Hospital Tübingen
Tübingen
Germany

Professor Alexander-Friedrich completed her PhD, investigating factors involved in the pathogenesis of rheumatoid arthritis disease, at the Department of Orthopaedic Surgery of the University Hospital of Tübingen in 2003 and continued her post-doctoral studies at the faculty. She has held the position of Head of the Research Laboratory of the Department of Oral and Maxillofacial Surgery, University Hospital of Tübingen since 2005 and was awarded a professorship in 2017. The focus of Professor Alexander-Friedrich's research is bone tissue engineering.

CONTACT

E: dorothea.alexander@med.uni-tuebingen.de
W: http://www.med.uni-tuebingen.de/zzmk/cms/

Dr Avci-Adali studied a Diploma in Pharmaceutical Technology and then received an MSc in Biomedical Engineering in 2006. She was awarded her PhD in Biology from the Eberhard Karls University of Tübingen in 2010. Since 2010, she has led the groups, 'In vivo Tissue Engineering' and 'Aptamers' at the Department of Thoracic and Cardiovascular Surgery, University Hospital Tübingen. Her research interests include tissue engineering and the use of aptamers with a view to developing new methods for applications in the cardiovascular field.

CONTACT

E: meltem.avci-adali@uni-tuebingen.de
W: http://www.medizin.uni-tuebingen.de/Patienten/Kliniken/Thorax__+Herz_+und+Gef%C3%A4%C3%9Fchirurgie-p-805/Klinisches+Forschungslabor/Arbeitsgruppe+in+vivo+Tissue+Engineering.html

FURTHER READING

M Avci-Adali, G Ziemer and HP Wendel, Induction of EPC homing on biofunctionalized vascular grafts for rapid in vivo self-endothelialization – A review of current strategies, Biotechnology Advances, 2010, 28, 119–29.

X Guan, M Avci-Adali, E Alarçin, H Cheng, SS Kashaf, Y Li, A Chawla, H Jang, A Khademhosseini, Development of hydrogels for regenerative engineering, Biotechnology Journal, 2017, 12, doi: 10.1002/biot.201600394.

H Steinle, A Behring, C Schlensak, HP Wendel and M Avci-Adali, Concise Review: Application of In Vitro Transcribed Messenger RNA for Cellular Engineering and Reprogramming: Progress and Challenges, Stem Cells, 2017, 35, 68–79.

EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN

ASSOCIATION OF MEDICAL RESEARCH CHARITIES

Over 30 years ago, a small group of diverse medical research charities formed the Association of Medical Research Charities (AMRC) to unite the sector and provide it with a leading voice. Since then, their membership has grown to 146 charities and they continue to lead and support the sector in delivering high-quality research that saves and improves lives. The AMRC is now the the UK's national membership organisation for health and medical research charities. In this exclusive interview, we speak with Aisling Burnand, AMRC's Chief Executive, to hear about their vital work.

Aisling Burnand,
Chief Executive Officer of the Association of Medical Research Charities

As the Chief Executive of AMRC, what are your vision and aims for the future?

As CEO, my vision is a future where the voice of patients, who are experts by experience, is given equal parity in shaping research and innovation priorities in a world class UK research environment.

The AMRC will be able to ensure that research charities are better prepared for future challenges and provide them with more collaborative opportunities that will deliver benefits to patients sooner.

What specific roles are filled by medical research charities in the overall research landscape?

Charities play a crucial role in the UK's medical research landscape, creating a much more diverse funding landscape than most other countries and ensuring that excellent research can be funded. Collectively our member charities spent £1.3 billion on medical and health research in the UK in 2018. This accounts for 41% of all publicly funded medical research nationally: more than the Medical Research Council or the National Institute of Health Research.

Charities Supply the UK's Skills Pipeline

Medical research charities invest with a long-term vision in human capital and the development of skills. In 2018, AMRC member charities were funding 17,000 researchers which span all essential roles in the research process, including 1,700 PhD students in universities across the UK. Charities are committed to growing the skills pipeline to deliver future generations of researchers trained in new and emerging fields, who are equipped to work across disciplines in order to tackle complex research challenges.

Charities Spark Further Investment in UK R&D

Charities are often the only funders in early-stage, preliminary research – de-risking ambitious projects for future investors and paving the way for other funders such as industry. In the last decade, funding from medical research charities leveraged over £196 million in funding from UK and international companies.

Charities Fund from the Patient's Perspective

Patients are at the heart of many medical research charities. The research that charities fund is often in response to patient need and aims to communicate and meet these needs. Thanks to this close relationship, many medical research charities are funding research that could result in truly transformative outcomes for patients.

Charities are Entrepreneurial and Innovative

Charities are increasingly seeking innovative and novel approaches to investing in research, in order to maximise the benefits for patients. They often act like entrepreneurs and seek collaborative partners to achieve transformative goals. In the last decade, researchers funded by AMRC charities created over 61 spin out companies; and contributed to the production of 550 medical products including drugs, medical devices and diagnostic tools.

Charities are Honest Brokers

Charities can bring together university researchers, funders, small and medium-sized enterprises, regulators, patients and others in areas of unmet need. Working together, they can more efficiently define problems, craft research proposals and identify funding sources to expedite solutions through cross-disciplinary, lateral and radical thinking.

How do you support member charities in promoting the delivery of high-quality health and medical research?

We equip our member charities with regular guidance and training, provide quality standards and carry out an audit every five years to ensure they are supporting the best, most impactful research and researchers. This in turn helps them to deliver the changes that really matter to patients.

With our member charities funding £13 billion in medical research over the last 10 years it's been essential for us to help them maximise the impact of their investment. In order to do this, we play a vital role in influencing the research environment - from forging partnerships, to voicing our member charities' concerns and ideas to policy makers, to developing position statements - we ensure medical research charities get the recognition and voice that they deserve and need.

Researchers are increasingly required to demonstrate the impact of their research. How do you feel we should best measure the impact of research?

Medical research charities are committed to funding research that positively impacts people living with health conditions or diseases. For many charities this is only made possible through public donations and so they must let the public know how their money is being spent and what impact it is having.

The pathway to impact is rarely linear. Instead, it often involves many different funders and research teams over a long period of time. This cumulative nature of impact makes it challenging to track in an effective way.

We're helping a number of our member charities use an online tool called Researchfish that allows them to collect data on the outcomes of their research funding over time. Many of our charities also choose to gather outcomes from research through other methods.

The member charities that do collect data on research outcomes through Researchfish enable us to pool their data and perform an in-depth cross-sector analysis of their impact. We categorise their outcomes into five impact areas:

- o Generating new knowledge
- o Translating research ideas into new products and services
- o Influencing policy and other stakeholders
- o Stimulating new research via new funding or partnerships
- o Developing the human capacity to do research

You can find out more in our sector impact report which is available online for anyone to read. By developing this impact report, we hope to demonstrate the many different ways that impact can be evidenced.

Why do you feel patients are so critical to the research process?

It's vital that people with health conditions, and their carers, are given the opportunity to influence and participate in research. There is a building body of evidence of the positive impact that involving patients and the public can have. It ensures that the right research is done, and that the research is done right.

No matter how complicated the research, or how brilliant the researcher, patients are experts by experience and always offer unique invaluable insights. Their advice when designing, implementing, and evaluating research invariably makes studies more effective, more credible and often more cost effective as well.

The experience of living with a health condition or caring for a loved one can provide a vital and different perspective to researchers when shaping or evaluating new programmes of research.

What challenges do you see on the horizon for health and medical research charities in the United Kingdom? How might these challenges be best managed?

If there is one guaranteed thing in today's world it is uncertainty, change, and unpredictability.

Disruption to EU research networks and funding streams as well as to the flow of skills and talent from the EU are already being felt as a result of the UK's decision to leave the EU. The shape of the UK's future relationship with the EU will impact many aspects of the UK research and innovation landscape and medical research charities may have to adapt to a new state of play.

According to the World Economic Forum we have already entered an intense period of unprecedented, exponential change. The rapid advances in, and fusion of, technology and biotechnology, the rise in algorithms and automation, and big increases in data collection, storage, and AI manipulation – all will combine and lead to a scale of transformation never before experienced. Charities need to get ahead of the curve.

The UK has an ageing population and people are increasingly living with a number of different conditions. In order to pursue research that can truly have a meaningful impact on patients, charities must react to the increasing incidence of multiple long-term conditions. This could require the traditional approach to health research to change, with collaboration taking on a new level of importance.

Data driven technologies are likely to become the mainstay of our future health care and research system. Data currency is likely to become significant. Charities, being closely connected with patient communities and highly trusted, have great responsibility to approach this space with transparency and openness, making sure that patient data is protected with rigorous ethical frameworks whilst the opportunities of new technologies are capitalised on.

We've developed a report which uncovers some of the excellent ways charities are taking the initiative and responding to the changing environment around them. This is intended to help our member charities reflect on their preparedness for challenges on the horizon.

W: www.amrc.org.uk
@AMRC

amrc
ASSOCIATION OF MEDICAL RESEARCH CHARITIES

INNOVATION IN TREATING DISEASE

FROM BENCH TO BEDSIDE: RECENT ADVANCES IN DRUG DEVELOPMENT

Advances in drug discovery and development are critical for medical care. There are many diseases, such as cardiovascular disease, that have increased in prevalence due to our increasingly ageing society. Other diseases have simply remained extremely challenging to treat throughout the history of medicine, and for which, there remains no cure. New diseases, such as HIV, may emerge, and finally, diseases which had declined in prevalence may re-emerge, potentially in drug-resistant forms, such as tuberculosis. This ever changing landscape of infection and disease necessitates the continual development of new and effective interventions. In this important section, we showcase the impressive work of researchers working to advance drug discovery, development and delivery for the benefit of human health.

Tuberculosis continues to be a leading cause of death resulting from infectious disease worldwide. We meet Professor Ulrich E. Schaible and Dr Tobias Dallenga at the Research Center Borstel in Germany, who are working to develop alternative treatments to the multi-drug and drug-resistant forms of tuberculosis. We read how the researchers are using experimental models to test compounds at various stages of drug development and how they are informing a personalised precision medicine approach to the disease.

Acute-on-chronic liver failure is another serious and often fatal disease. We read how Dr Cornelius Engelmann and Professor Thomas Berg at the University of Leipzig are conducting a large scale multicentre clinical trial to test their novel treatment strategy. Having identified a hormone that can stimulate stem cells, the researchers now hope to transform clinical practice in the treatment of liver disease.

Lassa virus is most prevalent across West Africa, with around 59 million people estimated to be at risk of Lassa fever. Although the symptoms can be mild and reasonably short-lived, for one-fifth of patients, they can be much more serious, and even fatal. We meet Dr Matthew Boisen, Director of Diagnostics Development at Zalgen Labs, who is tackling the problem of Lassa fever by improving diagnostic approaches, designing new therapeutic approaches, and finally, developing an effective vaccine against the virus.

Calder Biosciences is a molecular engineering company pioneering the development of safe and effective vaccines against viruses such as Respiratory Syncytial Virus and influenza. We meet their Chief Executive Officer, Dr Chris

Marshall, and read how Calder Biosciences has utilised a natural chemical reaction known as di-tyrosine crosslinking to increase the stability and potency of vaccines.

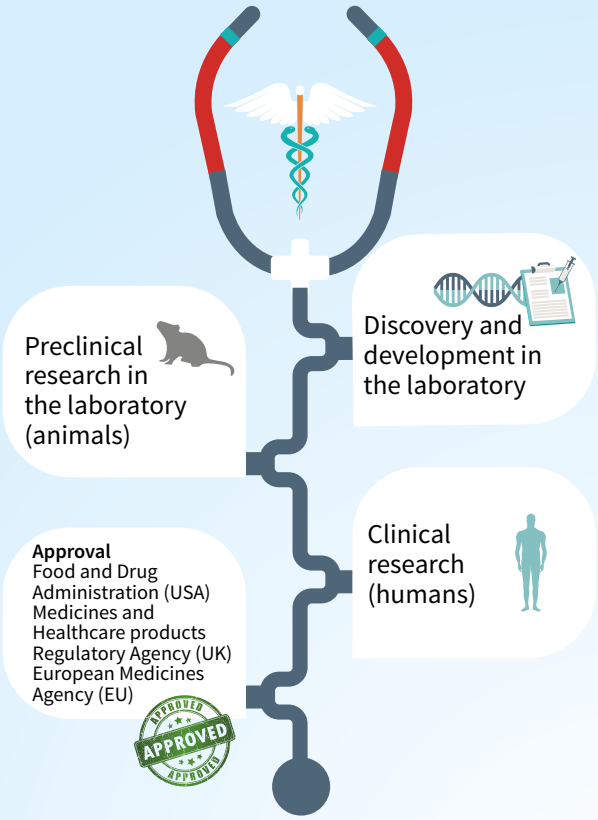
We then turn to the work of Dr Brian Peerce and Dr Slomowitz, co-founders of DuoPhos, a biotechnology company focused on developing treatments for chronic renal failure. Approximately 2.2 million people are affected by chronic kidney disease worldwide, and we read how by blocking dietary phosphorus absorption, DupPhos is driving forward the development of more effective interventions with fewer side effects than the treatments currently available.

Atherosclerosis is an important cause of cardiovascular disease and mortality, and is also closely associated with type 2 diabetes. It involves a multitude of different pathways and systems throughout the body, but for Dr Elena Galkina at Eastern Virginia Medical School, understanding the inflammatory immune response is critical. We read how her work is informing the development of much-needed novel therapies for atherosclerosis and also type 2 diabetes.

We then consider advances in treating one of the complications associated with diabetes in adults, eye disease – diabetic retinopathy. Dr Tammy Movsas at the Zietchick Research Institute, USA, is transforming treatment for this retinal disease, as well as retinopathy of prematurity, which affects premature infants. We read how Dr Movsas and her team at the Zietchick Research Institute have undertaken an ambitious plan to develop more readily accessible and even preventative treatments for these conditions.

We conclude this section with an exclusive interview with Kiki Syrad, Director of Grants at Sparks Children's Medical Research Charity. We read how Sparks is committed to funding the research needed to find the new tests, treatments, and cures that are desperately needed by critically ill children.

DRUG DEVELOPMENT: FROM BENCH TO BEDSIDE



Phase 1:
Safety trials of small doses in small numbers of healthy volunteers

Phase 2:
Trials in larger groups of patients to test efficacy and safety

Phase 3:
Larger scale trials to confirm efficacy and safety

Phase 4
Post-approval long-term monitoring of safety and efficacy in the real world

KEY FACTS AND FIGURES

It typically takes **10-15 years** to develop a new medicine

As many as **90% of drugs** under development fail to reach approval and the healthcare market

Life expectancy in the UK has increased by **10 years** since the 1960s – helped by medicines and vaccines

Medicines and vaccines have helped **double cancer survival** over the past forty years

1970 ~25%
2018 ~50%

Pharmaceutical companies invest **~£140 billion** globally each year researching and developing new medicines

The cost of developing each new medicine is **~£1.2 billon**

Sources: US Food and Drug Administration, Multiple Sclerosis Trust, Association of the British Pharmaceutical Industry

A NEW APPROACH TO TUBERCULOSIS TREATMENT

With the growing challenge of antibiotic resistance, developing alternative treatments for tuberculosis is vital. Elegant research led by **Professor Ulrich E. Schaible** and **Dr Tobias Dallenga** at the Research Center Borstel in Germany suggests that innate host immune cells could be potential targets for an exciting new therapy for the disease.

Tuberculosis (TB) is the leading cause of death from infectious diseases worldwide. According to the World Health Organization, 10.4 million people worldwide contracted TB in 2016 alone, and 1.7 million died from complications related to the infection. Especially alarming is the rising numbers of cases of multi-drug and extensively drug-resistant forms of the disease, which constitutes half a million cases annually. For patients infected with extensively drug-resistant forms of TB, the survival rate is only 28%.

Presenting a major global healthcare challenge, the emergence of these resistant forms of the disease prompted the G20 leaders to single out TB within the emerging problem of antibiotic-resistant bacterial infections in their 2017 summit declaration.

The Importance of the Host Immune System

TB is caused by infection from a bacterium called *Mycobacterium tuberculosis* (or *M. tuberculosis*). Most infections of TB do not have symptoms, and these are known as latent tuberculosis. About 10% of latent infections progress to active disease, which if left untreated, kills about half of those infected. In its active form, the illness is characterised by a massive influx of inflammatory host immune cells into the lungs, followed by destruction and loss of tissue. This leads to the patient coughing up contagious particles, and the infection spreads from person to person through inhalation of the infectious particles.

One immune cell, in particular, has recently fallen into the spotlight of TB research: the neutrophil. Circulating in the bloodstream, these white blood cells represent the first line of defence against bacterial infections. Upon sensing signals that an infection is present, neutrophils quickly migrate to the infection site in large numbers to begin killing the invading germs.

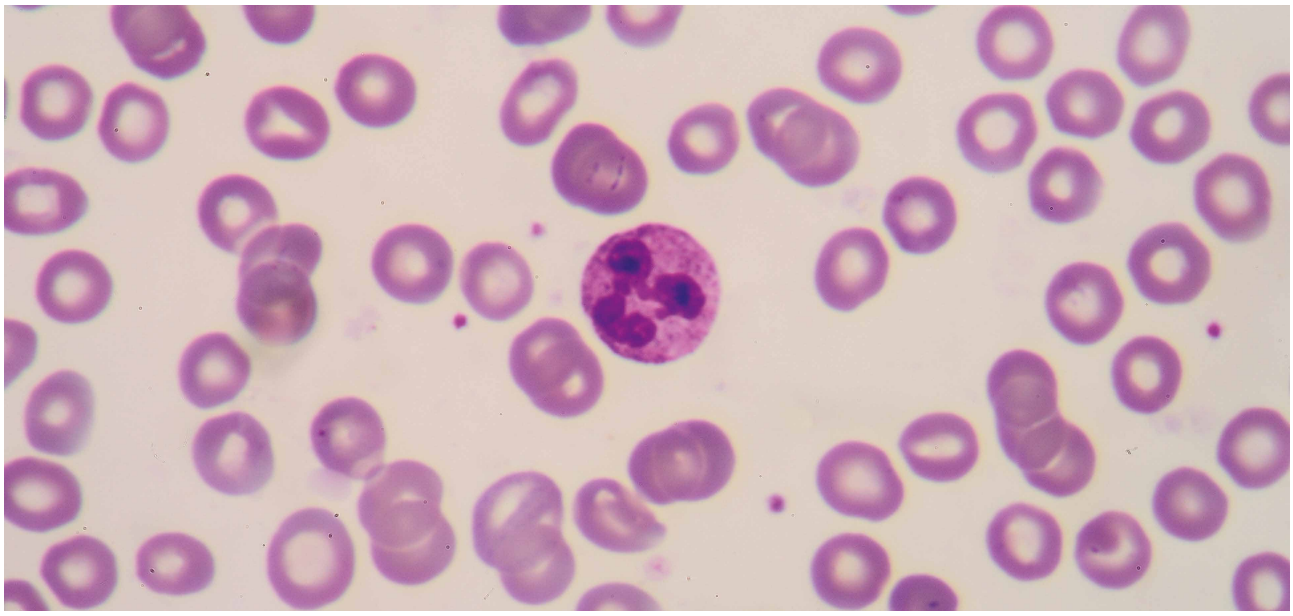
Until recently, the role of neutrophils in TB has been poorly understood because they are hard to detect in commonly used animal models of the disease. In the last ten years, however, it has become apparent that many animal models do not accurately reflect the pathology of the disease in humans. In contrast, in human TB, neutrophils are the main infected cell type in the lungs of patients with active TB.

Professor Ulrich E. Schaible and Dr Tobias Dallenga at the Research Center Borstel set out to better understand the role of neutrophils in TB, and to explore the hypothesis that these white cells exacerbate TB pathology. The team's ultimate aim is to find potential new treatments for the disease.

TB Induces Cell Death in Neutrophils

In an early set of experiments, the team infected human neutrophils with *M. tuberculosis* under laboratory conditions and found that although the neutrophils quickly engulfed large numbers of *M. tuberculosis*, they failed

‘We aim to design host-directed therapies to make antibiotic treatment more effective, to curb pathology and long-term lung damage and respiratory deficiencies of cured patients.’



to kill the mycobacteria. Intrigued by their observation that these highly effective defence cells could not clear the *M. tuberculosis* infection, Professor Schaible and Dr Dallenga took a closer look at exactly what happens to the infected neutrophils. They found that human neutrophils isolated from the blood of healthy donors quickly succumbed to a certain type of cell death known as necrosis starting only six hours after infection with virulent *M. tuberculosis* under laboratory conditions.

The researchers found that this necrotic cell death is associated with release of both, neutrophil cell contents and *M. tuberculosis*, which were freed into the extracellular space. They showed that induction of this type of cell death was dependent on one specific small virulence factor, called ESAT-6, that contributes to the function of the bacterial secretion system ESX-1. Using a mutant version of *M. tuberculosis* lacking this virulence gene and therefore, lacking a functional ESX-1 secretion system, the team found that these mutant bacteria failed to cause neutrophil necrosis.

Interestingly, the team also found that *M. tuberculosis*-induced neutrophil necrosis was dependent on the neutrophil’s own generation of reactive oxygen species. Not only did they show that inhibiting reactive oxygen species prevented necrosis, but neutrophils from patients with impaired reactive oxygen species production were protected from necrotic cell death when infected with *M. tuberculosis*.

The team hypothesised that neutrophils provide a short-lived environment for *M. tuberculosis* bacteria. The vast influx of neutrophils, engulfment of *M. tuberculosis*, and subsequent neutrophil death appeared to provide a niche for the bacteria. Setting out to examine this further, they looked closely at what happened to neutrophils when infected with mutant *M. tuberculosis* that lacked ESX-1. Here, the neutrophils underwent default apoptosis (normal programmed cell death) similar to that seen in uninfected neutrophils.

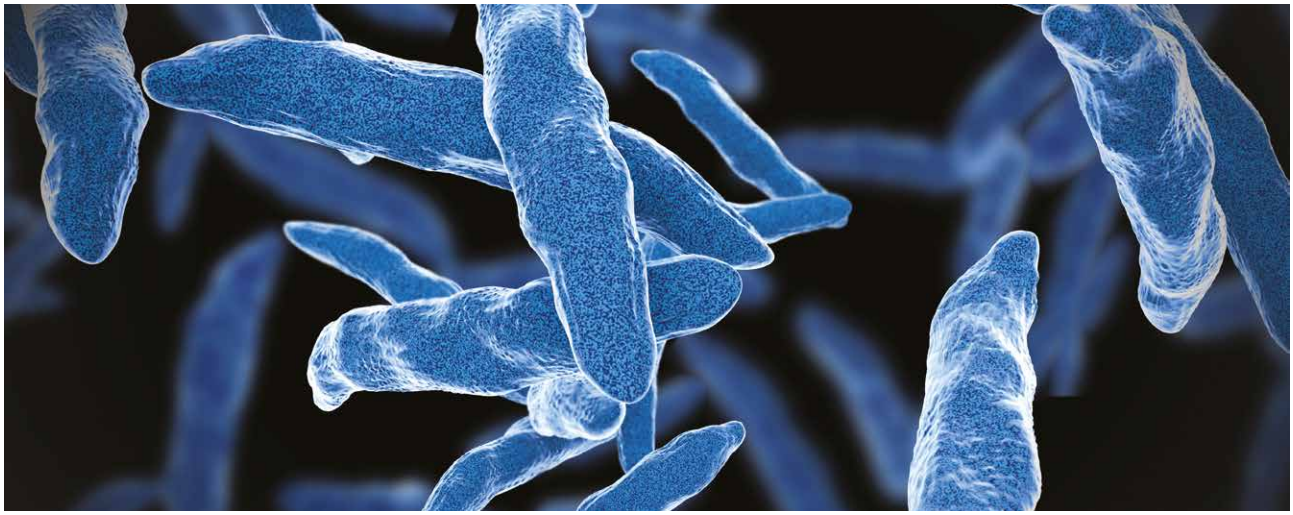
TB Infection – A Vicious Cycle

The team’s finding that the type of neutrophil cell death – either apoptosis or necrosis – is determined by the virulence of the infecting *M. tuberculosis* agent, led the researchers to analyse the fate of *M. tuberculosis*-infected neutrophils in macrophages.

Macrophages are another type of white blood cell in the immune system, which engulfs and digests bacteria and other foreign substances, in a process called phagocytosis. These large protective immune cells are found throughout our body, and constantly patrol for potentially harmful threats. Professor Schaible and Dr Dallenga’s work showed that these cells play an important part in the fate of *M. tuberculosis* in disease.

Recently published work by the team in the journal Cell Host & Microbe reports that removing necrotic neutrophil debris together with virulent *M. tuberculosis* promotes the survival of *M. tuberculosis* and its proliferation in human macrophages. This was in contrast to apoptotic neutrophils infected with an attenuated mutant *M. tuberculosis*.

In this research, the team cultured human neutrophils and macrophages together under safe and carefully controlled laboratory conditions, known as cell co-culture. In this system of infected neutrophils and uninfected



human macrophages, the latter became infected through taking up necrotic neutrophils.

Subsequently, only virulent *M. tuberculosis* bacteria were able to grow within these macrophages and, ultimately, also drove those into necrotic cell death. Notably, the team demonstrated that when they pharmacologically prevented neutrophil necrosis, virulent *M. tuberculosis* were not able to subsequently grow in macrophages.

Therefore, neutrophil necrosis is necessary for the growth of the infectious agent within macrophages. This starts a vicious cycle: the infected necrotic neutrophils are taken up by the macrophages, in which *M. tuberculosis* grows, leading to a necrotic cell death of the macrophages, the TB infection then spreads and the infection is maintained. The switch point, either necrosis or apoptosis, determines the subsequent fate of *M. tuberculosis*.

Controlling *M. tuberculosis* Growth

In the same paper, the team found that human neutrophils fail to kill virulent mycobacteria due to *M. tuberculosis* infection-induced production of reactive oxygen species, confirming their hypothesis that the reactive oxygen species production sends neutrophils into necrotic cell death – allowing *M. tuberculosis* to escape elimination. Notably, when necrosis of infected neutrophils was pharmacologically inhibited, the macrophages could then control *M. tuberculosis* growth by taking up infected neutrophils.

These data suggest that host cell necrosis, driven quickly upon infection of neutrophils, is beneficial for the fate of *M. tuberculosis* in the subsequent host cell, such as a macrophage. Consequently, interfering with neutrophil necrosis restored the ability of macrophages to control *M. tuberculosis* growth.

The researchers hypothesised that consecutive cycles of infection and host cell necrosis, interspersed with periods of *M. tuberculosis* proliferation, ultimately results in TB-associated tissue damage, which leads to coughing and the infection spreading. Excitingly, the team suggests that their results could lead to a novel host-directed therapy that could be used alongside antibiotic treatment to effectively control the illness, even with drug-resistant forms of *M. tuberculosis*.

Neutrophils as Therapeutic Targets for TB

The importance of neutrophils as markers for TB disease progression and their role in the pathology of TB makes them potential biomarkers for point-of-care diagnosis. Neutrophils can indicate disease severity and also enable treatment monitoring in patients with TB. As such, they also constitute a promising target for host-directed therapy. Here, a patient's immune system is specifically influenced to mitigate potential tissue damage and deterioration of symptoms whilst restricting the spread of the bacteria.

'We aim to design host-directed therapies to make antibiotic treatment more effective, to curb pathology and long-term lung damage and respiratory deficiencies of cured patients,' Professor Schaible explains.

The team's research focuses on identifying new targets to interrupt this vicious circle and re-equip the immune system with the ability to control *M. tuberculosis* infection. Excitingly, they are looking for suitable predictive diagnostic biomarkers to determine the correct time window and appropriate host-directed therapeutic drug. In this way, their aim is to develop a personalised precision medicine approach.

'We are using our experimental tuberculosis models to screen a wide range of compounds currently either at the experimental stage, safety-approved for clinical trials, or clinically (FDA) approved stage – preferentially those that can be re-purposed as they are in routine clinical use for other diseases,' Professor Schaible explains. Ultimately, neutrophils represent key targets for host-directed therapy in TB, a disease that remains a significant health challenge worldwide.

Meet the researchers

Professor Dr. rer. nat. Ulrich E. Schaible
Division of Cellular Microbiology
Research Center Borstel - Leibniz Lung Center
Borstel
Germany

Professor Ulrich E. Schaible completed his doctoral thesis at the Max Planck Institute of Immunobiology in Germany. Following postdoctoral studies in St. Louis and Berlin, he became Professor of Immunology at the London School of Hygiene & Tropical Medicine, UK, in 2006. Subsequently, he became Director of the Department of Molecular Infection Research at the Research Centre Borstel and Professor of Immunochemistry and Biochemical Microbiology at the University of Lübeck. Professor Schaible is currently Director of the Priority Area Infections, head of the Cellular Microbiology division at the Research Centre Borstel and chairman of the international Masters course in Infection Biology at the University of Lübeck. Professor Schaible has long-standing expertise in experimental infection research, evidenced through a list of over 125 publications and six patents.

CONTACT

E: uschaible@fz-borstel.de
W: http://www.fz-borstel.de/index.php/de/sitemap/programmbereich-infektionen/zellulaere-mikrobiologie-prof-dr-ulrich-schaible/mission#innercontent
W: http://www.tbsequel.org/personnel/ulrich-e-schaible/
W: http://www.leibniz-infections21.de/en/research/objectives-tasks/

Dr. rer. nat. Tobias Dallenga
Division of Cellular Microbiology
Research Center Borstel - Leibniz Lung Center
Borstel
Germany

Dr Tobias Dallenga is an infection biologist with a diploma in neuroanatomy from the University of Oldenburg. He spent several months as an intern at the Center for Molecular Neurobiology in Hamburg, Germany, before completing his doctoral studies in multiple sclerosis research at the Institute for Neuropathology, University Medical Center, Göttingen, Germany in 2011. He was appointed senior scientist and is now Deputy Group Leader of the division Cellular Microbiology at Borstel. Since 2015 he has been a lecturer in Molecular Life Science at the University of Lübeck. Dr Dallenga is co-founder and section officer of the Leibniz Postdoc Network, the first nation-wide association representing postdoctoral researchers, their needs, and careers, in Germany.

CONTACT

E: tdallenga@fz-borstel.de
W: http://www.fz-borstel.de/index.php/de/sitemap/programmbereich-infektionen/zellulaere-mikrobiologie-prof-dr-ulrich-schaible/mission#innercontent
W: https://www.linkedin.com/in/tobias-dallenga/
W: https://biography.omicsonline.org/germany/research-center-borstel/tobias-dallenga-834378
fa @TDallenga

KEY COLLABORATORS ON THIS PROJECT

Professor Gareth Griffiths, Dr Urska Repnik, University of Oslo, Norway
Professor Alexander Apt, Professor Vladimir Yeremeev, Central Institute for Tuberculosis, Moscow, Russian Federation

Research Center Borstel
Leibniz Lung Center

FUNDING

German Science Foundation / Deutsche Forschungsgemeinschaft (SPP 1580, IRTG 1911, German-Russian Coproject)
German Ministry of Research and Education (ANTI-TB, Deutsches Zentrum für Infektionsforschung - TTU-TB, BMBF-Afrika TB-Sequel)
Leibniz Association (Leibniz Research Alliance INFECTIONS'21)

THE HORMONE MAKING LIVER FAILURE TREATMENT SUCCESSFUL

Liver cirrhosis causes many harmful symptoms that develop as time goes on. These symptoms can culminate in a severe condition, with currently few treatment options, known as acute-on-chronic liver failure (ACLF). **Dr Cornelius Engelmann** and **Professor Thomas Berg** at the University of Leipzig in their ongoing multicentre GRAFT trial funded by the German Research Foundation (DFG), will evaluate a hormone showing a lot of promise for the future treatment of ACLF.

The Chemical Factory of the Human Body

The liver acts as our very own chemical processing plant and also acts to keep us safe from harmful toxins. In fact, the liver is one of the human body's most important organs, converting nutrients from the foods we eat into substances that the body can later use. They receive approximately 30% of our blood supply each minute and are responsible for the vital processes of metabolism or energy regulation. It is through this process that dietary nutrients are broken down to either be used or stored by specific liver cells, called hepatocytes.

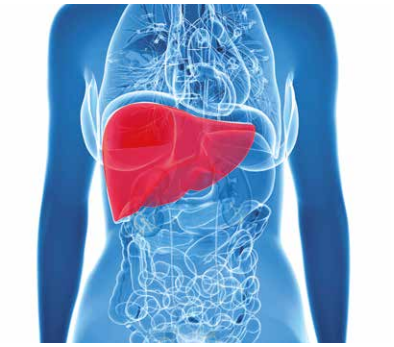
Whether its carbohydrates, proteins or fats, your liver processes anything and everything. Take, for instance, when you eat a chocolate bar – the carbohydrates within that bar get broken down into glucose by the gastrointestinal tract (the stomach and intestines). This glucose then enters the bloodstream, where it is transported to the liver to be stored and regulated – releasing more sugar into your body when you need energy and stopping its release when you don't.

The liver performs similar regulatory functions for fats as well, breaking them down to again provide our bodies with energy when we need it most. It also performs an important function to remove unwanted toxins from the body and can also remove excess amino acids produced by breaking down proteins for energy. In the liver, hepatocytes remove nitrogen from amino acids to create a highly toxic substance called ammonia. The liver then quickly acts to convert this ammonia into urea, which is safely released via your urine.

Failure to Deliver

It is no surprise then that, like with any other of our body's organs, when the liver fails it can cause a host of problems. However, liver failure is arguably more life-threatening than most conditions, demanding urgent medical care when it becomes apparent.

Most forms of liver failure tend to gradually develop over time, usually as a result of excessive alcohol intake, autoimmune conditions where the immune system targets the patient's



own body, or other health conditions such as viral infections with hepatitis B or C. This type of liver failure is classed as 'chronic', often occurring as a result of another liver-related condition called cirrhosis. During cirrhosis, the liver fails to function as a result of long-term damage. This leads to the steady onset of symptoms which might typically include tiredness, itching, yellow skin, and spider-like blood vessels on the skin.

Forms of Failure

Sometimes cirrhosis can lead to another more severe and acute form of liver disease. This condition, known as 'acute-on-chronic liver failure', is a more serious form of liver disease, usually

‘Patients with chronic liver diseases and cirrhosis can develop a severe worsening of their disease, known as acute-on-chronic liver failure. Unfortunately, many of these patients die as a result of their liver and other organs losing their functionality.’

only becoming diagnosed when the symptoms of somebody with chronic liver disease get significantly worse.

Dr Cornelius Engelmann, a Senior Research Fellow at University College London (UCL) and leading expert on all things liver failure, describes the disease: ‘Patients with liver cirrhosis (failure) can develop a severe worsening of their disease, known as acute-on-chronic liver failure (ACLF). Unfortunately, many of these patients die as a result of not only the liver but also their other organs, losing their functionality.’

Finding new treatments for ACLF is therefore potentially life-saving. But how do you go about treating a condition that worsens rapidly, and is difficult to treat? In order to understand ACLF researchers are looking at the role of the immune system in the development of this deadly disorder.

Immune System Overload

When your body perceives a threat, or a foreign substance like bacteria or a virus, it kickstarts your immune system into action – unleashing an immune response to combat the danger. This is great most of the time, in that it removes threats that would make you feel a lot worse if left to roam free.

However, the immune system can sometimes overreact and starts to incorrectly target healthy tissue. This can occur in a condition called sepsis when the immune response to a bacterial infection starts to damage the organs of the body. This kind of immune system activation against cells in your own body plays an important role in ACLF and is a major characteristic of the disease’s profile. Dr Engelmann explains that, ‘the immune system plays a major role in this disease as it is massively activated, similar to what we observe in sepsis.’

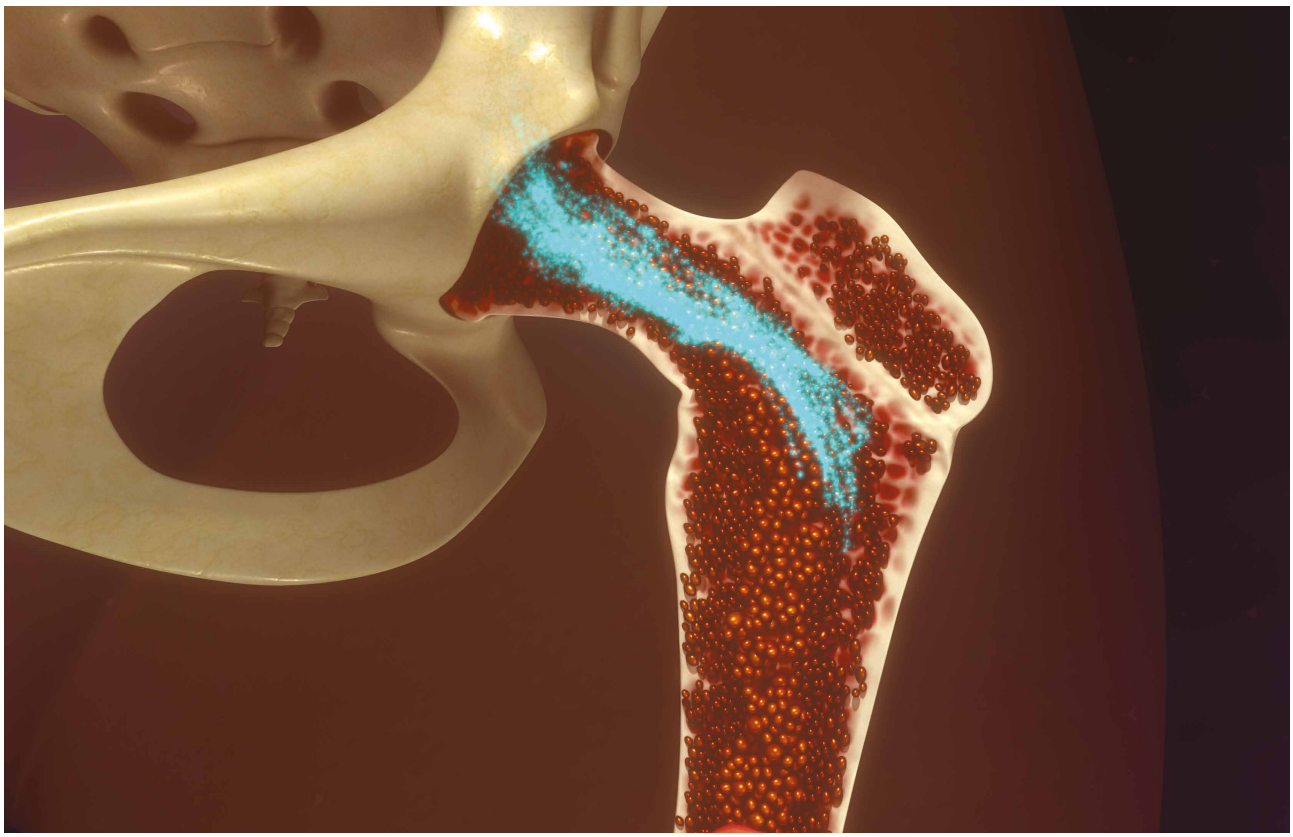
Dr Cornelius Engelmann, working collaboratively with Professor Thomas Berg – a fellow leading hepatology expert at the University of Leipzig, Germany – have been working on the role of the immune system in ACLF and are currently conducting a study, called the GRAFT trial, to examine a new treatment for the disease.

The GRAFT Trial

Working together, Dr Engelmann and Professor Berg’s work investigates a hormone that can stimulate stem cells, that are typically contained within our bone marrow, and that possess the ability to replicate rapidly and produce any type of immune cell required for an immune response.

Dr Engelmann explains: ‘Stem cells have the ability to appease the immune system but are very costly to isolate and reinfuse into the body as a treatment.’

Fortunately, there is a naturally occurring hormone available which acts as a cytokine and which can promote the release of these stem cells. This is known as granulocyte



colony stimulating factor, or G-CSF for short. Small studies by the research team have already shown positive results for ACLF patients after treatment with G-CSF. The team is now conducting a much larger study – the GRAFT study – that will look to continue on with that research and take it to the next level to investigate the effectiveness of the G-CSF treatment across a number of liver specialist centres in Germany.

Getting to Know G-CSF

G-CSF is produced by a number of different tissues that act to stimulate bone marrow – the material within the hollow part of your bones – to produce, stimulate and release protective white blood cells, known as granulocytes, and stem cells into the bloodstream. The reason these stem cells are of such interest comes from their ability to develop into different cell types, depending on the function required and their huge regenerative potential.

Within ACLF, this stem cell release promotes liver regeneration and acts to support the immune system that has become compromised. Therefore, this hormone could prove highly effective in developing new treatments for this dangerous condition.

The Trial

During the trial, over 1200 participants will be assessed across many liver specialist centres for eligibility. However, only 22% (262) of these will make it into the trial itself, before being split up into two groups. One group, known as the 'experimental' group, will receive the G-CSF treatment alongside typical treatments for liver failure, whilst the second group, known as the 'control' group, will only receive typical liver failure treatments. The researchers will then follow up with individuals from both groups regularly throughout the first year. After four years the influence of using G-CSF to treat ACLF can finally be evaluated.

The multicentre trial's success will then be determined according to the number of surviving participants after 90 days, who haven't needed to undergo a liver transplant – the typical treatment option given to ACLF patients. The trial is currently actively recruiting ACLF patients to take part.

'We are currently recruiting patients for the GRAFT study and, within the next year, we hope to finalise our enrolment period,' Dr Engelmann and Professor Berg recently said. 'If the results are positive, we hope that this therapy will become a new standard treatment in ACLF.'

From Failure to Success

ACLF is a debilitating condition with a worryingly high mortality rate. Developing effective new treatments is therefore paramount to combat this deadly disease. Fortunately, through Dr Engelmann and Professor Berg's research, G-CSF is now showing a lot of promise in treating the condition. Working together they are developing a new treatment strategy that has the potential to change clinical practice and save many lives in the future.

Meet the researchers

Dr Cornelius Engelmann
Section of Hepatology, Clinic of Gastroenterology and Rheumatology
University Hospital Leipzig
Leipzig
Germany

Institute for Liver and Digestive Health
University College London
London
United Kingdom

Dr Cornelius Engelmann is a German national, born in Ruedersdorf (near Berlin). He currently works as a Senior Research Fellow at University College London's Institute for Liver and Digestive Health. However, prior to that he studied medicine at the University of Leipzig in Germany between 2000 and 2006. During that time, he worked in a number of hospitals including the Departments of Neurosurgery and General Surgery at Royal Hallamshire Hospital, UK, and the Department of Anaesthesiology and Intensive Care Medicine at University Hospital Leipzig, Germany. After receiving his licence to practice medicine, Dr Engelmann went on to work as a Resident in Internal Medicine at Park-Hospital Leipzig before moving to University Hospital Leipzig in 2011. He later worked as a Medical Specialist at the Royal Free Hospital, London, before becoming a Research Fellow at UCL in October 2017.

CONTACT

E: cornelius.engelmann@medizin.uni-leipzig.de
W: http://gastroenterologie.uniklinikum-leipzig.de/mk2gastro.site,postext,hepatologie.html

Professor Thomas Berg
Section of Hepatology, Clinic of Gastroenterology and Rheumatology
University Hospital Leipzig
Leipzig
Germany

After completing his medical training at the University of Tübingen, Freiburg and Berlin, Germany, Professor Thomas Berg specialised in Internal Medicine in 2001, becoming a lecturer in the subject in 2002. During the same year, Professor Berg also became Associate Director and Professor of Medicine at the Department of Hepatology and Gastroenterology of Charité, Campus Virchow-Clinic, University of Medicine in Berlin, where he was Head of the Liver Out-Patient Clinic and the Laboratory for Molecular Hepatitis and Viral Diagnostics. Since December 2009 he has worked as the Head of the Section of Hepatology at the University Hospital in Leipzig, as well as of the Liver- and Study-Centre Checkpoint in Berlin, Germany.

CONTACT

E: thomas.berg@medizin.uni-leipzig.de
W: http://gastroenterologie.uniklinikum-leipzig.de/mk2gastro.site,postext,hepatologie.html

FUNDING

The study is funded by the German Research Foundation (DFG), EN 1100/1-1

FURTHER READING

C Engelmann, T Berg, Effects of granulocyte-colony stimulating factor (G-CSF) on stem cell mobilization in patients with liver failure, European Journal of Internal Medicine, 2016, 36: e37–e39.

UNDERSTANDING LASSA VIRUS

For many years, **Dr Matthew Boisen**, Director of Diagnostics Development at Zalgen Labs, has focussed on trying to understand Lassa fever. Part of the Viral Hemorrhagic Fever Consortium, his group's objectives are threefold: first, to develop fast and accurate diagnostics for Lassa fever; second, to design new therapeutic approaches; and third, to create an effective vaccine providing long-term protection against this condition.

It is estimated that there are 59 million people at risk of contracting Lassa fever, primarily in West Africa, with 3.3 million potentially suffering severe morbidity after contracting the disease. Following an incubation period of 7 to 21 days, most people experience mild symptoms including fever, headache, and general weakness. However, about 1 in 5 patients can develop a more severe condition, with hypotension, facial oedema, and vomiting.

In a third of these cases, symptoms may progress to general internal bleeding, which is fatal for most patients. Death occurs 10–14 days after presentation of the initial symptoms, usually due to multi-organ failure. Pregnant women are particularly at risk, with mortality reaching 90% for mothers, and spontaneous abortion occurring in the majority of cases.

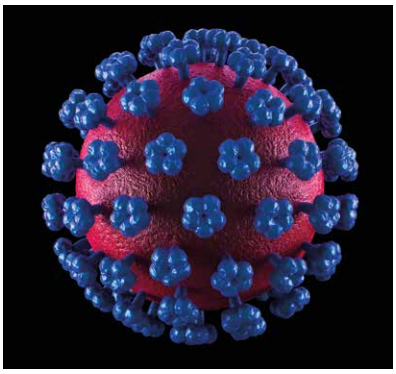
The Lassa virus is one of more than 30 known pathogens from the arenavirus family. The family has an Old World branch, including the Lassa virus as well as lymphocytic choriomeningitis virus, which causes fever and birth defects; and the haemorrhagic fever virus Lujo, first detected in 2008. New World viruses include the Machupo virus and Junín virus, responsible for the Bolivian and Argentinian haemorrhagic fever, respectively, as well as numerous other viruses, such as Sabía and Guanarito.

This virus infects more than 300,000 individuals per year, with Sierra Leone, Guinea, Liberia, and Nigeria reporting the highest incidences. Benin, Togo, Burkina Faso, and Mali have also reported cases over the past few years, and the virus has been exported from its normal geographic region, with cases identified recently in Germany, Sweden, the USA, and the United Kingdom.

Despite the seriousness of this condition, diagnosis can be difficult. The problem is that there are many other conditions which present similar symptoms, including malaria, typhoid fever, leptospirosis, and arbovirus diseases. To further complicate matters, the only available treatment – ribavirin – must be carried out during the first six days of illness to be effective. 'Rapid diagnosis is imperative for proper patient management, which includes patient isolation, treatment with ribavirin when available, and supportive care such as the replacement of fluids and electrolytes,' explains Dr Boisen.

Due to high mortality and ease of infection, the Lassa virus is at the centre of many attempts to accelerate the development of a new vaccine. Dr Boisen is a leading example, and his team at Zalgen Labs has been working hard to better understand the virus with the aim to develop simpler and more accurate diagnostic tools, as well as new therapeutic approaches.

ReLASV® Pan-Lassa Antigen Rapid Test Kit



Lassa virus particle

Antigen and Antibody

Our always vigilant immune system reacts quickly to chase and kill any

foreign substance entering our body. These substances – which can be present in bacteria, fungi, or viruses – are known as antigens and are usually the first port of call when scientists are trying to find a target to develop a new vaccine: find a way to enhance this response from the immune system and it will be harder for the invading pathogen to cause disease. The response from the immune system takes the form of antibodies, which are large proteins circulating in the bloodstream always on the look-out for pathogen by recognising and binding to their antigens.

In the Lassa virus, the antigen is a protein complex located in the viral surface. Researchers know this is the protein that the immune system recognises, but the mechanism is still unknown. Keen to unveil the mystery, Viral Hemorrhagic Fever Consortium (VHFC) collaborators at Tulane University and Scripps Research characterised 113 human antibodies derived from Lassa fever survivors and identified 16 that were able to neutralise Lassa virus. It turns out these antibodies can't spot small sections in the target protein but instead need to recognise its full structure. As this information is vital to design a new vaccine, the researchers had to confirm the results by introducing several mutations which caused minimal conformational changes in the viral protein but severely disrupted antibody activity.

For Dr Boisen, 'these studies provide the initial foundation for understanding the molecular mechanisms of antibody development, antibody binding and antibody-mediated neutralisation of Lassa in humans. These results will facilitate development of vaccines or antibody-based therapeutics, both urgently needed in this region of the world.' Dr Boisen is the principal investigator in a new National Institutes of Health grant supporting the development and optimisation of new laboratory tests to measure antibodies resulting from vaccinations. The ReLASV® Pan-Lassa GP Antibody ELISA test will serve as a companion diagnostic in future vaccine studies and aid in Lassa fever surveillance throughout West Africa.

How do Antibodies Neutralise the Lassa Virus?

After determining which antibodies work, the VHFC team wanted to explore how they recognise and neutralise the Lassa virus. 'Antibodies can be exploited to gain valuable insights into immune responses to pathogens, explains Dr Boisen. 'Determining the sites at which antibodies bind to pathogens and mediate reaction by the immune system, can reveal mechanisms of immune surveillance, evasion or escape.'

The Scripps Research collaborators quickly realised that the best way to achieve their aim was to study the structure of a virus bound to an antibody. This was not the first time this had been attempted but previous studies had been unsuccessful. The main problem was that, under normal circumstances, using the natural viral protein *in vitro* always led to unstable changes in conformation. These changes mimic how the protein 'unfolds' during the virus invasion of target cells. After 10 years spent trying to solve this problem, Dr Sapphire and her team at Scripps finally identified a few mutations to stabilise the protein to keep its structure prior to binding to target cells (prefusion) without affecting how it binds to the antibody. Interestingly, this approach allowed the identification of certain areas acting as shields, namely on the side and lower portion of the protein, leaving only a few regions vulnerable to antibody binding.

Developed ReLASV RDT assays. Negative result (left) and Positive result (right). Control Line signal (top line) indicates correctly developed rapid test. Test Line signal (bottom line on positive) indicates detection of Lassa virus nucleoprotein antigen.

The immune systems antibodies have found a way to maximise their power: this is to act before these shields are put in place, which occurs when the virus enters the target cell. By binding to the viral protein and locking it into the prefusion conformation, the antibody can prohibit viral invasion of the target cells and stop the infection. Indeed, *in vitro* studies showed a healthy 80% reduction rate in viral activity in the presence of these neutralising antibodies.

'This structure provides the initial foundation for understanding the molecular mechanisms of neutralisation of the Lassa Virus,' explains Dr Boisen. Furthermore, it's possible that other diseases caused by this family of viruses follow a similar pattern of infection and this information may prove valuable to develop new treatments. After all, this antibody also recognises lymphocytic choriomeningitis virus but does not recognise more distantly related Old World Lujo Haemorrhagic fever nor New World arenaviruses.

New Therapeutic Approaches

Several research groups are currently attempting to develop a vaccine, but any breakthroughs may still be years away and Lassa fever patients urgently need a treatment now. From their previous *in vitro* work, Dr Boisen and his team know that antibodies can target and destroy Lassa viruses. The next obvious step is to test whether the same approach will work in animals.

It turns out that macaques exposed to Lassa virus and then treated with human antibodies didn't get sick, whereas animals not treated showed a series of symptoms, including problems with liver, spleen, and brain functioning. The researchers further showed that even if treatment was delayed up to a week, when animals were already showing signs of illness, it was still possible to reduce the viral load and clear symptoms.

'The current studies present a novel alternative for the treatment of Lassa fever infections, using an approach that relies on the use of fully human antibodies derived from convalescent donors,' adds Dr Boisen. 'Antibodies targeting the Lassa virus might be of clinical benefit for individuals with Lassa fever, and thus warranted further preclinical study of antibody efficacy.' Zalgen Labs has developed a human antibody therapeutic (Arevirumab™) which is advancing through preclinical studies in the hope it will eventually be approved as a frontline Lassa fever therapeutic.

Reliable Diagnostic Tools

Another problem that physicians based in remote areas must overcome is that of difficult diagnosis. The laboratory diagnosis of Lassa fever can be achieved using multiple methods but most are not suitable for use as a routine method in the rural endemic regions of West Africa. From Dr Boisen's perspective, the best possible alternative for use in remote locations relies on detecting the presence of viral antigens in suspected Lassa fever patients using rapid diagnostic tests (RDTs). Similar types of tests are already used to diagnose malaria, dengue, AIDS, and influenza.

Comparing different diagnostic methods, the team showed that Zalgen's ReLASV® RDT was at least as good as any other standard method, and in some cases, even surpassed the performance of other molecular testing methods such as polymerase chain reaction. The good news is that RDTs do not rely on any equipment and can be completed quickly and with limited resources. 'Widespread implementation could provide health care workers at remote locations with a reliable diagnostic tool rather than reliance on the less accurate non-specific clinical symptoms that have been the cornerstone of Lassa fever diagnosis for decades,' notes Dr Boisen.

How to Diagnose and Treat a Patient

The Kenema Government Hospital in Sierra Leone, and the Irrua Specialist Teaching Hospital in Nigeria, are the only two facilities in the world continuously testing for and admitting Lassa fever patients. This is an area well-known to Dr Boisen – the team evaluated the field performance of their ReLASV® RDT with individuals suspected of having Lassa fever from the Kenema Hospital and from control populations living in this area.

Upon arrival at the hospital, if patients present with suspected symptoms, they're screened for Lassa using Zalgen's RDT. This provides an initial diagnosis and allows physicians to start ribavirin treatment for positive results. If the result is negative, there is a second approach with more time-consuming methods to confirm the original result.

The benefits of Dr Boisen's research are thus already apparent with the translation of work to date from lab to clinic bringing practical benefits to the care of Lassa fever patients. Efforts such those undertaken by Dr Boisen and his team hold much-needed promise for reducing the burden and devastating impact of Lassa fever in West Africa.

Meet the researcher

Dr Matthew Boisen
Director of Diagnostics Development
Zalgen Labs, LLC,
Aurora, CO
USA

After completing a PhD in Biomedical Science from Tulane University in 2015, Dr Boisen joined Zalgen Labs as the Director of Diagnostics Development. The researcher has successfully obtained and managed multiple grants as a principal investigator, including management of international clinical research in collaboration with the Viral Hemorrhagic Fever Consortium (VHFC.org). With over 25 years' experience, Dr Boisen has unparalleled expertise in the development of rapid, accurate, point-of-care diagnostic tests for infectious diseases and bio-warfare pathogens.

CONTACT

E: mboisen@zalgenlabs.com
W: http://www.zalgenlabs.com/; www.vhfc.org

KEY COLLABORATORS

Dr Robert F Garry, Tulane University
James E Robison MD, Tulane University
John Schieffelin MD, Tulane University
Donald Grant MD, Kenema Government Hospital, Sierra Leone
Dr. Christian Happi, Redeemers University, Nigeria
Dr Luis M Branco, Zalgen Labs LLC
Dr Erica Olmann-Saphire, La Jolla Institute for Allergy and Immunology
Dr Kathryn M Weinell, La Jolla Institute for Allergy and Immunology
Dr Thomas W Geisbert, Galveston National Lab, University of Texas Medical Branch
Dr Robert W Cross, Galveston National Lab, University of Texas Medical Branch

FUNDING

National Institute of Allergy and Infectious Diseases (NIAID)
Coalition for Epidemic Preparedness Innovation (CEPI)
Bill & Melinda Gates Foundation

FURTHER READING

ML Boisen, et al, Field validation of recombinant antigen immunoassays for diagnosis of Lassa fever, Scientific Reports, 2018, 8, 5939.

KM Hastie, et al, Structural basis for antibody-mediated neutralization of Lassa virus, Science, 2017, 356, 923–928.
C Mire, et al, Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever, Nature Medicine, 2017, 23, 1146–1149

J Robinson, et al, Most neutralizing human monoclonal antibodies target novel epitopes requiring both Lassa virus glycoprotein subunits, Nature Communications, 2016, 7, 11544.

Zalgen Labs
GLOBAL CHALLENGES. SOLUTIONS

CALDER BIOSCIENCES: ENGINEERING SOLUTIONS FOR IMPROVED VACCINES

Designing better vaccines is the end goal for Calder Biosciences Inc., a company that has found a new way to engineer vaccines, ensuring greater stability, prolonging their duration in the body and thereby enhancing protection. Using a natural chemical reaction known as di-tyrosine (DT) crosslinking, Calder is creating safe and potent vaccines against viruses.

Harnessing the Power of Di-Tyrosine Crosslinking

Di-tyrosine (DT) crosslinking is a type of molecular bond that occurs in nature in materials such as silk, keratin in hair, and the exoskeletons of insects. Occurring when two tyrosine molecules – one of 20 amino acids that form the building blocks of proteins within cells – come together in close proximity and bond together, these bonds serve to increase the strength and elasticity of proteins.

Calder Biosciences has figured out a way to use this chemistry to target di-tyrosine crosslinks within the structure of a protein, and thereby to strengthen and lock into place the protein structures of viral vaccines. This increases the stability and potency of antibody-antigen recognition and specificity.

An antigen is a foreign protein, for example from a virus, which induces an immune response in the body. Vaccines are often made from inactivated forms of a virus or one of its surface proteins (antigens) that stimulate the body's immune system to produce antibodies that neutralise the virus. 'We optimise the potency of vaccines by introducing natural "molecular staples" into proteins that will be present on the surface of a virus – we thereby lock these proteins in their 3-D structures that most accurately represent the structure of the proteins on the surface of the virus,' says Dr Chris Marshall, Calder's founder and CEO.

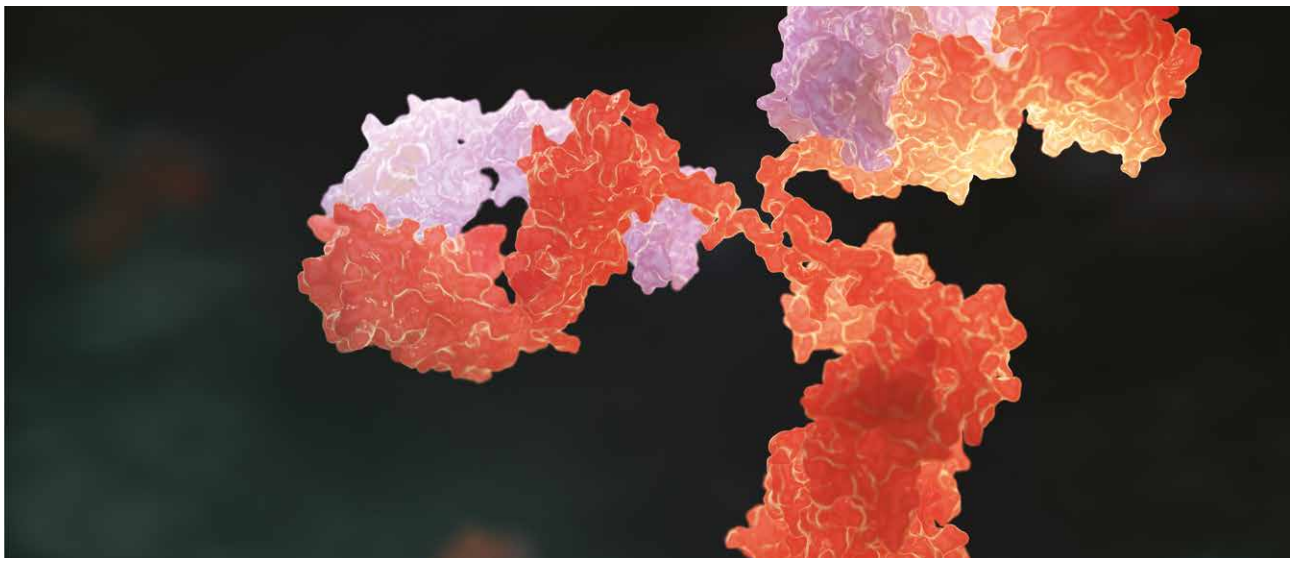
This approach, says Dr Marshall, has the potential to further the development of vaccines that are difficult to produce and can potentially eliminate the need for adjuvants – agents that are combined with vaccine formulations to enhance the immune response.

Calder's primary focus is on the development of a vaccine against the Respiratory Syncytial Virus (RSV), a common childhood infection, as well as a universal influenza vaccine. Calder's research team, headed by Dr Marshall and Dr Mark Yondola, has now, together with their collaborators, successfully stabilised vaccines towards both of these viruses using their DT-crosslinking technology, and studies to further test them in animal models are now underway.

Respiratory Syncytial Virus

Respiratory Syncytial Virus, or RSV, is a disease common in babies and young children. In the US, RSV infects nearly 100% of children within three years of life. In most children it passes as a common cold, but in those with immature immune systems, it can lead to a particular polarisation of the immune system, causing breathing problems throughout life: wheezing, asthma, and chronic obstructive

‘We optimise the potency of vaccines by introducing natural “molecular staples” into proteins that will be present on the surface of a virus-we thereby lock these proteins in their 3-D structures that most accurately represent the structure of the proteins on the surface of the virus.’



pulmonary disease. For their first six months, infants are vulnerable due to the immaturity of their immune systems.

RSV vaccines have been developed in the past. However, they present a problem in that if babies are vaccinated and then re-infected at a later date, their systems can react with an extreme response due to a phenomenon known as enhanced respiratory disease.

There is no antiviral therapy or vaccine for RSV. The only currently approved protective therapy for RSV is the administration of an antibody therapy called Synagis, made by MedImmune/AstraZeneca. Due to its high cost, use of Synagis is limited only to premature infants and to infants less than 24 months old with underlying conditions. Elderly and immune-compromised adults are also at risk of significant RSV-related illness and mortality. It is evident that an effective and safe RSV vaccine is urgently needed.

Engineering a Safe and Effective RSV Vaccine

When the RSV virus encounters a target cell, it fuses its membrane with that of the cell membrane, and thereby infects the cell. This fusion is mediated by the spring-loaded and metastable viral fusion protein, the F protein, which triggers when it encounters a target, and undergoes a major change in shape in order to infect the cell.

When pre-fusion (preF) and post-fusion (postF) structures of the F protein were compared using an imaging technique called x-ray crystallography, it was revealed that preF is the form that best binds to – and that will cause the immune system to make – protective neutralising antibodies. However, preF suffers significant instability. Other research groups have attempted to design preF vaccines in the past using alternate engineering techniques. However, these vaccines have continued to lack the stability needed to make a successful RSV vaccine.

After engineering a number of DT-crosslinked preF designs, Drs Marshall and Yondola and their team, together with their collaborators and advisors, Drs Jason McLellan of the University of Texas, Austin, Barney Graham of the National Institute of Allergy and Infectious Disease and Barry Buckland of BiologicB consulting group, screened a number of the designs. They found that DT-crosslinking stabilised preF and improved the ability of this vaccine to generate protective immune responses in mice and cotton rats. Having verified the potency, specificity and stability of their new DT-crosslinked RSV vaccine, the team is now testing formulations and strategies that will eliminate any chance of enhanced respiratory disease occurring in newborns.

Because enhanced respiratory disease only ever occurs in children with immature immune systems, vaccinating mothers during pregnancy is believed to be the safest approach in protecting infants from RSV, allowing mothers to transfer their protective antibodies to the unborn foetus through the placenta and the mother’s milk. In collaboration with Dr Kerry Empey at the University of Pittsburgh, Calder has developed a maternal-to-infant vaccination mouse model that is able to elicit immune responses and pathology in mice that emulate many of the characteristics of enhanced RSV disease observed in humans.

Using this model, Drs Marshall and Yondola and their team, together with the Empey lab, will test a number of

formulations and identify the one that most effectively protects postpartum mothers, new-born pups and newly weaned mice following maternal vaccination, and which does not lead to enhanced respiratory disease in infant mice. Calder's research team will then perform similar studies in cotton rats before initiating clinical trials.

Influenza – A Costly Global Affliction

Influenza is one of the most dangerous human pathogens. Spreading around the world in yearly outbreaks, it results in about 3–5 million cases of severe illness every year, and tens of thousands of deaths in the US alone, with a cost burden of over $10 billion per year. It is estimated that a future pandemic could cause untold deaths and suffering, and hundreds of billions of dollars in direct and indirect costs.

Vaccines are available but since the virus mutates from season to season, vaccines need to be reformulated every year. A vaccine that protects against all strains of influenza would provide an important safeguard against the threat of an outbreak of a pandemic influenza virus.

A Novel Approach to Designing a Universal Influenza Vaccine

Haemagglutinin (HA) is also a fusion protein found on the surface of influenza viruses. The HA protein is primarily responsible for inducing protective antibody responses. The structure of all HA molecules is comprised of a stalk and a globular head. The stalk is highly similar across strains, and most antibodies that protect against all strains of influenza target the molecular groups of HA's stalk. The globular head, however, varies from season to season, and further presents a barrier to vaccine design in that it prevents the immune system from reacting to the stalk.

Therefore, the development of a stable 'Headless HA' as an agent capable of stimulating an immune response that protects against all strains of influenza has been a major goal of the researchers for decades, but so far it has been impossible to design a Headless HA that assumes the native stalk conformation with sufficient stability.

In designing a stable Headless HA, Dr Marshall's and Yondola's team first locked the conserved stalk of HA in its native conformation by introducing DT-crosslinks that were targeted to the stalk of a complete HA protein. Then, once the stalk was stabilised with the DT bonds, the researchers removed the globular head using engineered cleavage sites. The resulting vaccine is a 'DT-Headless HA'.

The Potential of Headless HA Antigens

In preliminary data, the scientists showed that Calder's engineered influenza vaccine, DT-Headless HA, presents the stalk in its fully native conformation. They predict that its stability will also prolong the period that the protein holds its native conformation in the body, giving it enough time to teach the immune system to generate strong immune responses that protect against all influenza pathogens.

Going forward, Calder's research team along, with Dr James Crowe of the Vanderbilt Vaccine Center, will test and verify the potency of their DT-Headless HA vaccine in mice and ferrets, before initiating clinical trials.

Vaccines for the Future

DT-crosslinking provides a promising platform to overcome obstacles associated with the design of many vaccines. Comprised of a world-class research team made up of experts in vaccine design, Calder Biosciences is currently focused on protective solutions for RSV and influenza, however, this technology has the potential to be transferred to other difficult-to-design vaccines such as HIV, Dengue Fever and Herpes Simplex Virus. If so, it could save countless lives and give those afflicted and their families hope for the future.

Meet the researcher

Calder Biosciences Inc.
Brooklyn, NY
USA

Calder Biosciences Inc. is a molecular engineering company that aims to solve common vaccine design obstacles, using a unique approach based on the introduction of targeted di-tyrosine (DT) crosslinks. DT-crosslinking technology locks key viral proteins in place to elicit the most protective antibody response while improving vaccine thermostability and shelf life. Headed by CEO Chris Marshall, PhD, a New York-based researcher and entrepreneur, Calder has verified the design of both a DT-crosslinked Respiratory Syncytial Virus (RSV) and a universal influenza vaccine.

CONTACT

Dr Chris Marshall, CEO
E: cmarshall@calderbiosciences.com
W: www.calderbiosciences.com

KEY COLLABORATORS

Barney Graham, MD, PhD, Vaccine Research Center, NIH
Jason McLellan, PhD, Dartmouth College
James Crowe, MD, Vanderbilt Vaccine Center

FUNDING

Gates Foundation and International AIDS Vaccine Initiative
National Institute of Allergies & Infectious Diseases
National Institute on Aging

calderbiosciences

FIGHTING CHRONIC KIDNEY DISEASE WITH 2FP

According to the National Kidney Foundation, ten per cent of the world's population is affected by chronic kidney disease (CKD), and millions die each year from the condition. In response, **Dr Brian Peerce**, a biochemist, and Dr Slomowitz, a nephrologist, co-founded DuoPhos – a biotechnology company focused on developing treatments for chronic renal failure. One of the company's chief products, known as '2FP', is a new weapon in the fight against CKD.

The Battle Against Chronic Kidney Disease

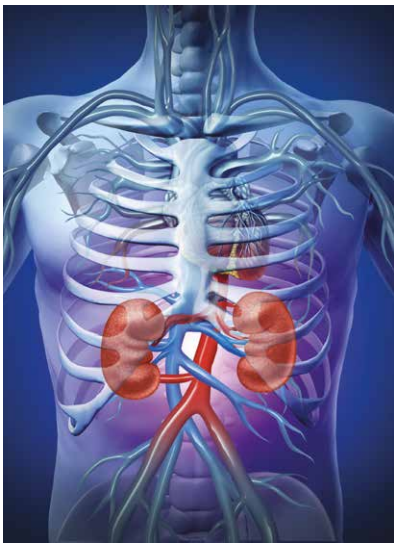
Chronic kidney disease (CKD) is a progressive condition resulting in the total loss of renal function. End-stage renal disease, which is the fifth and final stage of CKD where the kidney permanently fails, affects approximately 2.2 million people worldwide. In the United States, according to the National Kidney Foundation, more than 660,000 are being treated for end-stage renal disease – costing billions of dollars in the process. Common treatment options for end-stage renal disease include dialysis (the process of removing waste products and excess fluid from the body) or a full kidney transplant.

Drugs are often used to ensure that such treatments are not required, or if they are, that they are as effective as possible. In addition, drugs are often a more economically viable solution, and in many cases, can be accessed more readily than dialysis equipment or a kidney transplant. As a result, scientists and medical practitioners are seeking to improve the drug-based treatment options available to patients. They also aim to deliver these safely and efficiently to patients who are battling CKD, especially those who have limited access to healthcare.

Biochemist Dr Brian Peerce and his colleagues from DuoPhos have been developing a drug known as '2-fluorophosphophloretin', or '2FP', for treating CKD. Since streamlining the synthesis of 2FP, their research and development goals have been to assay the safety of 2FP, to determine the chronic toxicity of 2FP in animal testing and to examine the chronic toxicity of 2FP on human cells in vitro, in the laboratory outside the human body. Completion of these goals will establish the drugs suitability for human trials and accelerate its transition to clinical use.

The Problem with Elevated Phosphorus

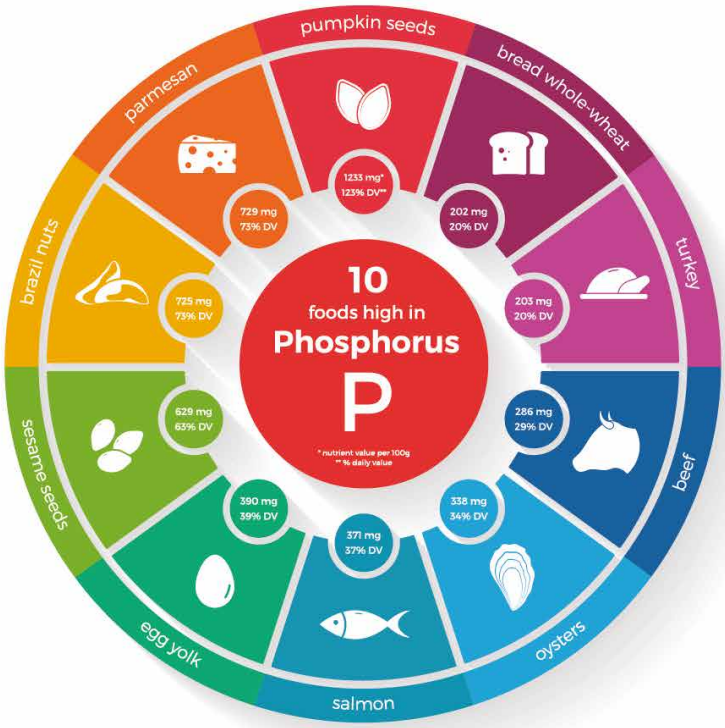
Although phosphorus is needed for energy production, muscle and nerve function, and bone growth; elevated levels in the blood predict poor patient outcomes. As Dr Slomowitz explains: 'Your kidneys remove all the excess phosphorus, and when they are compromised, high phosphorus levels or hyperphosphatemia ensues. Dialysis treatments remove phosphorus from blood quite effectively, but most excess phosphorus is stored in other body compartments, so the amount of phosphorus removed is less than the amount absorbed for many patients.'



Hyperphosphatemia portends higher mortality rates in both kidney patients not on dialysis and those receiving dialysis therapy. Hyperphosphatemia causes a variety of serious health problems because all the endocrine glands involved in bone and calcium homeostasis also become dysfunctional. This leads to weak and fragile bones prone to fractures and calcification of blood vessels, which leads to a greater risk for heart attacks and strokes.

The National Kidney Foundation recommends that a patient's level of phosphorus should be between 3.5 mg/dL and 5.5 mg/dL of blood.

‘The current standard of care for late-stage CKD is phosphorus binder drugs. However, binders are poorly effective and have multiple gastro-intestinal side effects. We have developed an alternative, known as ‘2FP’, which is water-soluble and blocks phosphorus uptake in the intestine. It has proven to be effective in milligram quantities, instead of the grams of phosphorus binders normally used to treat patients. Not only that, it appears to have no side effects.’



Unfortunately, most patients suffering from end-stage renal disease have phosphorus blood levels above what is recommended, and improving these levels is a major focus of kidney healthcare providers.

Present Therapies and Associated Challenges

Restricting dietary phosphorus is the cornerstone in treating hyperphosphatemia. Unfortunately, changing one’s diet is always difficult and low phosphorus diets are especially cumbersome. For example, meat, eggs and dairy products are high in phosphorus. Dr Slomowitz explains that, ‘the current standard of care for late-stage CKD is phosphorus-binder drugs. However, binders are ineffective (phosphorus intake is reduced by 20%) and have multiple side effects. Because of palatability issues, patient compliance is relatively poor.’

A more effective method to reduce phosphorus intake, or remove phosphorus from blood is needed and that is exactly what Dr Peerce and his colleagues have been working on. He relates, ‘we have developed an alternative, known as “2FP”, a pill which is poorly absorbed and blocks phosphorus uptake in the intestine. It has proven to be effective in milligram quantities, instead of the grams of phosphorus binders normally used to treat patients. Not only that, it appears to have no side effects.’

2FP and How It Works

After synthesis, Dr Peerce and his team at DuoPhos assessed the potential therapeutic action of 2FP. 2FP was found to inhibit the action of the sodium (Na+)/phosphate cotransporter (NaPi2b) – an intestinal protein responsible for 50 to 70 per cent of dietary phosphorus absorption. The ability of 2FP to limit the uptake of dietary phosphorus was indeed a critical discovery, as it could be used to lower blood phosphorus levels in patients with CKD. The next step, therefore, was to conduct animal tests to establish its real-world efficacy in doing exactly that, as well as any potential side effects.

Animal Tests Yield Positive Results

In animal testing, 2FP was demonstrated to reduce blood phosphorus levels by 40 to 55 per cent, at daily doses of five micrograms per kg of body weight over a two to 12-week period. Rats, for example, exhibited a 50 per cent decrease in blood phosphorus levels with 2FP. The result of this decrease was the recovery of renal function and the delayed progression of renal failure. Furthermore, rats treated with 2FP presented with no adverse health effects. In other tests, dogs were given 2FP in varying doses to assess the potential health effects on larger animals. After three days of repeat doses of ten micrograms, 100 micrograms, and one milligram per kilogram of body weight, no adverse side effects were observed.

In summary, at doses up to 1000 times the minimal efficacious dose, animals exhibited no side effects related to the drug – either after a single dose, or when the drug was given for up to four weeks. Bearing this in mind and given that in animal testing 2FP proved to be effective at hindering phosphorus absorption and lowering blood phosphorus levels, the next step was to conduct in vitro testing of toxicity on human cell samples in the laboratory.

Initial Tests Show No Toxic Effects

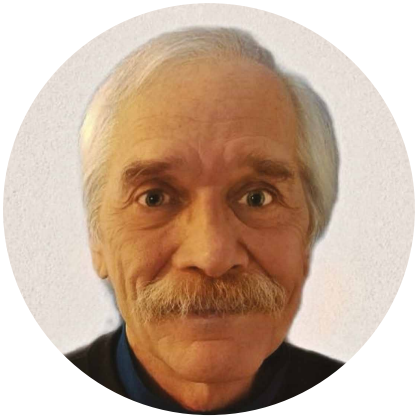
2FP's potential human toxicity was evaluated in vitro using several standard industry assessments, in both short-term settings and over longer periods of time at concentrations 100 times the anticipated maximum therapeutic dose for humans. Under both testing conditions, no toxicities were observed. Interestingly, the major by-product of 2FP metabolism and the 'backbone' of the 2FP is phloretin, an antioxidant found in apples. The team at Duophos is excited that 2FP showed no side effects in vivo (in the body) or during in vitro testing.

Another reason for 2FP's lack of toxic effect on humans is that, after ingestion, only a very small percentage of the drug is absorbed into the body (0.03 – 0.1 per cent). Despite this, the drug is still incredibly effective at limiting phosphorus absorption in the intestine, and thus, treating CKD.

Establishing Real-world Viability of 2FP

2FP has been tested on animals and an appropriate dosage has been determined. The toxicity of the drug on human cells has also been ascertained by in vitro testing and found no adverse side effects. 2FP has not been tested in human clinical trials to establish its real-world efficacy and associated side effects. That is the next step for Dr Peerce and the team at DuoPhos. In fact, they are currently preparing an application for FDA approval for the first human clinical trials.

2FP has the potential to be an effective treatment for CKD, with little to no side effects. Both animal and in vitro testing on human cells has confirmed this to be the case. In light of these positive results and with human clinical trials on the horizon, this new treatment could be moving from the laboratory to the clinic in the near future. The DuoPhos team is excited that if the drug works in humans as it does in animals, it would solve the problem of hyperphosphatemia in dialysis patients and could potentially eliminate the need for dietary restrictions. In CKD patients not on dialysis, it could help maintain their kidney function and hopefully avoid or delay the need for dialysis.



Meet the researcher

Dr Brian Peerce
DuoPhos
Friendswood, TX
USA

Dr Brian Peerce received his PhD in biochemistry from the University of Alabama at Birmingham. Since then, he has held faculty positions at the University of California Los Angeles (UCLA) and the University of Texas Medical Branch in Galveston. His research focuses on the mechanisms of membrane protein-mediated ion transport and developing pharmaceutical derivatives for disease treatment. In 2009, Dr Peerce and Dr Larry Slomowitz founded DuoPhos – a biotechnology company focused on developing new treatments for chronic renal failure. After conducting in-depth studies into the action of intestinal Na+-phosphate cotransporter (NaPi2B), the team at DuoPhos developed an inhibitor of NaPi2B known as '2FP' which, in turn, blocks dietary phosphorus absorption. Given that high phosphorus is a contributor to the progression of kidney disease, this is a significant step towards better treatment of the condition.

CONTACT

E: bpeerce@gmail.com

KEY COLLABORATORS

Dr Larry Slomowitz
Dr Pamela Joy Barton

FUNDING

National Institutes of Health
United States Department of the Treasury

IMMUNE CONTROL OF INITIATION AND PROGRESSION OF ATHEROSCLEROSIS

Atherosclerosis is a global health issue. Atherosclerosis is a multifactorial chronic inflammatory disease characterised by the accumulation of modified lipoproteins and immune cells in the aortic wall, vascular dysfunction, low-grade chronic inflammation, and formation of dangerous atherosclerotic plaques within the medium and large size vessels. Atherosclerosis is a prominent cause of cardiovascular diseases and mortality in many countries and this disease is closely associated with type 2 diabetes. **Dr Elena Galkina**, Professor of Microbiology and Molecular Cell Biology at Eastern Virginia Medical School, USA, has been working to determine the immune processes involved in an attempt to identify much-needed novel therapies.

The Immune Link with Atherosclerosis

A host of immune cell types have been associated with the initiation and progression of atherosclerosis. Dr Elena Galkina, Professor of Microbiology and Molecular Cell Biology at Eastern Virginia Medical School, USA, has identified the presence of many immune cell types, including both B- and T-lymphocytes, within normal aortic tissues as well as atherosclerotic tissue.

Furthermore, Dr Galkina has demonstrated that at least partial control of both T- and B-lymphocytes is managed by L-selectin, a molecule known to be involved in the 'homing' of T-cells to specific lymph nodes.

Function of B-lymphocytes in Atherosclerosis

Atherosclerosis, the leading cause of cardiovascular disease, accounted for approximately one-third of deaths in the USA in 2010. In an effort to reduce the rate of atherosclerosis development and progression, drugs or lifestyle changes are prescribed clinically. Unfortunately, this strategy only delivers an incomplete reduction of atherosclerosis.

Dr Galkina's team indicate the imperative nature of further investigation into the pathways that may confer protection against initiation and progression of atherosclerosis. There is a substantial body of evidence that demonstrates the involvement of both the innate and adaptive immune response in the chronic inflammation that initiates and promotes atherosclerosis. Different cell types and even different sub-groups of the same cell type may be either pathological or protective with regards to atherosclerosis.

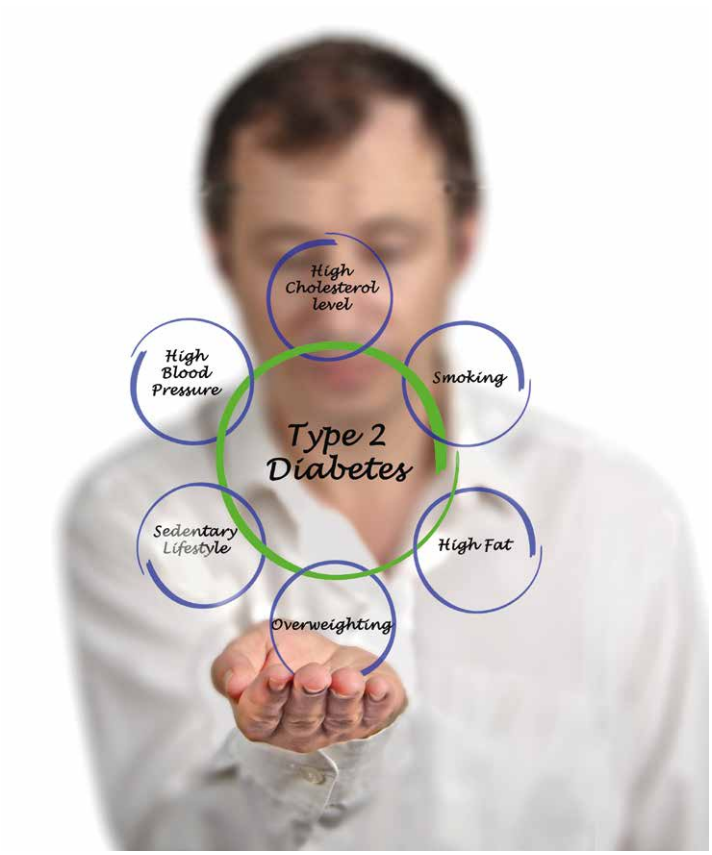
As the team has shown, B-cells are found in both atherosclerotic and normal aortic tissue, with differing ratios of B-lymphocytes of a specific sub-type being associated with atherosclerosis, both protective and disease-promoting.

Dr Elena Galkina and Colleagues

It is largely accepted that B-cells do have a role in the initiation of atherosclerosis; however, the activation mechanism and the balance between protective versus disease promoting B-cell types remains to be elucidated.

B-Cells and mLDL Uptake: Pathways and Cell Function Alterations

The research team is proposing to extend their work in the area of B-cell function and specific pathways for mLDL uptake, defining the pathways and, critically, the effect of this uptake on B-cell function. Initial research supports the induction of inhibitory pathways and changes in B-cell phenotype.



GRAIL and other E3 ligases are known to play a role in inducing tolerance in some immune cells, that is, they prevent the immune cell from responding to its own antigen target. Having demonstrated the presence of GRAIL and E3 ligases in B-cells, a collaborative team led by Dr Galkina and Dr Nurieva now proposes that a GRAIL-dependent mechanism is an inhibitory pathway that assists in regulating the B-cell activation threshold and induction of B-cells which do not respond to their antigen (anergic B-cells).

In their future plans, the team aims to identify the molecular mechanisms by which GRAIL exerts these effects on B-cells and the association with atherosclerosis.

Effector T-cells and Their Influence on Atherosclerosis Development

Dr Galkina and her colleagues have investigated the mechanism by which T-lymphocytes are involved in progressing atherosclerosis. Specifically, they interrogated the role of a receptor present on the surface of a sub-group of T-cells, CXCR6. They detected a significant correlation between this receptor expression on T-cells and atherosclerosis development.

In the absence of CXCR6, aortic lesion formation was found to be half that of CXCR6 sufficient mice. In addition, CXCR6 was positively associated with INF-γ, that is, where CXCR6 was low or absent, the concentration of pro-inflammatory cytokines was also reduced. Conversely, higher levels of CXCR6 induced production of INF-γ which, in turn, increased the recruitment of T-cells and macrophages (large white blood cells which are part of the immune system and a key component of atherogenesis) via up-regulation of a number of expressed proteins. Indeed, the team found that a population of CXCR6 deficient mice had a 90% reduction in one important sub-group of macrophages within the aorta.

Controlling Diabetes-associated Cardiovascular Disease

Cardiovascular disease related to atherosclerosis is increasing. Given the established link between inflammation of fat tissue, loss of beta cell function in the islets, and insulin resistance, the team of Dr Galkina and Dr Nadler team now intends to interrogate the link between loss of beta cell function and cardiovascular disease (CVD). They propose that identification of a shared pathway opens the door to developing dual-purpose therapies, that is, a methodology that addresses both beta cell decline and CVD.

Atherosclerosis is recognised as a major factor related to CVD in diabetic patients. As we have seen here, it is clear that atherosclerosis is a highly complex condition, involving a multitude of different pathways and systems throughout the body, and critically, includes the inflammatory immune response. Atherosclerosis-related inflammation is driven by a complex interaction and balance of

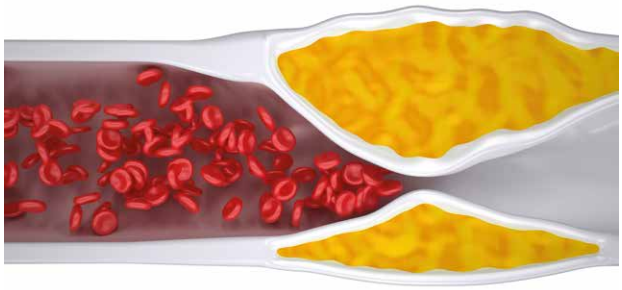


Diagram of Atherosclerosis: A Clogged Artery

immune cell subsets. However, within this myriad of immune cell interactions, there is a body of evidence that highlights the important role of myeloid cells in this damaging pathology.

Initiation of Atherosclerosis in Insulin Resistance

Investigating the link between atherosclerosis, obesity, insulin resistance, and type 2 diabetes, the research team of Dr Galkina and her EVMS collaborators Dr Nadler and Dr Dobrian established a link between STAT4, an immune regulatory factor, and increased inflammation in atherosclerosis and insulin resistance. Thereafter, Dr Galkina's team focused their efforts on the effect of STAT4 on initiation of atherosclerosis, and specifically, the formation of plaques in cases of insulin resistance.

Using an animal model that displays both atherosclerosis and insulin resistance, the team found that low/no STAT4 levels leads to reduced atherosclerosis and enhances the metabolic response. Looking directly at the numbers of plaques in STAT4-deficient mice compared to diet-aligned controls, they found significantly fewer lesions – up to a 36% reduction. Furthermore, the plaque area at the aortic root was significantly smaller in the STAT4-deficient mice compared to controls.

The team extended the study timeline to account for the later stages of plaque formation and found that STAT4-deficient animals continued to show reduced plaque burden (at a rate of approximately 45% reduction) compared to matched controls.

Cellular Level Effects of STAT4 on Atherosclerosis Initiation

When the research team focused on the role of STAT4 with respect to immune cell infiltration and immune reactions, they found that the initiation of atherosclerosis was affected via pro-inflammatory macrophage activity, altering the ratio of two important sub-groups of T-lymphocytes, leading to local immune response modulation within the aortic cell wall in insulin resistance-accelerated atherosclerosis.

Importantly, these findings suggest a possible therapeutic role for the modulation of STAT4 expression in reducing the accelerated atherosclerosis that is associated with insulin resistance and type 2 diabetes.

In their future work, Dr Galkina's and Dr Nadler's research groups plan to untangle the pathways that drive myeloid cell activation and differentiation in atherosclerosis. They have previously investigated the important role of STAT4 in the mouse model, and they have demonstrated that deficiency – or blocking – of this important molecule conveys protection against atherosclerosis. They found that this occurs via control of macrophage activation and disruption of the delicate balance of cell recruitment, death, and proliferation. These areas of cellular activity are vital to the progress of their work.

The team will extend the reach of their work by investigating the role of STAT4 activation of neutrophils, which is known to lead to beta cell dysfunction, decreased metabolic activity, and increased atherosclerosis. The team hypothesises that there is a common pathway, linked to STAT4, which targets macrophages and neutrophils to progress the dual pathologies of type 2 diabetes and atherosclerosis. Importantly, this research will inform the development of innovative treatments to maintain beta cell mass and simultaneously reduce the accelerated cardiovascular disease which is strongly correlated with pre-diabetes and diabetes.

Lymphocyte Populations and Pro-inflammatory Cytokines in Islet functions

It is well established that metabolic stress from excessive nutrition provokes islet inflammation and dysfunctions via lipotoxicity, and oxidative and endoplasmic reticulum stress, resulting in type 2 diabetes. While the role of the immune system in adipose tissue inflammation in type 2 diabetes is becoming more and more clear, very little is known about the impact of the immune system on islet health and functions in the conditions of type 2 diabetes. Thus, Dr Galkina and Dr Imai, an endocrinologist at the University of Iowa, initiated a project to determine lymphocyte and cytokine profiles in islets from normal human donors and type 2 diabetics. They found higher levels of CCL2, a protein that recruits specific immune cells to a site of inflammation, and TFN-α, an inflammatory cytokine, in type 2 diabetic derived islets when compared to normal islets.

In moderately functioning type 2 diabetic islets, some specific types of immune cells were increased in number when compared with poorly functioning islets. A range of both T- and B-lymphocytes was found in both healthy and damaged tissue; however, the team notes that the ratio of B-cells was significantly increased in the type 2 diabetic derived islets. The altered cell ratios point to a role of the adaptive immune response in islet damage. In terms of future plans, Dr Galkina and Dr Imai will focus their efforts on the characterisation of B lymphocyte functions in the regulation of islet dysfunction in pre-diabetic and type 2 diabetic patients.

Meet the researcher

Dr Elena V Galkina
Professor of Department of Microbiology and Molecular Cell Biology
Eastern Virginia Medical School
Norfolk, VA
USA

Dr Elena Galkina received her PhD in 1999 from the Saint-Petersburg Institute for Experimental Medicine, Russia. After completing a post-doctoral fellowship with the National Institute of Medical Research in the UK, she made the move to the University of Virginia, where she completed a postdoctoral fellowship before undertaking a series of academic position cumulating in her attainment of professorship in 2018. The focus of Dr Galkina's research is the role of the immune system in cardiovascular disease, and in particular, on improving the understanding of immune cell involvement and efficacy of therapeutic agents with a view to reducing mortality. As a fellow of the American Heart Association, Dr Galkina is frequently invited to share her expertise at cardiovascular disease conferences and seminars, attended by international audiences.

CONTACT

E: galkinev@evms.edu
W: https://www.evms.edu/directory/profiles/elena-v-galkina.php

KEY COLLABORATORS

Dr Jerry Nadler, New York Medical College, Valhalla, USA
Dr Mark Kaplan, Indiana University, Indianapolis, USA
Dr Roza Nurieva, MD Anderson Cancer Center, University of Texas, Houston, USA
Dr Larry Sanford, Eastern Virginia Medical School, Norfolk, USA
Dr Yumi Imai, University of Iowa, Iowa City, USA
Dr John Cambier, University of Colorado, Boulder, USA
Dr Anca Dobrian, Eastern Virginia Medical School, Norfolk, USA

FUNDING

NIH National Heart, Lung, and Blood Institute (NHLBI R01 HL139000 and NHLBI R01 HL142129)
American Heart Association (AHA AIREA33960546)

FURTHER READING

E Galkina, A Kadl, J Sanders, D Varughese, I Sarembock, K Ley, Constitutive lymphocyte recruitment into the aortic wall prior to development of atherosclerosis is partially L-selectin-dependent, Journal of Experimental Medicine, 2006, 203, 1273–82.

E Galkina, B Harry, A Ludwig, E Liehn, C Weber, K Ley, CXCR6 promotes atherosclerosis by supporting T cell homing, interferon gamma production and macrophage accumulation in the aortic wall, Circulation, 2007, 116, 1801–11.

MJ Butcher, D Hallinger, E Garcia, Y Machida, S Chakrabarti, J Nadler, EV Galkina, Y Imai, Association of proinflammatory cytokines and islet resident leucocytes with islet dysfunction in type 2 diabetes, Diabetologia, 2014, 57, 491–501.

P Taghavie-Moghadam, T Wassem, L Glenn, A Dobrian, M Kaplan, Y Yang, R Nurieva, J Nadler, E Galkina, STAT4 regulates CD8+Treg/Tfh cell axis and promotes atherogenesis in insulin-resistant Ldlr-/- mice, Journal of Immunology, 2017, 199, 3453–3465.

TOWARDS A BRIGHTER FUTURE: HOW ZIETCHICK RESEARCH INSTITUTE PLANS TO TRANSFORM TREATMENT FOR RETINAL DISEASE

Both diabetic adults and premature babies are at risk for a similar type of eye disease that involves the growth of abnormal, blood vessels in the retina, the photosensitive layer of the eye. When this eye disease occurs in diabetics, it is called diabetic retinopathy and when it occurs in premature infants, it is called retinopathy of prematurity. The pathologic vessels, seen in both of these diseases, can pull on the retina and cause it to detach, leading to blindness. **Dr Tammy Movsas** (Executive Director and Principal Investigator) and Dr Arivalagan Muthusamy (Chief Scientist) at the Zietchick Research Institute, USA, are developing new therapeutics to treat these serious retinal diseases that affect both premature baby eyes and mature adult eyes, such as those of diabetic women.

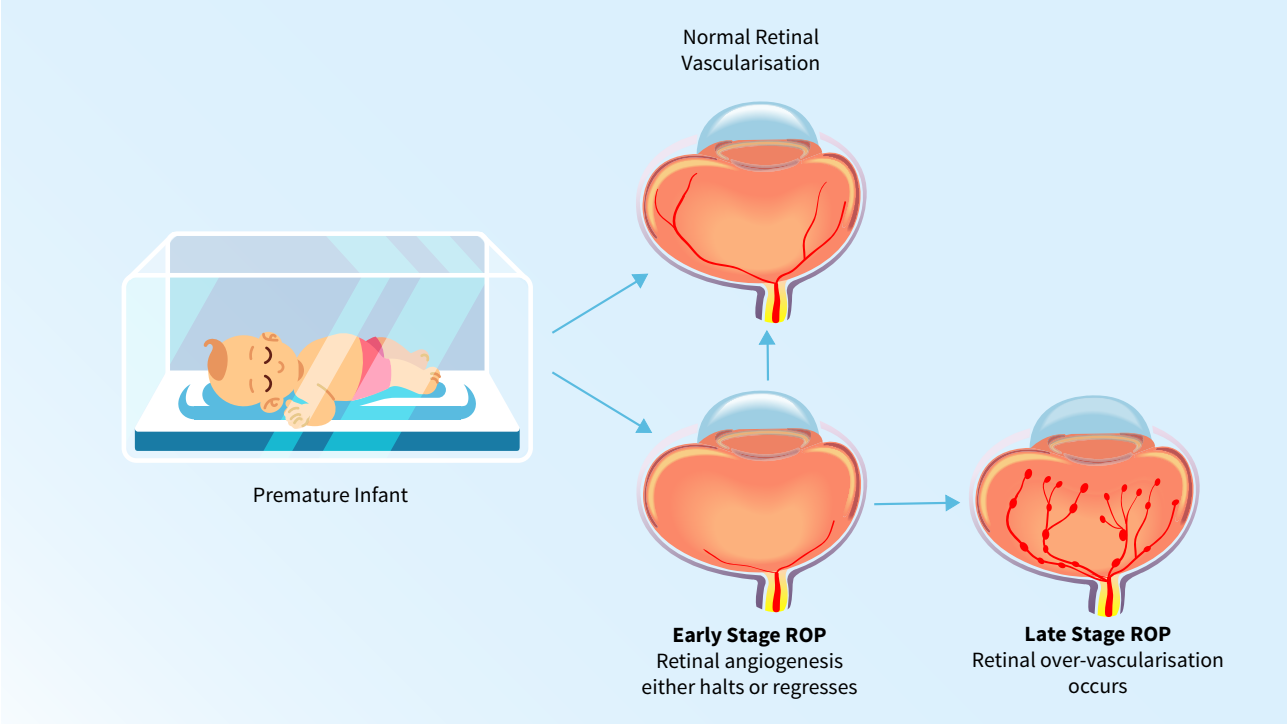
Diabetic Retinopathy

Diabetes mellitus is a global problem and one that is increasing. Currently, approximately 5% of the global population is diagnosed as being diabetic. However, the World Health Organisation predicts that by the year 2030, we can expect there to be more than 360 million people afflicted by diabetes worldwide. Type 1 diabetes occurs when the body's immune system attacks the pancreatic cells that are responsible for producing insulin and this is often diagnosed early in life. In contrast, type 2, the most common subtype of diabetes is often associated with obesity and typically onsets later in life. However, because of rising rates of obesity in youth, there has been a steep rise in the incidence of type 2 diabetes during the teenage and early adulthood years. In Type 2 diabetes, the body is either unable to produce enough insulin or becomes insensitive to the effects of insulin.

Diabetic retinopathy is the most common complication arising from diabetes, afflicting almost 35% of diabetic patients in developed countries. For diabetic women with retinopathy prior to pregnancy, there is a significant likelihood of their eye disease considerably worsening over the course of pregnancy. Due to the increasing number of young people with type 2 diabetes, there are more child-bearing aged women who are now affected by this. Despite the well-documented clinical awareness of this phenomenon, our knowledge regarding the underlying mechanisms of this exacerbation during pregnancy remains very limited.

Gonadotropins and the Eye

Dr Tammy Movsas (Executive Director and Principal Investigator) and Dr Arivalagan Muthusamy (Chief Scientist) at the Zietchick Research Institute (ZRI) are working with their colleagues to



Stages of Retinopathy of Prematurity, Credit Zietchick Research Institute

overcome the significant challenges posed worldwide by retinopathy. Their current work focuses on two related gonadotropin hormones. These are known as luteinising hormone (LH) and human chorionic gonadotropin (hCG). Both these hormones bind to the same receptor in the human body.

Gonadotropins are hormones that classically target reproductive organs such as the uterus and ovaries in females, and testes in males. For example, LH stimulates ovulation in teenage girls and women of childbearing age. Yet the importance of these hormones to the function of non-reproductive organs seems to be much greater than was first thought. On the basis of previously published research, Dr Movsas made the intriguing prediction that these hormones would also play a key role in retinal angiogenesis, that is, the development of new blood vessels in the eye.

Dr Movsas and colleagues were the first to demonstrate that a deficiency of LH receptors in the mouse eye negatively affects the vision enabled by the cone photoreceptors in the retina. Cone cells, named after their conical shape, are the retinal cells responsible for colour vision and for sharp central vision.

In an already landmark study, Dr Movsas and colleagues continued to show that LH is reliably present in the living human eye. In confirming their initial predictions, they also demonstrated that proliferative diabetic retinopathy is associated with increased levels of LH. In such cases, the researchers also noted that females seem to have high higher levels of LH relative to their male counterparts. These studies conducted by the Zietchick Research Institute in collaboration with university scientists converge to suggest a critical role for LH in the eye and for vision.

Retinopathy of Prematurity (ROP)

Retinopathy of prematurity (ROP) is one of the primary causes of visual disability in children. ROP is indiscriminate in its affliction of premature babies; it affects infants of all races, ethnicities, and socioeconomic groups. Premature babies of very low birthweight (that is, less than 1,500 grams) are at the highest risk and approximately 60,000 babies in the USA alone are at risk of the development of ROP every single year. It is problematic that the currently available treatments are typically either laser surgery or drugs that need to be injected directly into the eye. These are expensive and invasive for patients and have several side effects associated with their use.

Dr Movsas and her colleagues hope to dramatically change the horizon in terms of available treatments for ROP. They aim to develop a much-needed treatment that can be administered using eye drops. Not only would this provide a much simpler and less distressing intervention for young children, but such an approach would be more readily adopted in countries where eye surgeons are not as available as in the USA.

In a study published in 2018, Dr Movsas and colleagues investigated the contribution of gonadotropins to regulating levels of vascular endothelial growth factor (VEGF) in the developing eye. VEGF encourages the growth of new blood vessels. Premature infants are born before their eyes have fully developed, and sufficient levels of VEGF are needed for their retinal vessels to develop normally after birth. Low

Zietchick Research Institute is located in the Michigan Life Science and Innovation Center in Plymouth, MI

Dr Arivalagan Muthusamy, Chief Scientist of Zietchick Research Institute (ZRI)

levels of this growth factor in the first weeks of life in babies are associated with early-stage ROP. In early-stage ROP, there is a halting of new blood vessel formation in the retina. Dr Movsas and her team found that young mouse eyes without gonadotropin receptors had lower levels of VEGF as well as a lower density of retinal blood vessels. Their work suggests a vital role for gonadotropins in the vascular maturation of the retina. This finding is now being used to support the development of novel strategies by the Zietchick Research Institute to prevent the onset of ROP.

Gaining a Better Understanding

Since as far back as the 1940s, we have known that high oxygen levels used in the care of premature babies can cause ROP. For this very reason, as little oxygen as medically necessary is now administered. However, there are many other factors, in addition to oxygen, that also influence development of the eye. To better understand these factors, Dr Movsas and colleagues conducted a study of 113 babies who were born extremely prematurely and weighed less than 1,500 grams (under 3.3 lbs) at birth.

Often known as the 'pregnancy hormone', hCG helps promote the development of blood vessels in the uterus during pregnancy. Without adequate levels of hCG, pregnancy cannot be sustained by the mother, and low levels of the hormone are often used to diagnose miscarriage. Dr Movsas hypothesised that hCG also plays a key role in the development of blood vessels in the developing eye.

In premature babies, Dr Movsas and colleagues found that at 4 weeks old, blood levels of hCG are approximately the same as within the first days after birth. This suggests that premature babies make their own version of the pregnancy hormone – this was previously unknown. Moreover, they found that hCG levels tend to be lower in premature infants who develop ROP compared to those who do not, and that this is the case for both girls and boys. These findings have critical implications. This may be the first suggested role for hCG in human development, namely, its association with retinal vascularisation. Finally, given the presence of hCG receptors in other organs such as the gut and the brain, Dr Movsas points to the possibility that dysregulated hCG levels may be associated with other diseases associated with premature birth. Investigating this key hormone beyond vision may ultimately lead to improving the health of children who are born prematurely.

Onwards and Upwards

Having exciting evidence, thus far, for the potential involvement of gonadotropins in the development and maintenance of healthy vision, Dr Movsas and her colleagues now have an ambitious, evidence-based programme of research set in place. Dr Movsas explains, 'Most vision scientists working in the field of vasoproliferative retinal disease concentrate on either diabetic retinopathy or retinopathy of prematurity. At the Zietchick Research Institute, we feel that we can best advance our understanding of these disorders by studying them both in conjunction.'

In addition to continuing her work on preventing blindness in premature infants, Dr Movsas aims to uncover the reasons why pregnancy presents such a significant risk factor for the worsening of diabetic retinopathy. Building upon her team's findings to date, Dr Movsas predicts that hCG may increase VEGF in the maternal eye and this is what leads to the progression of diabetic eye disease during pregnancy. Dr Movsas proudly points out that the Zietchick Research Institute has undertaken the unique mission to develop therapeutics to optimise the eye health of both mothers and babies.

It is without a doubt that Dr Movsas and her colleagues have embraced a critically important challenge. By better understanding the underlying biological mechanisms of retinopathy in both mothers-to-be and premature babies, their research has the potential to transform the lives of many across the globe. By aiming to develop more readily accessible and even preventative treatments for conditions that eventually lead to blindness, the research undertaken by the Zietchick Research Institute provides a beacon of light in healthcare innovation worldwide.

Meet Zietchick Research Institute's Founder

Dr Tammy Movsas, MD, MPH
Executive Director and Principal Investigator
Zietchick Research Institute, L3C
46701 Commerce Center Drive
Plymouth, MI
USA

Dr Tammy Movsas, Founder of Zietchick Research Institute (ZRI), has always had a passion for innovation and experimentation. Scientific ingenuity and constant questioning of assumptions have been an integral part of her life for as long as she can remember. Over the years, she has studied biochemistry, medicine, and epidemiology at some of world's top universities: Harvard University (Cambridge, MA), Washington University School of Medicine (St Louis, MO), and University of Michigan School of Public Health (Ann Arbor, MI). She has completed a variety of residencies and fellowships at Washington National Eye Center (Washington DC), St Christopher's Hospital for Children (Philadelphia, PA), Scheie Eye Institute, University of Pennsylvania (Philadelphia, PA), University of Michigan (Ann Arbor, MI), and Michigan State University (East Lansing, MI). She practiced clinical ophthalmology for 12 years before devoting her career to public health and research. Then, for six years, she served as Medical Director of the Midland County Department of Public Health in Midland, MI. During her tenure as Medical Director, Midland County's health rankings rose each year reaching as high as #7 out of 83 Michigan counties. Filled with entrepreneurial spirit, Dr Movsas founded the Zietchick Research Institute in 2012. Within a short time, ZRI has become a world-class research organisation with a unique focus on developing therapeutics for maternal and child eye diseases. Dr Movsas has served as principal investigator on several National Institutes of Health and State-of-Michigan grants aimed to help advance our understanding of serious retinal eye disorders.

CONTACT

E: tmovsas@zietchick.com
W: http://www.zietchick.com/

FURTHER READING

TZ Movsas, N Paneth, IH Gewolb, Q Lu, G Cavey, A Muthusamy, The postnatal presence of human chorionic gonadotropin in preterm infants and its potential inverse association with retinopathy of prematurity, Pediatric Research, 2019, doi:10.1038/s41390-019-0580-8.

TZ Movsas, KY Wong, MD Ober, R Sigler, LM Lei, A Muthusamy, Confirmation of Luteinizing Hormone (LH) in Living Human Vitreous and the Effect of LH Receptor Reduction on Murine Electroretinogram, Neuroscience, 2018, 385, 1–10, doi: 10.1016/j.neuroscience.2018.05.049.

TZ Movsas, R Sigler, A Muthusamy, Elimination of Signaling by the Luteinizing Hormone Receptor Reduces Ocular VEGF and Retinal Vascularization during Mouse Eye Development, Current Eye Research, 2018, 43, 10, 1286–1289, doi: 10.1080/02713683.2018.1495740.

SPARKS CHILDREN'S MEDICAL RESEARCH CHARITY

Sparks is a UK-based charity funded entirely by their supporters. Their current campaign, *No Time to Lose*, aims to raise £10 million in the next four years to find the treatments children with rare conditions urgently need. In this exclusive interview, we speak with **Kiki Syrad**, Director of Grants, to hear about the importance of their work and how they aim to transform the futures of children afflicted by disease.

Lucy, who has a starring role in the No Time to Lose Campaign. Credit Sparks.

To begin, please give us a brief introduction to Sparks.

Sparks is a children's medical research charity dedicated to helping children with rare and complex conditions. We do that by funding life-saving child health research across the UK.

Since 1991, Sparks has funded over £30 million of research into over 80 childhood conditions, from childhood cancers to difficult-to-treat epilepsy, and many more conditions besides. We have a strong reputation in the child health research community and have supported over 90 research institutions across the UK with our grants. Currently, around one in three children with a rare condition won't live to celebrate their fifth birthday; a statistic that we are determined to change.

What is the overarching vision at Sparks for supporting critically ill children over the next few years?

Many children are living with medical conditions for which there is no known treatment or cure. Some don't even have a diagnosis, as their condition is so rare. For all these children and their families, research offers hope; for the answers that a diagnosis can offer, a future with kinder treatments, and ultimately a future with a cure.

Right now, paediatric researchers have the seeds of ideas, that, with funding could help transform children's lives. But child health research is severely underfunded – just 5% of public funding and charity spending on research in the UK funds medical research for children. One in 17 of us will be affected by a rare disease at some point in our lives and 75% of rare diseases present in childhood.

As a small, but we like to think, mighty, funder of paediatric research, we are working to raise £10 million over the next four years to help us make a real difference to seriously ill children. We will fund research that helps

doctors diagnose conditions earlier and accurately, that helps improve treatment options, and research that works towards the long-hoped for cures that will transform lives.

Child health has improved dramatically over the past decades – what problems face children and their families in the current day?

Medical research has definitely transformed the outlook for some children. For examples, in the 1960s only about three out of every 10 children (30%) with cancer in the UK were successfully treated. Before that it was largely incurable. But today, thanks to improvements in care and sustained investment in research, the outlook for young patients is much more positive – more than eight out of every 10 children in the UK diagnosed with cancer will live for at least five years, and most of these children will be cured *according to official statistics.*

This proves that investing in medical research works, but it's really important that we keep up with that investment, because despite these welcome improvements, there are still no treatments or cures for many rare childhood conditions. Sadly, childhood cancers are still the leading cause of death in children under 14 in the UK.

Another consideration is that children are still growing and have the rest of their lives ahead of them, which means they need treatments especially tailored to them and their bodies – ones that don't leave them with lasting side-effects. In addition, diseases like childhood dementia, juvenile arthritis, and childhood cancers are not always the same, and do not have the same underlying causes, as they do in adults. This emphasises even more that children need bespoke diagnoses and treatments; discovered and created especially for them, not just handed down from adult medicine.

The only way we can change the odds for seriously ill children facing rare and complex childhood conditions, is through research. Without it, families will continue to face a battle to find the answers that research could provide.

How do you directly support researchers and research institutions?

We run an annual process – our 'National Call' in partnership with Great Ormond Street Hospital Children's Charity. This joint call is the largest single dedicated funding call for child health researchers in the UK. By partnering, we can increase the amount of money we make available – aiming to make around £2 million available each year. Through this call, we invite researchers from across the UK to submit funding applications for their research, which then go through a rigorous scientific assessment process before we select the highest-quality projects to fund. We are one of the leading national funders of child health research and for many child health researchers, Sparks is one of the few places they can turn to for project funding.

To give you an idea of the impact that fundraising for Sparks can have, between 2015 and 2018, thanks to donations from our supporters, we

Putting smiles back on children's faces. Credit Sparks.

funded research into conditions that affect 20,000 children in the UK each year. In the last year alone, we committed funding to 12 new projects which cover a spectrum of techniques and conditions – from pioneering gene therapy for children with difficult-to-treat epilepsy, to creating superpowered immune cells to treat a range of childhood tumours and finding better ways to diagnose and treat Vanishing White Matter Disease – a devastating brain condition with no cure.

Where does your funding for research come from?

All the money we invest into funding research has been donated by our incredible supporters. We receive no government funding, so without our supporters we couldn't help the brightest minds seek the treatments and cures of tomorrow.

Whether it's running a marathon, attending a golf day or gala dinner, voting for Sparks as their employer's charity partner, or leaving a gift in their will, every penny our supporters raise gets us a step closer to finding the tests, treatments and cures that critically ill children so desperately need. We're immensely grateful for their support.

How do you assess the beneficial impact of your funded research?

Researchers keep us updated on their progress, including when they reach key milestones such as completing laboratory experiments or publishing scientific papers that make valuable findings available to other researchers around the world. They also report to us annually, with a larger report due at the end of their projects. The impact of their work can be incredibly far-reaching as it may inform future research and ultimately impact on seriously ill children all around the globe.

One clear indicator of success is when a new treatment or test, developed with Sparks funding, reaches clinical trial or is approved for wider use. Every medical advance starts out as just an idea, and it takes a lot of time, effort, and money to gather the scientific evidence needed to prove it's safe and effective enough to offer children. It's incredible to see how our funding can make those ideas a reality – from changing clinical practice to giving seriously ill children access to promising new tests and treatments.

Our funding can also help researchers gather the vital evidence they need to win the backing of larger funders, to take their promising idea forward or implement their findings. We have numerous examples where our funding has been the stepping-stone needed to take research to the next level.

What are the key challenges facing child health in the United Kingdom and how might these challenges be best overcome?

The challenges facing child health in the UK are varied.

It's positive that more rare and complex diseases are being identified thanks to the ongoing revolution in genetics and the tests that are available to diagnose them. With a diagnosis it is easier to work towards treatment and support for that child, and also ends the so-called 'diagnostic odyssey' for families. But the challenge remains that not every rare condition can be diagnosed, and once parents get a diagnosis, they may have to face the reality that there is no treatment or cure for their child's condition.

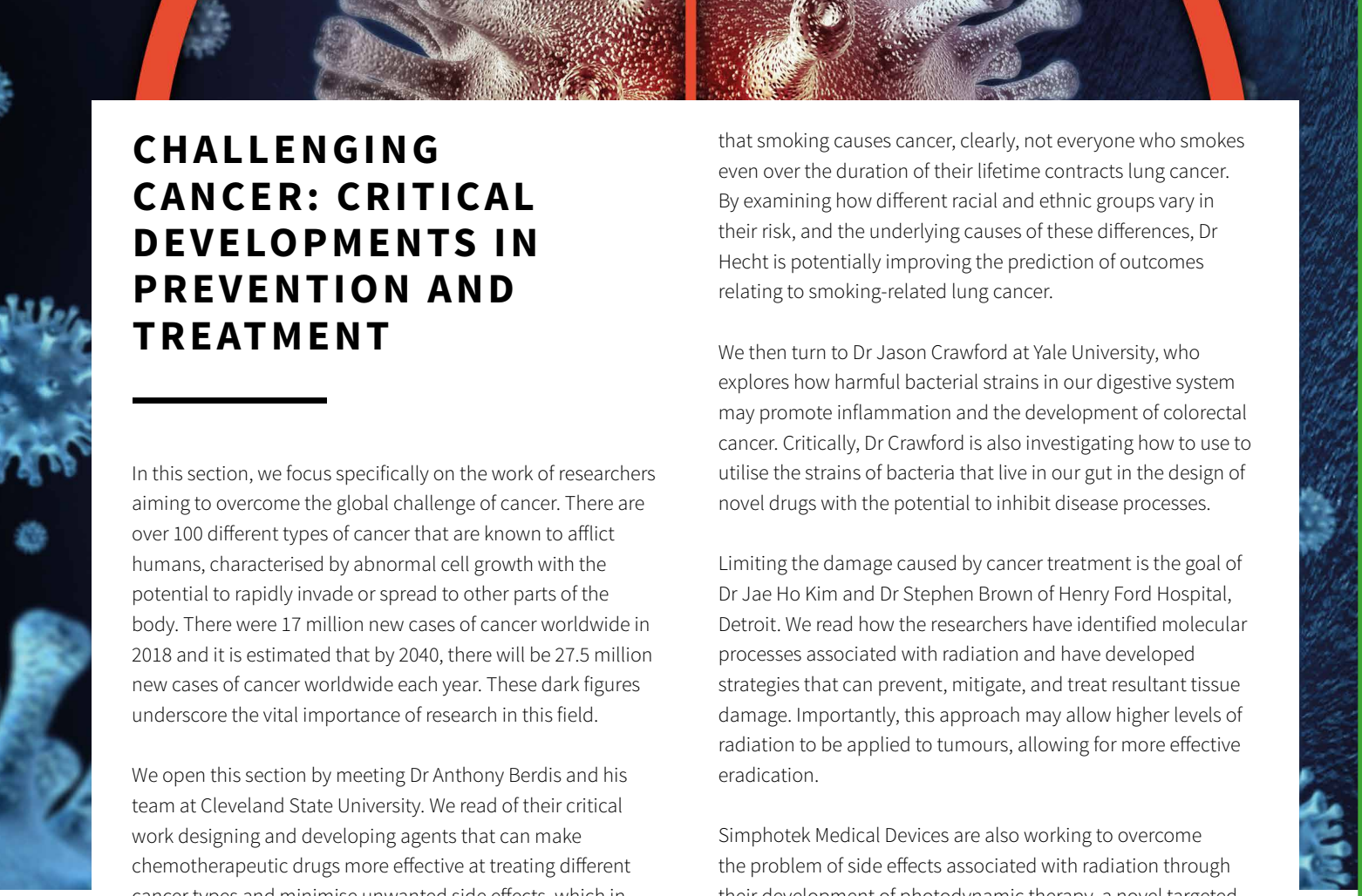
Our challenge, and our ambition, is to continue to inspire people to donate to us and to demonstrate the difference their money can and does make, as we support Sparks' funded researchers to find the new tests, treatments, and cures that are desperately needed. For critically ill children, there's *no time to lose*.

For more information on how you can support Sparks, visit www.sparks.org.uk

W: www.sparks.org.uk
@SparksCharity

Sparks
Children's Medical Research Charity

ADVANCES IN CANCER RESEARCH



CHALLENGING CANCER: CRITICAL DEVELOPMENTS IN PREVENTION AND TREATMENT

In this section, we focus specifically on the work of researchers aiming to overcome the global challenge of cancer. There are over 100 different types of cancer that are known to afflict humans, characterised by abnormal cell growth with the potential to rapidly invade or spread to other parts of the body. There were 17 million new cases of cancer worldwide in 2018 and it is estimated that by 2040, there will be 27.5 million new cases of cancer worldwide each year. These dark figures underscore the vital importance of research in this field.

We open this section by meeting Dr Anthony Berdis and his team at Cleveland State University. We read of their critical work designing and developing agents that can make chemotherapeutic drugs more effective at treating different cancer types and minimise unwanted side effects, which in cancer, can be nearly as damaging as the disease itself.

Dr Douglas McNeel at the University of Wisconsin's Carbone Cancer Centre is working to enhance the efficacy of immunotherapeutic drugs. We read how Dr McNeel is specifically targeting prostate cancer, a leading cause of male deaths from cancer through his laboratory and clinical research. His goal is to identify specific proteins of the prostate that can be used to generate anti-tumour vaccines and identify how these can be best used to treat prostate cancer.

Improving the treatment of cancer is also the aim of Dr Joseph Vetro and his group at the University of Nebraska Medical Centre. By decreasing chemotherapy resistance using a technique known as 'RNA interference,' other drugs targeting the tumour can work more effectively. We read how this early-stage work is showing promise in revolutionising cancer treatment in clinical practice.

Melanoma, one of the most common forms of cancer across the world, is the focus of research by Professor H. Peter Soyer and colleagues at the University of Queensland. We read how they are developing a range of specialist techniques to aid the identification of people at greatest risk of developing a melanoma. This precision early detection allows more timely intervention, and ultimately, can save lives.

Understanding the biological mechanisms that link smoking, lung cancer and ethnicity is the goal of Dr Stephen Hecht and co-workers at the University of Minnesota. Although we know that smoking causes cancer, clearly, not everyone who smokes even over the duration of their lifetime contracts lung cancer. By examining how different racial and ethnic groups vary in their risk, and the underlying causes of these differences, Dr Hecht is potentially improving the prediction of outcomes relating to smoking-related lung cancer.

We then turn to Dr Jason Crawford at Yale University, who explores how harmful bacterial strains in our digestive system may promote inflammation and the development of colorectal cancer. Critically, Dr Crawford is also investigating how to use to utilise the strains of bacteria that live in our gut in the design of novel drugs with the potential to inhibit disease processes.

Limiting the damage caused by cancer treatment is the goal of Dr Jae Ho Kim and Dr Stephen Brown of Henry Ford Hospital, Detroit. We read how the researchers have identified molecular processes associated with radiation and have developed strategies that can prevent, mitigate, and treat resultant tissue damage. Importantly, this approach may allow higher levels of radiation to be applied to tumours, allowing for more effective eradication.

Simphotek Medical Devices are also working to overcome the problem of side effects associated with radiation through their development of photodynamic therapy, a novel targeted light-based technique which has already been approved as an effective treatment for some forms of cancer. We read how photodynamic therapy has the potential to become a routine clinical procedure, avoiding the complications of chemotherapy, radiotherapy, and surgery.

Dr Frank Wuest at the University of Alberta, Canada, seeks to improve both the diagnosis and treatment of cancer using molecular imaging probes. We read how his multidisciplinary work is identifying novel diagnostic and therapeutic biomarkers, and how he intends to take this work from the laboratory to clinical practice.

Taking a different perspective in the challenge against cancer, we turn to Dr Elizabeth Ryan at Colorado State University. Dr Ryan and her team aim to prove the disease prevention efficacy and mechanisms for whole foods (such as grains and legumes) in humans that have been found to have a protective benefit against cancer in animals. With an increasing evidence base for efficacy, Dr Ryan also highlights the importance of improving the public awareness of whole foods for disease prevention.

We conclude this section by meeting Dr Jaime Modiano at the University of Minnesota in the Twin Cities. We read how by examining the development of cancer in our canine friends, his research is progressing our understanding of how cancer develops at a basic level. Dr Modiano aims to use these insights to help improve the health of humans and our companion animals by more effectively treating, delaying, and perhaps even preventing the onset of cancer.

COMBATTING CANCER – BREAKTHROUGH RESEARCH AGAINST THE DREADED DISEASE

In essence, cancer cannot be 'cured', but it can be vigorously treated. However, many of the treatments currently used to combat cancer often cause harmful side-effects, that are sometimes even more dangerous than the disease itself. **Dr Anthony Berdis** and his team at Cleveland State University look to address this through their research, by designing and developing agents that can make chemotherapeutic drugs more effective at treating different cancer types.

Cancer is a disease everyone knows and everyone dreads. It is a term that sends shivers down the spine, representing a currently incurable disorder that affects everyone, everywhere. Whether it be a family member, a friend or even you yourself, everyone knows someone who has been affected by cancer. It's a disease that's hard to avoid.

As such, it has earned itself a fearful reputation – and now, because people are living longer, we have reached a stage where almost one in two people will develop some form of cancer during their lives. In fact, the World Health Organization expect the worldwide number of cancer cases to double by 2030, with the death rate rising by 60% in that time as well. Research into cancer's physiology is therefore vital.

Curing the Incurable

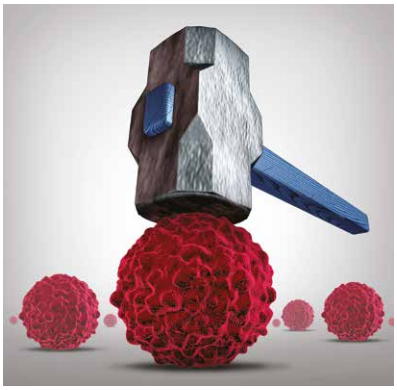
Herein lies the difficulty though. Cancer is not some sort of evil person or entity that can simply be destroyed – it represents a multitude of diseases, all of which require different treatment options. It will never be like the smallpox or polio viruses, which have both been effectively eradicated through straightforward vaccinations. Cancer is far too complex for that – and for two key reasons.

Firstly, cancer cells are highly intelligent, and are able to evade the body's immune system and adapt to form resistance against treatments over time. And secondly, there are over 200 different types of cancers, many of which break down into further subtypes as well. There can't simply be a 'one-for-all' cure against them all.

So, what can be done? Well, cancer can be researched, fought and continuously treated against, and that's exactly what scientists such as Dr Anthony Berdis at Cleveland State University (CSU) are currently doing.

Cleveland Cancer Research

Cancer research has been at the top of the agenda for Dr Berdis throughout his academic career. He and his team at CSU have uncovered several breakthroughs in understanding a number of specific cancers, including brain tumours, breast cancer and lung



disease. His research has largely looked at investigating methods to improve the effectiveness of currently-used anti-cancer drugs, through the design and development of supportive therapeutic agents.

As Dr Berdis stated himself in a recent *News 5 Cleveland* article: 'Thirty years ago, the goal really was to try to "cure" cancer. Realistically, the expectation now is to develop better treatments.' However, in order to understand the science behind Dr Berdis and his team's research more clearly, it is important to first understand the design of the drugs that are currently used to treat different types of cancer.

‘The risks and side effects from current cancer treatments can be nearly as bad as the disease itself.’

As cancerous tumours are often specific to one area of the body, drugs and treatments typically used to remove tumours are similarly specific in their targeting function. That said, these treatments don’t only target the tumour itself – they also affect the surrounding healthy tissue as well, leading to harmful side-effects such as heart disease and DNA damage. Dr Berdis and his team focus on reducing these side effects, by developing effective methods that can improve and sustain current anti-cancer drug mechanisms.

Two is Better Than One

Take temozolomide for example. This anti-cancer drug is used to treat a number of different cancers, including brain cancer – a condition that affects thousands of adults and children annually. In fact, according to Dr Berdis, two-thirds of those diagnosed with such a cancer die within five years. He and his team’s recent research has fought to change that statistic.

Temozolomide functions through its ability to penetrate the blood-brain barrier and kill cancer cells by damaging DNA. However, while the drug may target the cancer, it also leads to DNA lesions which cause the death of cells – both healthy and tumorous cells.

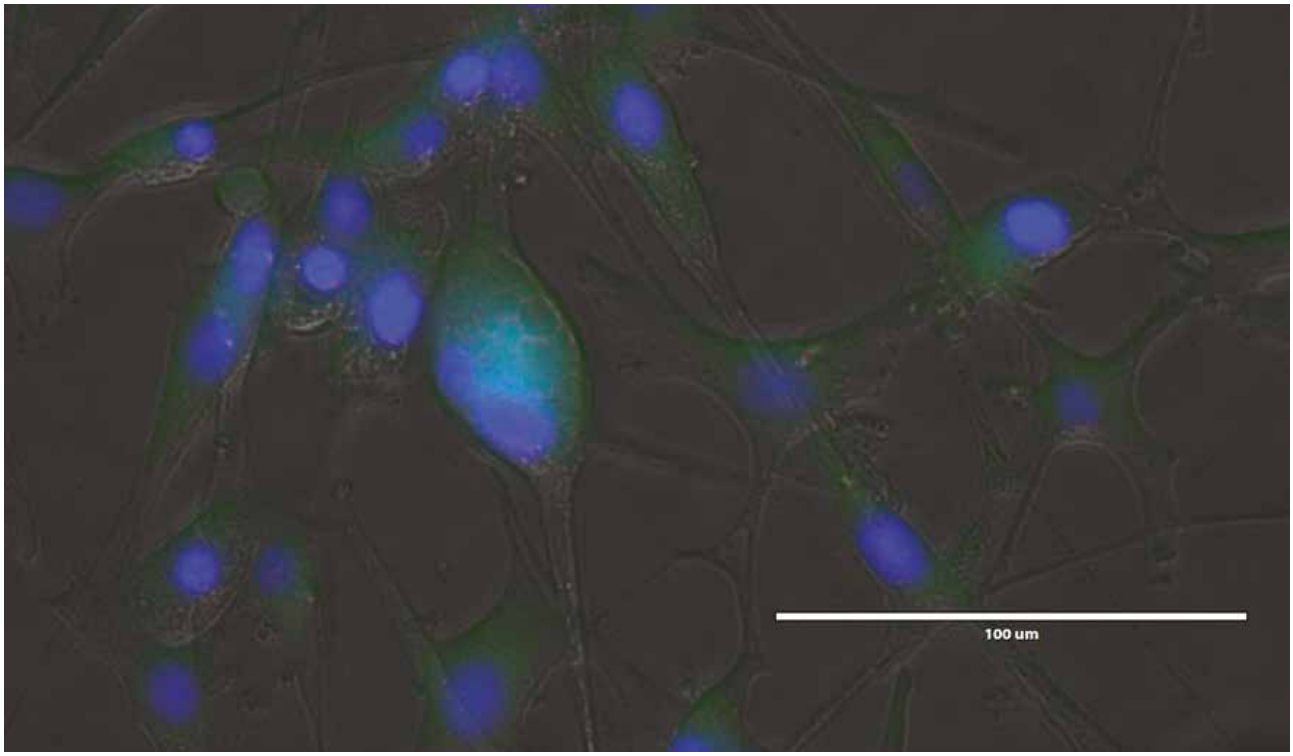
The team at CSU also discovered that cancer cells can gain resistance against temozolomide over time, through the functions of an important enzyme in the DNA replication process called DNA polymerase. This drug resistance can lead to the formation of more aggressive and harder-to-treat tumours, meaning that temozolomide’s effectiveness at treating brain cancer can become limited over time.

However, Dr Berdis and his team have now developed a novel therapeutic agent that can improve the effectiveness of temozolomide in treating brain cancer. This agent is known more scientifically as an artificial nucleoside called 5-NIdR (5-nitroindolyl-2’-deoxyriboside), which the team found to ‘efficiently and selectively inhibit the replication of DNA lesions generated by temozolomide’. To put it more simply, the researchers effectively found that when 5-NIdR was used alongside temozolomide, it proved more successful at treating brain cancers. ‘The risks and side effects from current brain cancer treatments can be nearly as bad as the disease itself,’ says Dr Berdis. ‘The combination of our drug with temozolomide has the potential to greatly improve both life expectancy and quality of life for patients. This research could have tremendous applications for addressing numerous types of cancers and, ultimately, help patients to live longer, better lives.’

The Importance of Nucleosides

When an anti-cancer drug is administered to a patient, it often acts by damaging and destroying the cancer cells’ DNA. In essence, this DNA is the ‘brain’ of the cell – take away the brain, and the cancer cell will die. However, DNA replication is highly complex and, through a variety of different methods, cancer cells can eventually gain resistance against anti-cancer drugs.

In order to understand the importance of nucleoside agents in combatting cancer resistance, Dr Berdis and his team conducted a study in 2017. In this study, they used their 5-NIdR nucleoside to not only visualise the replication of damaged DNA, but also to identify the cells that were able to acquire



resistance against anti-cancer drugs. The team then analysed the nucleoside to see what impact it had when used alongside anti-cancer drugs, such as temozolomide.

The researchers found that their artificial nucleoside was able to inhibit DNA synthesis (preventing the replication of cancer cells) and boost the therapeutic activity of anti-cancer drugs. It was also able to identify drug-resistant cancer cells, which could be highly valuable for the development of diagnostics and personalised medicine.

Future Research – Breast and Brain

Looking to the future, Dr Berdis and his team's research efforts will fundamentally focus on two key areas: breast cancer, and a brain tumour called glioblastoma multiforme.

In their breast cancer research, the team aims to use a similar method to their recent brain tumour work on temozolomide, evaluating the effect of the 5-NIdR nucleoside on the efficiency of currently-used breast cancer treatments.

Breast cancers can come in many forms, or 'subtypes', as they are commonly known. These mainly include 'invasive' forms, where cancers form after breaking through breast tissue before spreading to other areas of the body, and 'non-invasive' forms, where cancerous cells remain in a particular area of the breast without spreading.

Current breast cancer treatments typically include surgery, ionising radiation and chemotherapy. However, these methods all come with various side effects, such as the extensive inflammation that ionising radiation can cause and the lasting DNA damage that chemotherapy results in. Similarly, anti-cancer drugs such as doxorubicin can also produce severe heart failure and side effects that limit their efficacy, despite their initial effectiveness at inhibiting cancer cell growth. To this end, Dr Berdis and his team now hope to determine whether or not an artificial nucleoside could be used to improve the usefulness of such treatment options.

The team at CSU also aims to use their novel nucleoside knowledge to improve the treatment and outcomes of glioblastoma multiforme – a brain tumour commonly referred to as 'the deadliest of all cancers'. Their research will seek to prevent the production and replication of the harmful DNA lesions that currently form using typical anti-cancer agents.

Slow and Steady

There is still a long way to go in understanding how to treat cancer effectively, but Dr Berdis and his team at CSU have made great strides in tackling cancer research head-on. Their work into limiting the side-effects of current anti-cancer treatments, through the design, development and utilisation of artificial nucleoside agents, has paved the way for further novel scientific research into cancers of all areas.

However, that's not all. Dr Berdis has also provided cancer patients around the world with an emotion that is absolutely vital in the fight against the dreaded disease: the feeling of hope. Without hope, the battle is lost and cancer wins – and we can't let that happen. Fortunately, with Dr Berdis and his team at the helm, it doesn't look likely to.

Meet the researchers

Dr Anthony Berdis
Center for Gene Regulation in Health and Disease
Cleveland State University
Cleveland, OH
USA

Dr Anthony Berdis received his BSc in Chemistry from Gannon University in 1990, after which he was awarded his PhD in Biochemistry from the University of North Texas in 1993. He later went on to work at other universities, including Pennsylvania State University and Case Western Reserve University. Dr Berdis currently works as both an Associate Professor in the Center for Gene Regulation in Health and Disease at Cleveland State University, and as the co-founder/chief scientific officer of Red5 Pharmaceuticals. His team's research focuses on the development and biological testing of non-natural nucleosides and nucleotides that target DNA polymerase activity.

Dr Jung-Suk Choi
Academic Affairs
Ghent University Global Campus
Yeonsu-Gu, Incheon
Korea

Dr Jung-Suk Choi received her BS and MS degrees in Food Science and Nutrition from the Hallym University in Korea. She then received her PhD in Food Science and Nutrition in 2009 from the Hallym University. During her graduate education, she published 22 manuscripts, including several papers as first author that were published in high-ranking journals in the field of nutrition. Dr Choi joined the Berdis group for postdoctoral training. During this time, she published 10 research articles and two book chapters on the development and application of novel diagnostic and therapeutic approaches against various cancers including leukaemia and brain cancer. Using a multi-disciplinary approach, Dr Choi has integrated several research areas including bioinorganic chemistry, pharmacology, cancer biology, and imaging to produce new chemical entities and methodologies to provide more effective cancer treatments. Dr Choi is currently an academic study counsellor at the Ghent University Global Campus in Incheon, Korea.

CONTACT

E: a.berdis@csuohio.edu
W: http://www.csuohio.edu/grhd/faculty/anthony-berdis

KEY COLLABORATORS

Dr Irene Lee, Case Western Reserve University
Dr Stephen J. Benkovic, The Pennsylvania State University
Dr Thomas Gray, Case Western Reserve University

FUNDING

National Cancer Institute
National Science Foundation
Department of Defense
Ohio Third Frontier Foundation
Dr John C. Vitullo's Pilot Fund

FURTHER READING

J Choi, C Kim, and A Berdis, Inhibition of Translesion DNA Synthesis as a Novel Therapeutic Strategy to Treat Brain Cancer, American Association for Cancer Research, 2018, 15, 1083–1096.

J Choi, C Kim, E Motea, and A Berdis, Inhibiting translesion DNA synthesis as an approach to combat drug resistance to DNA damaging agents, Oncotarget, 2017, 8, 40804–40816.

J Choi, A Maity, T Gray, and A Berdis, A Metal-containing Nucleoside That Possesses Both Therapeutic and Diagnostic Activity against Cancer, The Journal of Biological Chemistry, 2015, 290, 9714–9726.

ENHANCING THE EFFICACY OF IMMUNOTHERAPEUTIC DRUGS FOR PROSTATE CANCER

Prostate cancer is a leading cause of male cancer deaths worldwide, with one man in every seven likely to contract the disease during his lifetime. As late-stage prostate cancer remains a fatal disease resistant to conventional treatment, the need for effective new therapies is dire. **Dr Douglas McNeel**, a Professor of Medicine in the Haemato-Oncology Division of the University of Wisconsin's Carbone Cancer Centre, has been working to meet this need. His team's goal is to identify specific proteins of the prostate that could be used to generate anti-tumour vaccines, and then evaluate the best means to deploy these vaccines to treat prostate cancers.

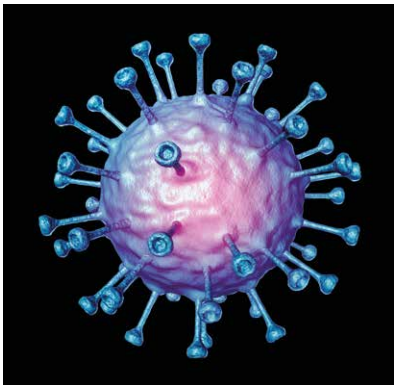
The Immune System and the Search for Prostate Cancer Antigens

Conventional treatments for prostate cancer include chemotherapy, radiotherapy, and strategies to decrease circulating levels of androgens (male sex hormones including testosterone) – which feed cancer cells. In metastatic castration-resistant prostate cancer, conventional drugs are of limited efficacy: the cancer soon develops resistance to treatment, causing death. Thus, the advent of immunotherapy drugs, which exploit the ability of the body's own immune system to fight cancer, constitutes a welcome advance in combating this disease.

Immunotherapy works by activating immune cells to recognise cancer tissue as different from normal body cells. Cancerous cells carry a specific protein marker on their surface, called an antigen, which the immune system recognises and attacks. T and B cells (also known as lymphocytes) are the main players in recognising and destroying such cells.

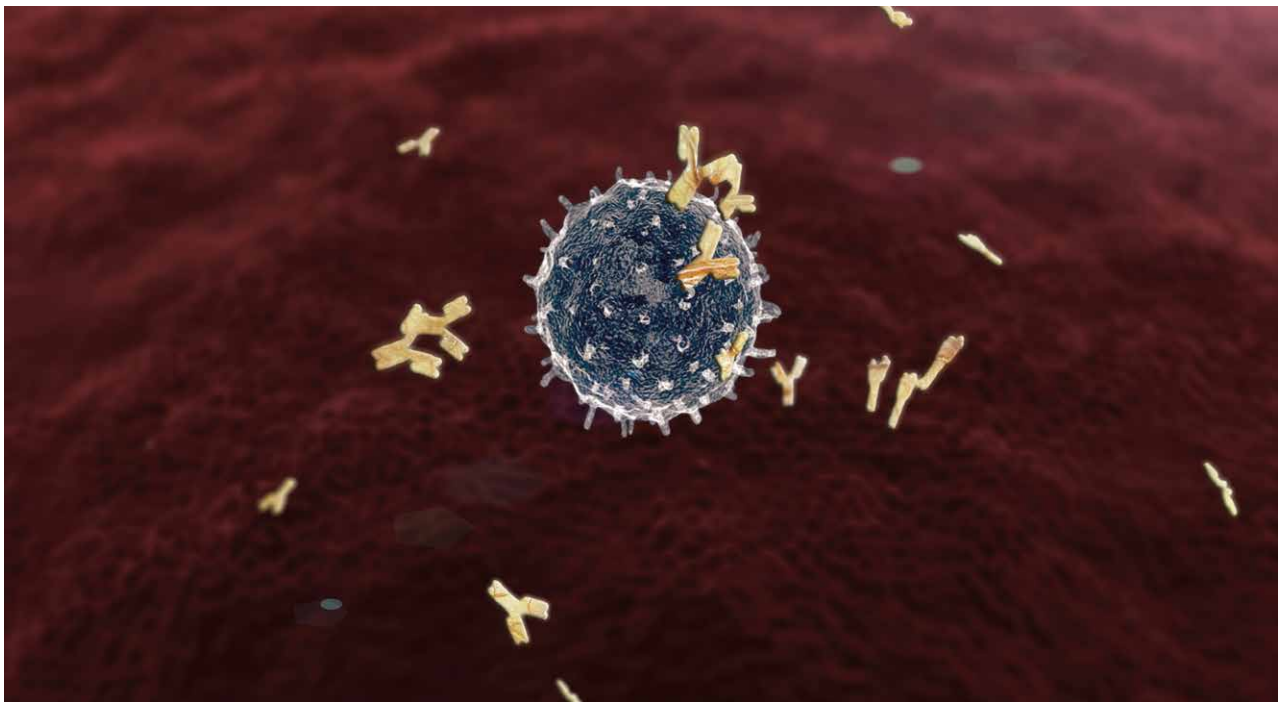
B-cells (which mature in the bone marrow) are characterised by a surface protein called the B cell receptor. They make Y-shaped proteins called antibodies that stick to antigens on the surface of cancer cells, creating cancerous clumps that other immune system cells then destroy. The antigen-antibody reaction is direct but the B cell does not itself interact directly with the target. An antibody does not recognise the entire protein antigen but rather a small (5–6 amino acid) section of it, called an epitope. Thus, for example, the human prostate-specific (PSA) antigen, which is 240 amino acids long, could carry several epitopes.

T-cells (so-called because they develop in the thymus) are characterised by their surface T cell receptors (TCR). Like B cells, they recognise antigens expressed by cancer cells. However, they can kill cancer cells directly. The killing is



carried out by a subset of T cells called cytotoxic T cells, killer T cells, CD8+ cells –or simply T8 cells. A T8 cell will only attack an antigen that is presented to it by another cell of the immune system, known as an antigen presenting cell (APC). The interaction involves yet another protein known as MHC, a membrane-bound molecule found on the surface of the antigen-presenting cell and by tumor cells. The function of MHC is to display 'foreign' or abnormal antigens, including cancer-specific antigens, so that T cells can recognise and destroy cells that contain these antigens.

‘Ultimately we would like to intervene with a therapy early in the course of disease to eradicate the cancer and spare men the side effects of testosterone-lowering therapies – therapies that are rarely curative.’



Lymphocytes and antibodies

Antigens specific to prostate tissue include PSA, prostatic acid phosphatase (PAP), and prostate-specific membrane antigen. These three proteins constitute the target of most cancer immunotherapy drugs. Early on in his research, Dr Douglas McNeel, currently based at the University of Wisconsin’s Carbone Cancer Centre, identified and prioritised three potential target antigens with complementary features for vaccine development and clinical diagnosis:

1. PAP – based on the fact that this antigen is specific to prostate cells and was also expressed by rodents (enabling studies in the laboratory before human clinical trial testing)
2. AR LBD – a portion of the androgen receptor, a protein critical to the development and progression of prostate cancer
3. SSX-2, a protein that the group had previously demonstrated was found exclusively in cancerous cells.

The Role of Checkpoint Inhibitors

CTLA-4 and PD-1 are protein molecules found on white blood cells. Known as immune checkpoint molecules, they suppress the disease-fighting action of the immune system and are useful for preventing collateral damage to healthy tissue. Scientists have discovered that blocking the action of these proteins (called a checkpoint blockade) releases the ‘brakes’ on the immune response, stimulating anti-cancer activity. The antibody-based drugs ipilimumab and pembrolizumab, which block CTLA-4 and PD-1, respectively, gained rapid FDA approval due to their remarkable effects against metastatic melanoma and a variety of other cancers. However, checkpoint blockade was relatively ineffective against prostate cancer. Dr McNeel postulated that combining immune checkpoint blockade with vigorous T8 cell activity would constitute a necessary and effective method of combating prostate cancer.

Augmenting Cancer-Specific T8 Cells

In an impressive series of publications, Dr McNeel and colleagues reported that a PAP-based vaccine which his laboratory had developed was able to trigger a robust, long-lasting, and specific killer T cell response against prostate cancer, first in rodent models and then in human patients. Other investigators had conducted vaccine trials, but the McNeel lab trial was the only one to progress beyond Phase 1 in clinical trials using DNA vaccines. A critical finding for the lab was that, despite the presence of killer T cells with durable immunity, the cancer persisted. This anomaly provided the impetus for further investigations into the mechanism whereby cancerous prostate cells were evading the elicited immune response against them.

Tumour Escape Mechanism Deciphered

The goal of anticancer vaccines is to generate a pool of tumour associated antigen-specific T cells, including killer T cells. In order to investigate ways in which prostate cancer cells could escape immune surveillance, Dr McNeel and colleagues developed a DNA vaccine based on the cancer-associated SSX-2 protein. After using it to vaccinate mice that had been genetically engineered to produce human immune system proteins, they found not only an increased killer T cell response but also that the tumours were expressing a protein called PD-L1. As a rule, PD-L1 is expressed on the surface of normal (non-cancerous) cells: when PD1, carried on T cells, binds to PD-L1 on normal cells, the interaction shuts off the immune response. By expressing PD-L1 on their surfaces, the cancer cells were mimicking normal cells and succeeding in avoiding the immune mechanism that would eliminate them.

The McNeel group also found that modifying a T8 cell epitope to permit longer contact between the antigen-presenting cell and the T cell led to persistent expression of PD-1 on T8 cells. These findings were highly significant in explaining the inferior anti-tumour immunity arising from high-affinity epitopes that others had similarly reported, and also shed light on the mechanism of checkpoint blockade drugs. Although others had found similar results with high-affinity epitopes, Dr McNeel and colleagues were the first to implicate the expression of immune checkpoints as the specific cause.

When the McNeel group blocked PD-1 expression, the T8 cells recovered their tumour-killing efficacy in both the mouse model and human patients. Thus, combining vaccine treatment with an antibody blocking PD-1 fought the cancer much more efficiently than treatment with either vaccine or anti-PD1 alone, as predicted.

The results of this experiment also presented a simple, quantitative approach to identifying tumour cell expression of PD-L1: far more convenient and less invasive than the previous gold standard of tumour biopsy, evaluating circulating tumour cells obtained from blood samples for PD-L1 expression is a useful method for monitoring the effects not just of anti-tumour vaccines but also of any therapy, such as conventional radiation therapy, that might affect tumour-associated lymphocytes.

Refining the Immunotherapeutic Strategy

Dr McNeel's work has provided ground-breaking insights into important aspects of the immune system as well as the mechanisms of immunotherapeutic action. For example, his finding that T8 cells express PD-1 in the absence of cancer or pathogen challenges the paradigm that this protein characterises T cell 'exhaustion,' appearing instead to constitute a marker of a distinct state of differentiation. Dr McNeel has pioneered the combination of an anti-tumour vaccine with PD-1 blockade in a clinical trial - the first report involving a DNA vaccine. Furthermore, he has shown for the first time that the strength with which a T8 cell binds its MHC-linked antigen (as presented by an antigen-presenting cell) is proportional to the amount of PD-1 expression. What this means for therapy is that genetically modifying antigen epitopes to lessen T cell: APC binding strength is a potential strategy for circumventing the immune avoidance tactics of cancer cells masquerading as normal cells by expressing PD-L1: with PD1 expression blocked on the T8 cell, there will be nothing for the rogue cell's PD-L1 to bind to.

While Dr McNeel's research has shed light on several aspects of immunotherapy, many questions remain unanswered. It is now of critical interest to determine whether it is more advantageous to use multiple vaccines to broaden the attack on the cancer, to test this approach in patients who are at earlier stages of the disease, and to identify the further mechanisms of tumour resistance to CD8+ cell attack. Dr McNeel notes that, 'Ultimately we would like to intervene with a therapy early in the course of disease to eradicate the cancer and spare men the side effects of testosterone-lowering therapies – therapies that are rarely curative.'

Meet the researcher

Dr Douglas McNeel, MD PhD
Carbone Comprehensive Cancer Center
University of Wisconsin
Madison, WI
USA

Douglas G McNeel, MD PhD, is a tenured Professor in the Department of Medicine, Division of Hematology/Oncology, at the University of Wisconsin-Madison. After receiving his BA in Chemistry and Music at Whitman College in 1986, he then pursued graduate training under a Medical Scientist Training Award at the University of Chicago, receiving his PhD (Biochemistry and Molecular Biology) in 1992 and MD in 1994. He then completed an Internal Medicine residency at the University of Washington and a Medical Oncology fellowship at the University of Washington and Fred Hutchinson Cancer Research Center. During his fellowship training, he worked in the laboratory of Mary (Nora) Disis, MD, prior to moving to the University of Wisconsin-Madison as an Assistant Professor in 2001. Dr McNeel is a genitourinary oncologist with a clinical focus on prostate cancer. His laboratory and clinical research programs are focused on prostate cancer immunology with the goal of developing anti-tumour vaccines as treatments for prostate cancer. This work has led to the publication of over 100 scientific articles, multiple patents, and the initiation of a company, Madison Vaccines, Inc, focused on the clinical development of prostate cancer vaccine therapies. He served as co-leader of the Experimental Therapeutics Program of the University of Wisconsin Carbone Cancer Center, and is currently the Director for Solid Tumor Immunology Research. Dr McNeel is a member of multiple professional societies, and is on the Board of Directors of the Society for the Immunotherapy of Cancer, the leading professional society focused on cancer immunotherapy.

CONTACT

E: dm3@medicine.wisc.edu
W: http://www.mcneellab.com/

FUNDING

Prostate Cancer Foundation
National Institutes of Health/National Cancer Institute
Department of Defence Prostate Cancer Research Program

FURTHER READING

DG McNeel, JC Eickhoff, E Wargowski, C Zahm, MJ Staab, J Straus, G Liu, Concurrent, but not sequential, PD-1 blockade with a DNA vaccine elicits anti-tumor responses in patients with metastatic, castration-resistant prostate cancer, Oncotarget, 2018, 22, 25586-25596.

CD Zahm, VT Colluru, DG McNeel, Vaccination with high-affinity epitopes impairs antitumor efficacy by increasing PD-1 expression on CD8+ T Cells, Cancer Immunology Research, 2017, 5, 630-641.

BT Rekoske, BM Olson, DG McNeel, Antitumor vaccination of prostate cancer patients elicits PD-1/PD-L1 regulated antigen-specific immune responses, OncoImmunology, 2016, 5, e1165377.

JT Becker, BM Olson, LE Johnson, JG Davies, EJ Dunphy, DG McNeel, DNA vaccine encoding prostatic acid phosphatase (PAP) elicits long-term T-cell responses in patients with recurrent prostate cancer, Journal of Immunotherapy, 2010, 33, 639-47.

DG McNeel, EJ Dunphy, JG Davies, TP Frye, LE Johnson, MJ Staab, DL Horvath, J Straus, D Alberti, R Marnocha, G Liu, JC Eickhoff, G Wilding, Safety and immunological efficacy of a DNA vaccine encoding prostatic acid phosphatase in patients with stage D0 prostate cancer, Journal of Clinical Oncology, 2009, 27, 4047-54.

WISCONSIN
UNIVERSITY OF WISCONSIN-MADISON

COMPLEXED DRUGS FOR COMPLEX DISEASES

RNA interference is a genetic mechanism for altering the expression of genes that is being developed to fight cancer. By increasing the activity of RNA interference molecules in solid tumours after administration into the blood stream, **Dr Joseph Vetro** and his group at the University of Nebraska Medical Centre are working to move a promising new approach to treating cancer into clinical application.

Cancer is one of the major killers of the developed world, so it is no surprise that hundreds of different therapeutic approaches have been developed to combat it. From poisons that kill cancer cells slightly faster than normal cells to immune cells reprogrammed to attack tumours, the treatments for cancer are many and varied.

Despite this, cancer is both deadly and tenacious – patients can relapse and die years after being seemingly cured and fast-spreading metastatic tumours can kill otherwise healthy people within weeks. Especially problematic is that tumours eventually become resistant to all current types of chemotherapies. Thus, there still remains a need for newer and improved treatment options. One promising approach is that of RNA interference.

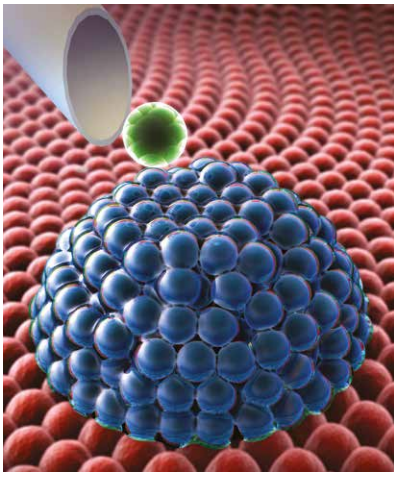
The Messengers of Protein Production

So, what exactly is RNA interference? One of the ways that cells use RNA is as a 'messenger' molecule where the gene encoded in our DNA is copied into specific messenger RNA (mRNA) within the cell's nucleus that is then transported into the cell interior where it is used as a blueprint to create specific proteins that help carry out specific functions for a given cell. These mRNA molecules are short-lived blueprints, constantly being created and destroyed in response to the cellular and extracellular environment. This is, in fact, just one way that the cell controls the levels of specific proteins.

Importantly, mRNA used by human cells is *single stranded* – a single copy of the genetic blueprint is contained on a single long molecule. RNA, however, also exists within the cell as short, double-stranded RNA. Double stranded RNA is synthesised in the cell's nucleus as well but, in contrast to mRNA, it is then exported and cut into smaller pieces by an enzyme that is known, appropriately enough, as Dicer.

These smaller double stranded RNA pieces, called siRNA – 'small, interfering RNA' – are then incorporated into a larger complex known as RISC. RISC then helps the siRNA find its mRNA target to subsequently inhibit, or interfere with, translation of that specific mRNA into protein. The siRNA has a matching genetic sequence to that of its target mRNA. Thus, given that the human genome has been completely sequenced, RNA interference molecules (RNAi) can be synthesised and used to decrease the expression of any specific protein at the mRNA level.

Unfortunately, there is a world of difference between the lab and the hospital, and RNA interference therapeutics have had a rocky start.



In particular, RNAi molecules are very difficult to administer to patients. They need to be injected directly into the blood through intravenous administration but are rapidly destroyed by the body's own enzymes and cleared by the kidneys.

It is also extremely difficult to get RNAi molecules from the bloodstream into cells. Double stranded RNAi is a comparatively large and charged molecule that has difficulty sneaking through the cell membrane.

Several different approaches have been proposed to avoid this problem, but all have their own strengths and weaknesses. As such, the effective and specific delivery of RNAi molecules into tumour cells in the clinic remains highly challenging to this day, and any group

‘An idea that started out on the lab bench is being developed into a new and exciting cancer therapy that may one day reach the hospital wards.’

that solves these problems will have pharmaceutical companies beating a path to their door.

Showing Early Promise

Facing this challenge is where researchers such as Associate Professor Joseph Vetro of the University of Nebraska Medical Centre come in. Dr Vetro’s group works on developing nanocarriers, nanometre-scale complexes formed through the self-assembly of negatively-charged RNAi molecules with positively-charged polymer molecules. Polymers are long molecules made from a chain of repeating units. Through incorporation into these complexes, RNAi molecules are protected from degradation or clearance through the kidneys, allowing them to circulate in the bloodstream for a longer duration so they have time to sufficiently accumulate in tumours throughout the body.

There are many different tumour-related proteins that can be targeted by RNA interference to treat cancer. The lead approach taken by Dr Vetro’s group is to decrease chemotherapy resistance by reducing the expression of tumour proteins that allow tumour cells to survive treatment with chemotherapy drugs. This does not ‘cure cancer’ by itself but can be given alongside other drugs to allow them to work more effectively.

The team has already shown much promise in their development of cholesterol-modified siRNA (Chol-siRNA) / poly-L-lysine-polyethylene glycol (PLL-PEG polymer) complexes. These complexes consist of Chol-siRNA mixed with small amounts of PLL-PEG. Negatively-charged Chol-siRNA binds to the positively-charged regions of PLL to create a region on the PLL-PEG polymer that is much less soluble in water.

This causes the normally separated PLL-PEG polymers to self-assemble in solution, creating a nanometre (1/100,000 of a metre)-scale polymer complex (‘nanocarrier’) that consists of a Chol-siRNA/PLL core and a PEG shell. The PEG shell of the nanocarrier then protects Chol-siRNA within the core from degradation and makes the entire complex large enough to remain in the bloodstream for a longer period of time.

To initially demonstrate the effectiveness of this approach, Dr Vetro’s group used breast tumour cells from mice that express the luciferase gene. The luciferase gene is normally found in fireflies. When other cells are genetically modified to express the luciferase gene you can make these cells glow when provided with the right chemicals.

This makes measurements of nanocarrier activity relatively easy – if nanocarriers that contain Chol-siRNA target luciferase mRNA to reduce the expression of luciferase protein, the amount of light that the breast tumour cells produce will also decrease.

Using this method, the group showed that creating a PLL-PEG complex increased the efficiency of Chol-siRNA in targeting mouse breast tumours after administration to the blood stream. They also found that Chol-siRNA efficiency was affected by the length of the positively charged PLL section of the molecule. If the PLL sections were too long or too short, this led to less-effective nanocarriers.

The Pathway to Therapy

At this point the group moved from luciferase to a more therapeutically relevant cancer gene – in particular, one known as Stat3. Stat3 is part of a cellular signalling pathway that is commonly mutated in cancer and leads to uncontrolled cell growth and migration to other tissues – metastasis. By blocking or reducing Stat3 activity it is possible to prevent tumour growth and spread as well as increasing the effectiveness of chemotherapy drugs.

The researchers showed that decreasing Stat3 mRNA with Chol-siRNA nanocarriers was effective in slowing tumour growth and metastatic spread in mice after administration into the blood stream. There was also initial evidence that the complexes are well-tolerated given that mice continued to gain weight over the course of the experiment.

At the same time, the group observed that there were some problems with Chol-siRNA nanocarriers. Specifically, gene suppression started to diminish 24 hours following the final treatment. Considering that longer-lasting protein suppression will be more effective for cancer treatment, increasing the duration of RNAi activity was a high priority.

Dr Vetro and his group achieved a longer duration of RNAi activity by using slightly larger DsiRNA – 'Dicer-substrate siRNA' – in their nanocarriers. Whereas the smaller siRNA directly binds to RISC, larger DsiRNA needs to be initially cut up by the Dicer enzyme. As before, these Chol-DsiRNA nanocarriers were able to significantly decrease luciferase expression in mouse breast tumours but maintained suppression around 48 hours longer than Chol-siRNA complexes after administration into a vein.

From Lab Bench to Hospital Ward

The long-term goal of Dr Vetro's group is to develop these nanocarriers for subsequent licensing by pharmaceutical companies to treat cancer patients with cholesterol-modified RNAi molecules (Chol-RNAi). Most pharmaceutical companies, however, will not consider licensing without evidence from a Phase I clinical trial that the Chol-RNAi complexes are likely to be active in tumours after administration into a vein, without serious toxicity. The financial costs of this process, however, are well beyond that of an academic research group. Thus, Dr Vetro founded a start-up company in 2013 called Actorius Pharmaceuticals to obtain the funding necessary for a Phase I clinical trial.

Actorius Pharmaceuticals has since received a Small Business Technology Transfer (STTR) grant from the National Institutes of Health that supports the transfer of research from the academic world into the business world as well as matching funding from the State of Nebraska. With this funding, they have demonstrated that Chol-DsiRNA nanocarriers are well tolerated at high levels in healthy mice after chronic administration to the blood stream. They are currently assessing how long the Chol-DsiRNA nanocarriers circulate in the bloodstream and how much localises to primary tumours and different organs after administration to mice. This information will be used alongside future independent toxicity studies to obtain permission for a Phase I clinical trial.

The Phase I clinical trial will determine a safe maximum dose of Chol-DsiRNA nanocarriers in breast cancer patients and, ideally, provide evidence that the nanocarriers are effective at targeting tumours. The remaining clinical trial phases and large-scale manufacturing to get a drug on the market, however, can take many years and many millions of dollars. This is not a process for the faint of heart.

Assuming positive results in the Phase I clinical trial, the start-up will be in a strong position to negotiate with larger pharmaceutical companies, licensing the eventual rights to develop the nanocarriers as part of future therapies against cancer.

Taken together, the research performed by Dr Vetro and his group is an excellent example of modern pharmaceutical development. An idea that started out on the lab bench is being developed into a new and exciting cancer therapy that may one day reach the hospital wards.

Meet the researcher

Dr Joseph Anthoney Vetro, PhD
University of Nebraska Medical Center (UNMC)
Department of Pharmaceutical Sciences
Omaha, NE
USA

Associate Professor Joseph Vetro of the University of Nebraska Medical Center has a long-standing interest in the clinical and academic sides of medical research. A biochemist by training, he completed his PhD in 2001 at Saint Louis University Health Sciences Center, Missouri, in the lab of Dr Yie-Hwa Chang as an American Heart Association Predoctoral Fellow. He then completed postdoctoral training in 2004 at the University of Kansas Department of Pharmaceutical Chemistry in the lab of Dr Russ Middaugh as an American Heart Association Postdoctoral Fellow. There he worked on developing new polymer complexes to improve the delivery of therapeutic DNA to microvascular endothelial cells that line the interior surface of blood vessels. This initial work guided his subsequent research using polymer complexes to increase the delivery of hydrophobically-modified RNA interference molecules into tumour cells that can greatly improve the treatment of cancer with existing therapies. Simultaneously working as an Associate Professor and as the Founder of the start-up Actorius Pharmaceuticals, Professor Vetro is also actively involved in teaching as well as the supervision of graduate students in his research team.

CONTACT

E: jvetro@unmc.edu
W: https://www.unmc.edu/pharmacy/faculty/pharmaceutical-sciences/vetro.html

KEY COLLABORATORS

Dr Rakesh K. Singh, PhD
UNMC Department of Pathology and Microbiology, College of Medicine https://www.unmc.edu/pathology/faculty/bios/singh.html
Dr Sam Cohen, MD, PhD
UNMC Department of Pathology and Microbiology, College of Medicine
https://www.unmc.edu/pathology/faculty/bios/cohen.html
Dr Don Coulter, MD
UNMC Division of Pediatric Hematology/Oncology, College of Medicine
https://www.unmc.edu/pediatrics/divisions/hemonc/faculty/coulter.html
Dr Tim McGuire, PharmD, FCCP
UNMC Department of Pharmacy Practice, College of Pharmacy
https://www.unmc.edu/pharmacy/faculty/pharmacy-practice/mcquire.html

FUNDING

National Institutes of Health / National Center for Advancing Translational Sciences
UNMC Pediatric Cancer Research Group
Nebraska Department of Economic Development

FURTHER READING

VV Ambardekar, RR Wakaskar, Z Ye, SM Curran, TR McGuire, DW Coulter, RK Singh, JA Vetro, Complexation of Chol-DsiRNA in place of Chol-siRNA greatly increases the duration of mRNA suppression by polyplexes of PLL(30)-PEG(5K) in primary murine syngeneic breast tumors after i.v. administration, 2018, International Journal of Pharmaceutics, 543, 130–138.

VV Ambardekar, RR Wakaskar, B Sharma, J Bowman, W Vayaboury, RK Singh, JA Vetro, The efficacy of nuclease-resistant Chol-siRNA in primary breast tumors following complexation with PLL-PEG(5K), 2013, Biomaterials, 34, 4839–4848.

VV Ambardekar VV, H-Y Han, ML Varney, SV Vinogradov, RK Singh, JA Vetro, The modification of siRNA with 3' cholesterol to increase nuclease protection and suppression of native mRNA by select siRNA polyplexes, Biomaterials, 32, 1404–1411.

University of Nebraska Medical Center
COLLEGE OF PHARMACY

BRINGING NEW TECHNOLOGIES TO THE FIGHT AGAINST MELANOMA

Melanoma is a worldwide killer that places a significant associated burden upon healthcare systems across the globe. **Professor H. Peter Soyer** and colleagues are part of a multidisciplinary team of experts at the University of Queensland, Australia, who are working to improve the early detection and management of melanoma skin cancer through patient-focused translational research. Their vision will be achieved utilising recent advances in genetic risk prediction, cutting-edge imaging and smartphone technologies combined with machine-assisted diagnostic algorithms, on a networked telehealth platform. The outcomes will afford rapid translation of research findings into routine practice and critically improve equitable access to health services in regional areas.

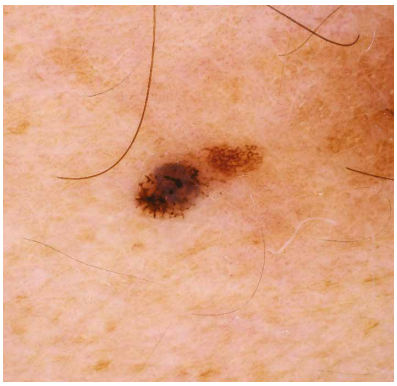
The Impact of Undetected Melanoma

Melanoma skin cancer is the third most commonly diagnosed cancer in both men and women in Australia, and the most common cancer in young Australians aged between 15 and 39 years. It affects over 12,500 Australians each year and results in over 1,800 deaths. This burden is not restricted to Australia, with occurrence rates of melanoma continuing to increase across the globe with a global incidence of 351,800 cases and 59,782 deaths in 2015.

In addition to this tragic loss of life, treatment of affected individuals, in particular those with advanced disease, places a large burden on the healthcare system. The estimated cost of treating melanoma varies internationally from approximately $45M to $930M, with recent data showing that the direct healthcare costs for new melanoma cases in Australia have now reached $200M per year, a seven-fold increase from $30M in 2008.

A melanoma may arise from existing moles or other skin blemishes and usually appears as a dark, irregular patch on the skin, which can be accompanied by itching or bleeding. A major contributor to the change from mole to melanoma is over-exposure to the sun's ultra-violet radiation, which can lead to damage to the DNA within skin cells. This can cause these skin cells to grow rapidly and out of control, forming a melanoma. If left to develop this can lead to metastasis and the spread of cancer widely around the body.

Melanoma is graded by medical practitioners from one to four, with one being the least serious and earliest stage, whilst grade four is more serious, has fewer treatment options and a five-year survival rate of only 15% – 20%. It is therefore crucial that strategies are developed that allow detection and treatment of melanomas before they develop into the later stages of this devastating condition.



CREDIT: The Dermatology Research Centre, The University of Queensland Diamantina Institute.

The challenge of early melanoma diagnosis is that these detrimental changes are not always related to sun-exposure. Individuals with lots of moles, a family history or personal history of melanoma, those with a particular skin or hair colour, and those with a specific genetic make-up are at a much higher risk of getting melanoma. These factors, combined with a lack of consensus on the optimal screening approach, further adds to the challenges in the early detection of this condition.

‘Targeted early detection strategies are essential to save lives, improve patient outcomes, and reduce the costs of melanoma diagnosis and care.’

CREDIT: The Dermatology Research Centre, The University of Queensland Diamantina Institute.

It is evident that diagnosing melanoma in its early stages is imperative to save lives, improve patient outcomes and reduce costs. There is a broad clinical consensus this will be achieved most effectively by targeting appropriate surveillance strategies to those deemed most at risk. In contemporary healthcare settings, early detection activities for melanoma are fragmented. They do not follow a logical or targeted plan for those at highest risk, and indeed are diluted by ad hoc and untargeted early detection efforts including low-risk patients and the ‘worried well’. The development of targeted detection strategies and early diagnosis, is as a result, vitally important to save lives, improve patient outcomes and reduce the associated burden of treatment on the healthcare system.

Modern Technology in the Fight Against Melanoma

The State of Queensland in Australia is the, ‘Melanoma Capital of the World’, with the highest rates of those affected in the world and also the greatest associated number of deaths. Professor Soyer and his colleagues Professor Monika Janda and Dr Anthony Raphael from the University of Queensland have teamed up to combine a range of specialist techniques to aid the identification of people who may be at high-risk of developing a melanoma.

They are working alongside a dedicated multidisciplinary team at The University of Queensland’s Dermatology Research Centre to create innovative new strategies for the screening of individuals at high-risk of developing a melanoma through the application of new imaging technologies, genetic risk prediction, artificial intelligence and patient behaviour.

The team is developing novel approaches to the early diagnosis of melanoma by pioneering the use of full body monitoring of the skin using specialist cameras. They describe how, ‘targeted early detection strategies are essential to save lives, improve patient outcomes, and reduce the costs of melanoma diagnosis and care.’

Cutting-edge technology is beginning to have an impact on diagnosis and treatment through the use of specialised cameras, telehealth networks and image processing systems. The research group is using the VECTRA WB360 whole-body 3D imaging system developed by US-based Canfield Scientific Inc. to capture highly detailed colour images of the whole body. This specialised imaging system uses 92 individual digital cameras to capture the whole skin surface that is then reconstructed onto an exact 3D model of the patient. An additional hand-held camera with an inbuilt magnifying lens is used to add a detailed up-close ‘dermoscopy’ image of the moles for the detection of those that may be cancerous.

This imaging system located at the Clinical Research Facility at Princess Alexandra Hospital, Brisbane, is one of the first of its kind in the world. Developing this photographic system from a research tool to a system used in clinical practice allows doctors to monitor moles or other skin problems over time. Whole body imaging technology is very fast and can capture a full data set of the entire skin surface in a fraction of a second. This can be stored and compared with surfaces captured previously to determine if there have been any significant changes.

By revealing which moles are changing in a suspicious way and which are stable and just strange-looking, it will also reduce the number of minor surgeries that need to be performed. The future development of artificial intelligent software that can apply risk criteria and classify the number, size, colour and shape of skin moles, assisting the diagnosing clinician, will allow better sensitivity and specificity in detection than that achieved by dermatologists alone.

Teledermatology

It has been shown that a significant proportion of melanomas were initially flagged as suspicious by the patient themselves or their partner. This fact combined with the 'era of the selfie' has prompted Professor Soyer's research team to focus on the innovative use of smart phones for patients to monitor their own skin. Patients can use their phone to photograph and transmit digital images of a suspected melanoma to a dermatologist for expert review in a process known as, teledermatology.

To improve accuracy, the patient's phone can be equipped with a magnifying lens and a special app to capture and store images of their skin. These images can then be sent automatically by the app to a local medical centre for screening and advice. Recent research on mobile teledermatology has shown that both patients and dermatologists reported high satisfaction and reduced waiting times, however, there were no firm conclusions regarding the diagnostic accuracy of this technology.

Currently, there are around 40 apps specifically for detecting melanoma. Some of which claim to be able to assess the risk of a mole on the skin by analysing a digital image and provide an action plan associated with that risk.

Professor Soyer and his team recently carried out research on three apps that assess the risk associated with skin moles and found that these apps did not perform very well. The aim of the apps was to classify suspected melanomas into two categories, suspicious or benign. A categorisation of suspicious would mean that some action would need to be taken, whereas the categorisation of benign meant that the issue could continue to be reviewed.

The team's study compared the outcomes of the apps with decisions made by a dermatologist and found the agreement to be poor. The team concluded that the apps could not be a substitute for a clinician's decision at this stage. It is expected that melanoma apps will continue to improve in the future – however, this smartphone technology will require further research and clinical validation before introduction to public use.

Screening of High-risk Individuals

Detecting melanoma at an early stage of the disease is very important to improve survival rates, but it is not possible to screen everyone, even in countries where melanoma risk is higher. Professor Soyer and the team are now focusing their research efforts on identifying individuals who are high-risk, so that they can be screened at regular intervals.

People with a personal or family history of melanoma are at greater risk of developing a further melanoma. This history together with the number of moles on an individual are the strongest known risk factors for melanoma. However, other factors including fair skin and red hair, in addition to a specific genetic make-up, contribute towards the overall risk an individual has of getting melanoma.

Identification of genetic risk factors in the general population through genetic sequencing could make screening of high-risk individuals more precise, so health resources can be targeted more accurately. There are currently no population-wide screening programs in Australia despite predictions that targeting high-risk individuals could potentially improve quality and length of life and reduce the overall cost burden of the disease.

Clinicians armed with knowledge about their patients' genetic risks will be able to monitor high-risk patients closely for changing moles and educate patients and their families about their melanoma risk and the importance of skin self-examination.

Professor Soyer states that, 'the next steps of the research are to validate and implement digital biomarkers obtained from our clinical, photographic and gene-based risk profiles through clinical trials involving a wide range of centres.' Following this, 'our research programme will provide much of the missing information needed to implement an early detection screening programme for people at high-risk of melanoma.'

The team has begun a Randomised Controlled Trial this year, with the aim of comparing health and cost outcomes of their surveillance program as compared to standard care for high-risk individuals, allowing them to further refine their melanoma risk assessment methods.

Their overall vision is to establish a state-wide program for the targeted detection of melanoma. This will also allow the training of the next generation of health service providers in early detection, provide expertise in 3D imaging for healthcare, and change current opportunistic screening of high-risk individuals to personalised surveillance, in order to improve the early detection of melanoma and save lives.

The research being carried out by Professor Soyer and his colleagues is aiming to, 'change clinical practice from that of current random screening to a personalised and targeted screening service using newly developed technologies for people at high-risk of melanoma.' Their vision of precision early prevention of melanoma requires a dedicated team of specialists in clinical imaging, genomics, artificial intelligence and behavioural science. Together they are searching for innovative solutions to improve early detection and treatment of melanoma reducing overall the loss of life and the cost to the healthcare system caused by this devastating disease.

Meet the researchers

Professor H. Peter Soyer
The University of Queensland
The University of Queensland
Diamantina Institute
Dermatology Research Centre
Brisbane
Australia

Professor Soyer is an academic dermatologist with over 30 years experience in the field, having obtained his degree in Medicine from the Karl-Franzens University, Graz, Austria in 1980. He was appointed as the inaugural Chair in Dermatology by The University of Queensland (UQ) in 2007 and as Director of the Princess Alexandra Hospital (PAH) Dermatology Department in 2008. He has a strong focus on translational skin cancer research in his dual role as Director of the Dermatology Research Centre (DRC), UQ Diamantina Institute, UQ Faculty of Medicine; and leadership of the Dermatology Department at the Princess Alexandra Hospital in Brisbane. Professor Soyer is considered a pioneer in dermatological imaging having led the clinical validation and implementation of dermoscopy. He was founding president of the International Dermoscopy Society and International Society of Teledermatology and is a current NHMRC-MRFF Practitioner Fellow.

CONTACT

E: p.soyer@uq.edu.au
W: https://dermatology-research.centre.uq.edu.au/profile/37/professor-h-peter-soyer
@hpsoyer

Professor Monika Janda
The University of Queensland
Centre for Health Services Research
Brisbane
Australia

Professor Monika Janda is a health psychologist with a research background in cancer prevention and quality of life research, with strong clinical collaborations. She gained her PhD in Psychology from the University of Vienna in 2002. She then worked as a research fellow for the Melanoma Screening trial with the Cancer Council Queensland up until 2006. Professor Janda was until recently a Principal Research Fellow and led the Health Determinants and Health Systems Theme at The Institute of Health and Biomedical Innovation, Queensland University of Technology. She is currently, Professor in Behavioural Science at the Centre for Health Services Research at the University of Queensland in the Faculty of Medicine.

CONTACT

E: m.janda@uq.edu.au
W: https://chsr.centre.uq.edu.au/profile/993/monika-janda
@MonikaJanda

Dr Anthony Raphael
The University of Queensland
The University of Queensland
Diamantina Institute
Dermatology Research Centre
Brisbane
Australia

Anthony has a PhD in Biomedical Engineering from the University of Queensland, Brisbane. He has spent the last decade working on transformative technologies within immunology and dermatology. After completing his postdoctoral research at the University of Queensland, he held appointments at Massachusetts General Hospital and Harvard Medical School until his return to Brisbane in 2017. Since then, Anthony has been working with academic dermatologist Professor Peter Soyer on the implementation of 3D total-body-imaging systems for early detection of skin cancer. He has been acknowledged as an emerging leader through his award of Australia's prestigious NHMRC Early Career Fellowship (2015) and 2016 Rolex Awards for Enterprise Finalist.

CONTACT

E: a.raphael1@uq.edu.au
W: https://dermatology-research.centre.uq.edu.au/profile/49/dr-anthony-raphael
@AnthonyRaphael_

FUNDING

NHMRC Early Career Fellowship

The Centre of Research Excellence for the Study of Naevi was funded by the National Health and Medical Research Council (ID: APP1099021) and The Princess Alexandra Hospital Private Practice Trust Fund.

BIOLOGICAL MECHANISMS LINK SMOKING, LUNG CANCER AND ETHNICITY

Cigarette smoking is a leading cause of cancer globally. **Dr Stephen Hecht** and co-workers at the University of Minnesota are investigating the substances present in cigarette smoke and in the urine and saliva of cigarette smokers that contribute to lung cancer. In a collaborative study with scientists at the University of Southern California and the University of Hawaii, they are studying the ways in which different ethnic groups take up, break down and detoxify these chemicals, leading to different levels of cancer-causing metabolites in the blood and varying rates of lung cancer.

Predicting Lung Cancer Risk

It is not completely clear why some smokers go on to develop cancer and others avoid it despite having smoked all their lives. Cigarette smoking is extremely addictive and the leading cause of fatal cancer globally. There are currently 38 million adult smokers in the US and one billion in the world. 'While cigarette smoking causes up to 90% of all lung cancer, the largest cause of cancer death in the world, only 10–20% of lifetime smokers will get lung cancer,' says Dr Stephen Hecht, a researcher at the University of Minnesota. Dr Hecht studies the chemical contributors to lung cancer in smokers in collaboration with a team of colleagues including Dr Sharon E. Murphy from the University of Minnesota and Dr Loic Le Marchand of the University of Hawaii Cancer Center.

Currently, lung cancer risk is gauged largely by cigarettes smoked per day and the number of years the individual has smoked. This leads to older rather than younger smokers being more likely to be screened. It would be useful for doctors to be able to determine how likely a younger smoker is to develop lung cancer, so that the disease may be prevented or treated at an early stage.

Nicotine is the main addictive substance in cigarettes. Although nicotine does not itself cause cancer, the cigarette is a terrible nicotine delivery device because it generates multiple carcinogens – substances capable of causing cancer in the body – that are inhaled along with the nicotine.

During metabolism, the body uses specialised proteins – enzymes – to breakdown cigarette smoke constituents into more readily excreted molecules called metabolites. In the eyes of researchers such as Dr Hecht, these metabolites would be a better way to gauge who is at risk, as they could be measured in the blood or urine and used to predict the outcome of disease.

Potential biomarkers, or metabolite predictors for lung cancer, might include carcinogens, or their cancer-causing breakdown products, as well as carcinogen DNA adducts – cancer-causing chemicals bound to segments of DNA. In their most recent research, the team of collaborators used biomarkers of carcinogen exposure and metabolism of nicotine to investigate the mechanisms underlying the racial differences in lung cancer risk.

Racial Groups Differ in their Risk for Lung Cancer

It is well established that the lung cancer risk of smokers varies by ethnic or racial group. In the mid-1900s it was noticed that Native Hawaiians experienced a greater rise in lung cancer deaths than other ethnic groups after the introduction of manufactured cigarettes.

‘While cigarette smoking causes up to 90% of all lung cancer, the largest cause of cancer death in the world, only 10–20% of lifetime smokers will get lung cancer.’

A study started in 1993 at the University of Hawaii Cancer Center called the Multiethnic Cohort Study followed hundreds of thousands of individuals over many years. This study found that the highest rates of lung cancer occurred in Native Hawaiians and African Americans, followed by Whites and Latinos, and even lower rates in Japanese Americans and Asians. These risk patterns could not be explained by the type of cigarettes smoked, levels of inhalation or diet. After additional studies, it was found that exposure to chemicals in cigarettes occurs to different extents in different ethnic groups.

Nicotine Metabolism and Carcinogen Exposure

Nicotine is metabolised by three main pathways in the body. The primary pathway for the breakdown of nicotine is called the C-oxidation pathway, whereby nicotine is broken down into the chemical cotinine by the enzyme CYP2A6. Cotinine is further metabolised to the molecule trans 3’-hydroxycotinine (3-HCOT), a reaction that is also mediated by CYP2A6.

The other two pathways produce the metabolites nicotine N-oxide and molecules called glucuronides. Nicotine, cotinine, 3-HCOT and their glucuronides plus nicotine N-oxide are collectively referred to as Total Nicotine Equivalents, or TNEs, and can all be identified and measured in urine. Other chemicals present in tobacco smoke include carcinogens such as nitrosamines, polycyclic aromatic hydrocarbons (PAH), 1,3-butadiene and compounds such as formaldehyde, acetaldehyde and acrolein.

Ethnic Groups Differ in Amounts of CYP2A6

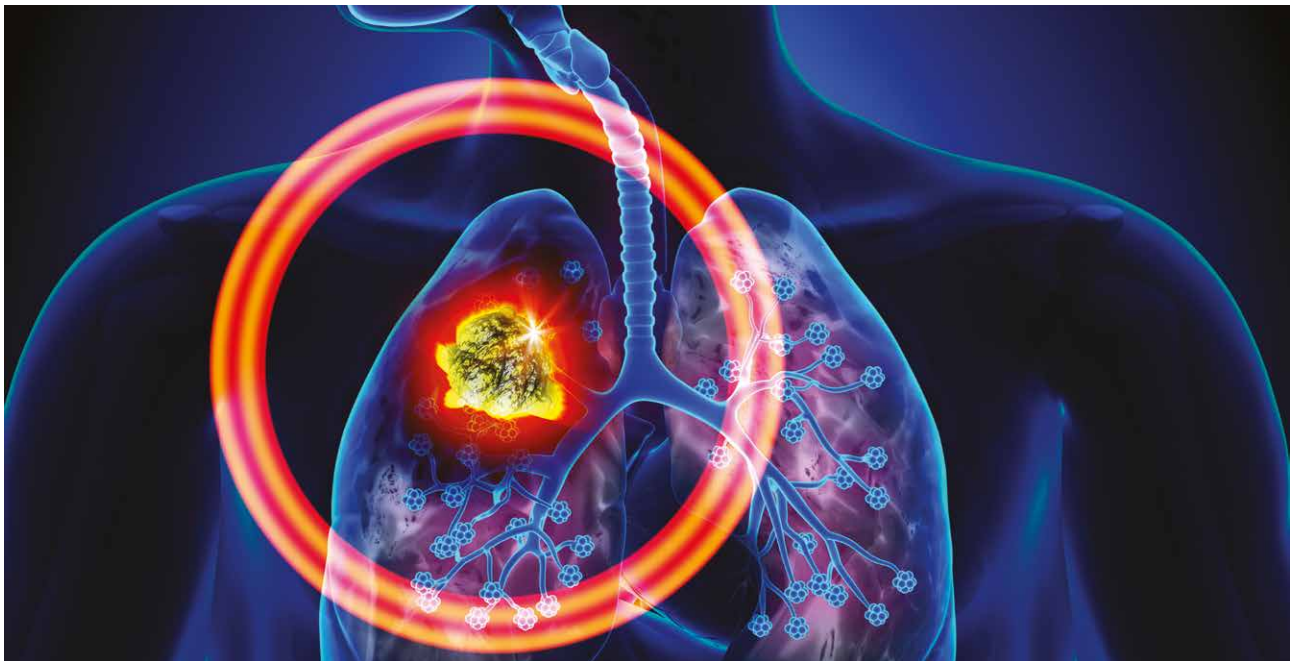
It has been found that, on average, African Americans take up greater amounts of nicotine than Whites per cigarette, while Japanese Americans take up less. The prevalence of low-activity or inactive CYP2A6 enzyme in Japanese Americans explains much of the lower nicotine uptake by this group. However, it is unclear what drives the greater uptake of nicotine by African Americans.

More than 50 variants of CYP2A6 have been identified in humans. Certain forms of this enzyme in Japanese Americans have been demonstrated to break down nicotine at much slower rates, leading to more unchanged nicotine in the body, and a decreased necessity to draw more nicotine from each cigarette. This in turn leads to lower carcinogen exposure and lower rates of heart and lung disease.

When reviewing the evidence, Dr Hecht’s team found that the higher risk for Native Hawaiians and the lower risk of Latino smokers for lung cancer could not be explained by differing extents of exposure to nicotine and carcinogens. They hypothesise that genetic differences might play a role in the varying rates of lung cancer in these groups.

Other Carcinogen Metabolites

Numerous compounds in tobacco smoke are known to be carcinogens and their metabolites have been found at different levels across ethnic groups. Dr Hecht’s team determined that Total Nicotine Equivalents (TNEs) in the Multiethnic Cohort Study were significantly higher in African Americans than in Whites, and significantly lower in Japanese Americans than in Whites. However Native Hawaiians had lower urinary TNE levels than Whites and Latinos.



NNAL is a potent lung carcinogen in rats and mice and is a urinary metabolite of a chemical called NNK – itself a tobacco-specific lung carcinogen. By investigating the smokers in the Multiethnic Cohort Study, Dr Hecht and his team found that the total NNAL in urine was consistent with the levels of TNE.

Two other compounds found in the urine, SPMA and MHBMA, which are the breakdown products of benzene and 1,3-butadiene, respectively, had levels that were highest in African Americans, intermediate in Whites, and lowest in Japanese Americans. The team noticed that both of these compounds are strongly influenced by differences in levels of an enzyme called GSTT1, with lower levels of the protein in those with zero or one copy of the gene compared to two copies. Japanese Americans tend to have fewer copies of the gene than other ethnic groups, and this was taken into account in the comparisons of SPMA and MHBMA in the ethnic groups.

The biological markers for the carcinogens NNK, PAH, 1,3-butadiene (MHBMA), and benzene (SPMA) were highest in African Americans, at intermediate levels in Whites, and at their lowest levels in Japanese Americans, similar to the TNE data and their relative risks for lung cancer. Native Hawaiians and Latinos followed the pattern of the TNE and NNAL data.

The team noticed that the metabolites of exposure to acrolein and crotonaldehyde (3-HPMA and HMPMA) did not follow this pattern, as the highest levels were found in Whites and Native Hawaiians, with lower levels in African Americans, Japanese Americans and Latinos. Acrolein and crotonaldehyde are not carcinogens, but both are toxic compounds which produce inflammation and other effects important in carcinogenesis.

Using Epigenetic Markers to Predict Risk

The field of epigenetics concerns itself with the addition or deletion of key molecular groups on specific areas of genes, a phenomenon that can alter the activity of a gene. One common epigenetic modification of DNA is called methylation. Methylation of specific sites on DNA called CpG sites is one of the most commonly studied epigenetic modifications and occurs when a methyl group – a carbon atom attached to three hydrogen atoms – is attached to a site on the DNA chain.

Smoking has been shown to cause high rates of DNA methylation, and the team hypothesises that these regions of DNA may serve as long term markers of lung cancer risk and help identify the genes involved in lung cancer development. Methylation of CpG sites in smoking-related genes has been shown to be associated with cancer risk in some studies. More research is needed, however, as studies of methylation across ethnicity remain sparse.

Future Studies

The study team is collaborating with other researchers who have collected blood or urine samples from large numbers of smokers over decades. The group is planning further studies to determine if smoking-related DNA methylation sites can be used to predict the risk of lung cancer.

Further investigations into the impact of smoking dose, intensity and duration on the epigenetic makeup of smokers (the epigenome) are needed. With the development of new techniques in the field of epigenetics the team expects new methylated CpG sites to be identified. This could provide additional knowledge on the impacts of smoking on the epigenome and identify genetic regions suitable for prediction of smoking-related lung cancer.

Meet the researchers

Professor Stephen Hecht
Masonic Cancer Center
University of Minnesota
Minneapolis, MN
USA

Dr Stephen Hecht is a Professor in the Department of Laboratory Medicine and Pathology and a member of the Medicinal Chemistry and Pharmacology graduate programs at the University of Minnesota in Minneapolis. From 1997 until 2014 he was head of the Carcinogenesis and Chemoprevention Program at the Masonic Cancer Center. Dr Hecht received his PhD in organic chemistry from the Massachusetts Institute of Technology in 1968, where he also did his postdoctoral work. From 2013 until 2017, he was the editor-in-chief of the journal *Chemical Research in Toxicology*. He is an expert on cancer-causing agents in tobacco products and his current research focuses on understanding the ways compounds in tobacco smoke cause cancer by studying how they enter the body, are metabolised, and cause mutations in human DNA.

CONTACT

E: hecht002@umn.edu
W: https://www.pathology.umn.edu/bio/lab-med-and-pathology-faculty/stephen-hecht
W: https://www.acs.org/content/acs/en/pressroom/experts/stephen-hecht.html

Professor Loic Le Marchand
Clinical Professor of Public Health
John A. Burns School of Medicine
University of Hawaii at Manoa
Hawaii
USA

Dr Loic Le Marchand is Professor of Population Sciences in the Pacific Program and a researcher at the University of Hawaii Cancer Center. His research interests include the interaction between genetic and lifestyle factors that underlie the differences in cancer risk among different ethnic and racial groups in Hawaii. He is carrying out research aimed at understanding the causes of cancer and the development of new prevention measures. He has played a key role in the Multiethnic Cohort study, which has collected detailed information on thousands of people to investigate the differences in lung cancer associated with smoking in different ethnic groups and the potential risk factors or mechanisms behind this.

CONTACT

E: loic@cc.hawaii.edu
W: http://manoa.hawaii.edu/ctahr/nutritionPhD/faculty/name/loic-le-marchand/

Professor Sharon E. Murphy
Department of Biochemistry, Molecular Biology and Biophysics
University of Minnesota
Minneapolis, MN
USA

Dr Sharon E. Murphy is Professor of Biochemistry, Molecular Biology and Biophysics in the University of Minnesota Medical School. She received her PhD in Chemistry from the University of Colorado, Boulder in 1982. Her research interest in tobacco related carcinogenesis began at the American Health Foundation and she joined the faculty at the University of Minnesota in 1996. Her research is focused on characterisation of the activation and detoxification pathways of nicotine and tobacco carcinogens and the role this plays in the development of smoking related cancer.

CONTACT

E: murph062@umn.edu
W: https://cbs.umn.edu/contacts/sharon-e-murphy

FUNDING

National Cancer Institute

UNIVERSITY OF HAWAI'I CANCER CENTER

UNIVERSITY OF MINNESOTA

COULD A BETTER UNDERSTANDING OF BACTERIA PREVENT COLORECTAL CANCER?

Our digestive system contains trillions of bacterial cells, constituting a highly diverse community of microorganisms living within us that can influence human physiology and cause disease. **Dr Jason Crawford** at Yale University has extensively researched some of the more harmful bacterial strains, looking at how they promote inflammation and colorectal cancer.

The Good, the Bad and the Ugly

Bacteria are literally everywhere. You only have to look as far as your hand to see some, albeit through a microscope rather than the naked eye. Whether they are on your dining table, on your person, or on your phone, avoiding bacteria is impossible.

That's not to say that all bacteria are bad. Bacteria get a bad reputation – they may be one of the major reasons behind why we become ill, but they can also do a lot of good for our bodies. Almost 100 trillion bacterial cells are currently living in your gut. Now, there's no reason to panic – most 'good' bacteria are vital to our continued survival. Not only do they help our bodies digest food and absorb nutrients, but they also produce vitamins in our intestinal tracts, including vitamin K needed for blood coagulation and vitamin B6 important for maintaining a healthy nervous system.

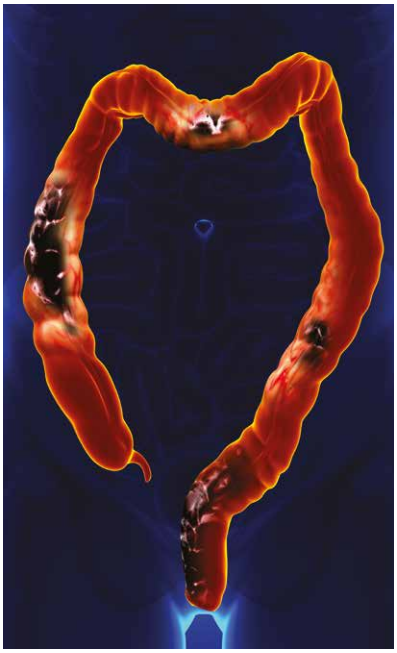
They can also help our immune systems out when responding to pathogens and harmful bacteria, by crowding them out in our gut, producing certain materials to prevent their growth and kickstarting our immune response into action.

Getting to Know Your Belly

The types of bacteria that live in your gut can vary massively over time. From probiotics such as *Lactobacillus*, *Bifidobacteria* and *Streptococcus*, to other forms of microorganisms such as *Bacteroides* and *Escherichia*, your gut is home to what is known as 'gut microflora'. Gut flora constitutes different bacterial species that vary in ratio and abundance according to time, diet and changes to overall health. The bacteria that live alongside us contain roughly 100 times more genes than the human genome itself representing a potentially huge number of biologically active small molecules that can be processed and synthesised by the bacteria living within their human hosts.

Small molecules produced by bacteria can affect both the numbers and types of microorganisms living within the gut, but they can also influence the physiology of their hosts by regulating a variety of different cellular processes.

Not much is known about how small molecules produced by bacteria influence human health and so their functions and how they are produced are an important area of biomedical research, especially as not all strains of

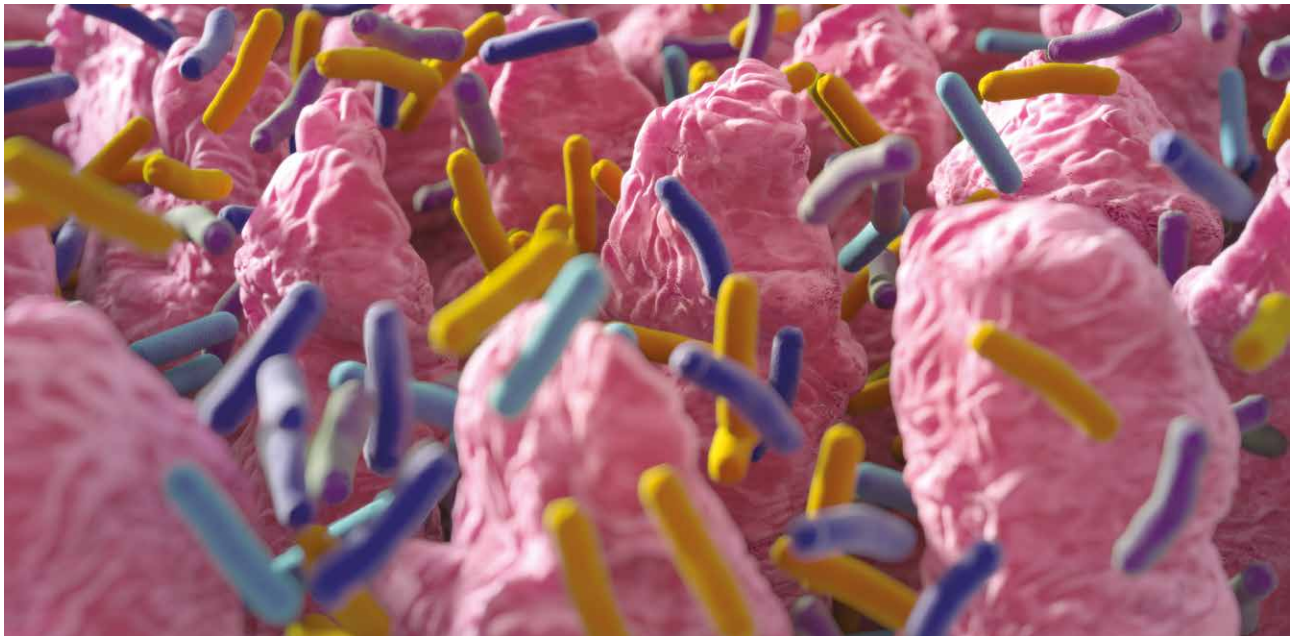


bacteria in the gut are harmless. Dr Jason Crawford and his team at Yale University have been investigating the strains of gut bacteria that have the potential to cause disease and pose a harmful risk.

Pondering Pathogen Potential

Dr Crawford and his team focus on the 'chemistry at the human-bacteria interface', predominantly looking at how bacteria regulate inflammation and cancer and how they could be

‘We at the Crawford Lab primarily focus on the molecular mechanisms of the interactions between humans and bacteria.’



exploited for human treatments. He describes how: ‘We primarily focus on the molecular mechanisms of the interactions between humans and bacteria. We specifically focus on how bacterial metabolism can regulate cancer initiation and treatment, immune regulation, and bacterial pathogenicity in the human host.’

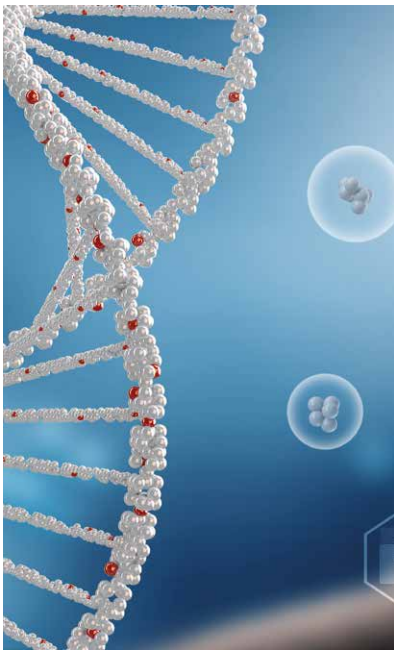
The team has researched and developed a number of methods to ‘mine’ the genomic sequences of pathogenic bacterial strains, to determine how they cause disease. The genomic sequences of bacteria encode the structures of the small molecules that they produce in the body, which can lead to disease. The team can then look to utilise this knowledge by designing drugs capable of inhibiting disease processes.

Through their research, Dr Crawford and his team have characterised a number of unusual clusters of genes suspected of synthesising or manufacturing important small molecules. As he states: ‘These small molecules often regulate complex interactions with their animal hosts, hold a rich history of being utilised as human drugs, and serve as excellent molecular probes for identifying new drug targets for a wide variety of diseases.’ As such, researching and identifying the gene clusters responsible for producing small molecules of this kind is a major focus of Dr Crawford and his team’s work.

The Bad Side of *E. coli*

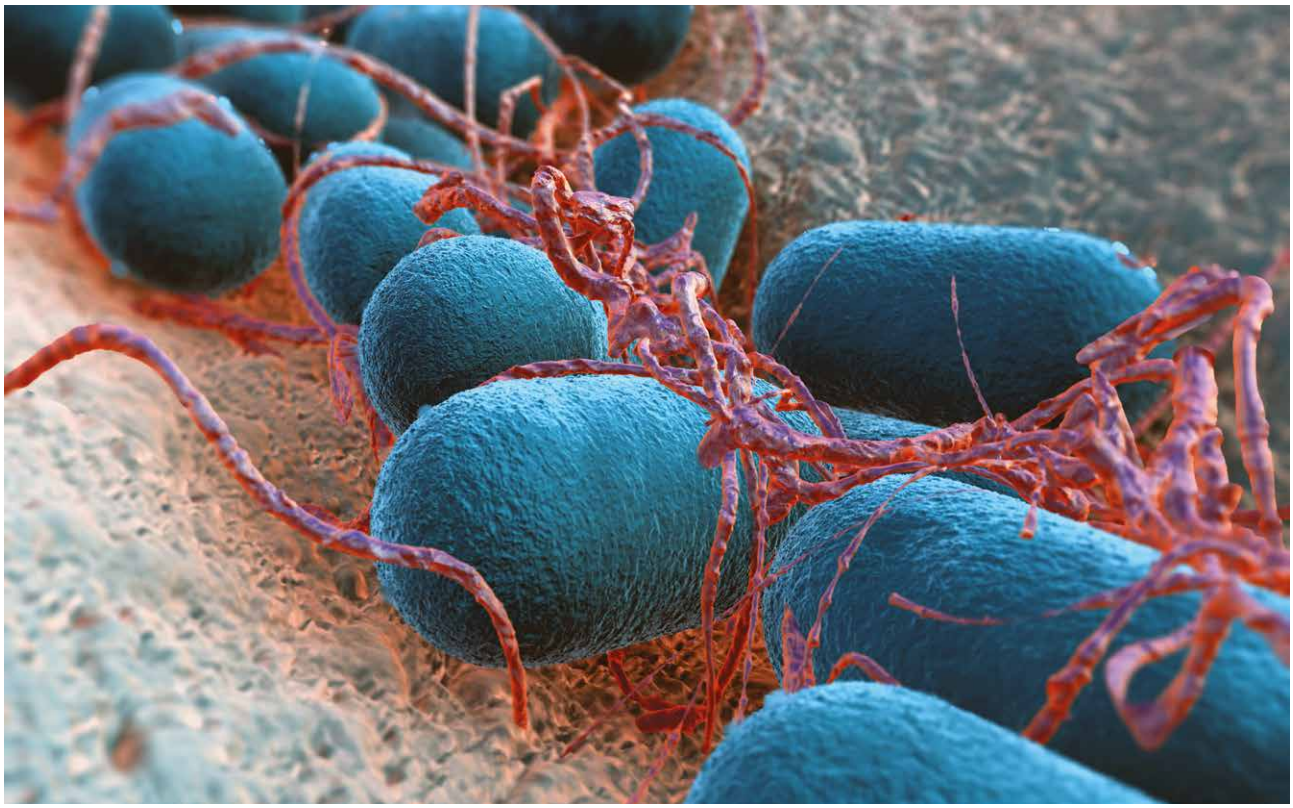
Over the past six years, Dr Crawford’s research team has focused a good portion of their research on gut bacteria called *Escherichia coli* (*E. coli*). *E. coli* is no stranger to the world of research, especially in babies and infants, as it is the first type of bacteria to colonise the gut after birth and is thought to live in about 90% of the human population.

It is usually a harmless type of bacteria. However, Dr Crawford’s team and his key collaborators study certain strains of *E. coli* that are linked to the onset of colorectal cancer – cancer of the intestines. Through their research, they have characterised a particular genetic pathway that encodes small biological molecules known as ‘colibactins.’ While its fully mature structure has been challenging to characterise, they have shown that fragments of the small molecule cause damage to DNA consistent with colibactin’s proposed role in causing colorectal cancer.



Introducing Colibactin

During their research, the team used a combination of techniques to characterise the structure of the ‘colibactin warhead’ – a critical component of the colibactin molecule responsible for damaging DNA and initiating tumour growth. The colibactin warhead participates in a process called DNA alkylation and cross-linking. DNA alkylation is a process where a specific section of a molecule (a reactive alkyl group) forms a bond with DNA.



DNA cross-links result from two bonds to the DNA duplex, locking DNA strands together. Modification of DNA with cross-links can be highly toxic, impairing cellular processes that use DNA as a template, such as the replication of DNA when cells divide or the production of RNA to produce proteins. Cross-links, for example, can induce multiple DNA repair mechanisms and can cause downstream DNA breaks in the double-stranded helix. The accumulation of DNA damage can lead to tumour formation.

The team's research demonstrated that certain forms of model colibactins, closely related to natural colibactins, are more efficient at initiating DNA damage than others. The pathway that makes colibactin uses giant modular enzymes called nonribosomal peptide synthetases and polyketide synthases to synthesise precolibactins in an 'assembly-line' manner that are later converted into colibactins, through an enzyme called a peptidase, dedicated to the task. Dr Crawford and his team looked at the effects of inactivating individual modules of the assembly line through a novel characterisation process called domain-targeted metabolomics.

The process involves investigating the synthesis pathways bacteria use to produce small molecules by combining gene editing of the different parts of each enzyme with analysis of the pathway and the products produced. The team then investigated the different steps in the assembly line in more detail and identified two new precolibactin products that could also potentially cause damage to DNA. A similar technical approach can be used in the future to decipher the many steps in the synthesis pathways of important small molecules produced by bacteria with very high precision.

Gutsy Work

The team's research has also looked at utilising the findings from their previous work to investigate potential molecules that could offer resistance against colibactin's ability to cause colorectal cancer. To continue their work, the products created by the DNA gene cluster that produces colibactin need to be investigated further. As Dr Crawford states in one of his research papers: 'Though advances toward elucidating (pre)colibactin synthesis have been made, the functions and mechanisms of several *clb* gene products remain poorly understood.'

Dr Crawford's team has more recently been investigating the molecular function of *ClbS* – a gene product that confers resistance to colibactin toxicity. Because *ClbS* neutralises the colibactin warhead, it could potentially be developed to prevent colibactin from causing cancer in the future.

Gut bacteria provide a fascinating area of scientific research and a huge potential reservoir of undiscovered drug-type molecules. Through the research of Dr Crawford and his team, science is now one step closer to understanding, combatting and preventing the onset of cancer in the intestine, utilising the strains of bacteria that live in our gut to our advantage. As their work supports, maybe the treatments to some of life's most debilitating conditions have been in the pit of our stomachs all this time.

Meet the researcher

Professor Jason M. Crawford
Yale University
West Haven, CT
USA

Professor Jason M. Crawford studied Biological Chemistry, graduating with a BS in Chemistry, with an emphasis in Biotechnology in 2001. He completed an MA in Bioorganic Chemistry in 2003, before receiving a PhD in Bioorganic Chemistry in 2008 at the Johns Hopkins University. He then went on to work as a Damon Runyon Cancer Research Foundation Postdoctoral Fellow at Harvard Medical School in the Department of Biological Chemistry & Molecular Pharmacology until 2012, before joining Yale University as an Assistant Professor of Chemistry and of Microbial Pathogenesis in 2012, where he has remained ever since. Jason is currently the Maxine F. Singer Associate Professor of Chemistry and of Microbial Pathogenesis, where he serves as a member of the Yale Chemical Biology Institute, the Comprehensive Cancer Center, and the Center for Pulmonary Infection Research & Treatment. He has also received a number of awards and honours along the way. In 2007, he received the Sarah and Adolph Roseman Achievement Award from Johns Hopkins University. In 2011, he received the NIH Pathway to Independence Award at Harvard Medical School. At Yale University, he has received the Searle Scholars, the NIH New Innovator, the Dale. F Frey Breakthrough Scientist, the Damon Runyon-Rachleff Innovation, the Camille & Henry Dreyfus Teacher-Scholar, and the Burroughs Wellcome Investigators in the Pathogenesis of Infectious Disease (PATH) awards over the last six years.

CONTACT

E: jason.crawford@yale.edu
W: https://crawfordlab.yale.edu/

Yale

KEY COLLABORATORS

For the selected project, Jason Crawford is indebted to the following key collaborators.
Seth B. Herzon, Yale University, USA
Steven D. Bruner, University of Florida, USA
Christian Jobin, University of Florida, USA

FUNDING

NIH National Cancer Institute (1DP2-CA186575 & R01-CA215553)
Burroughs Wellcome Fund (1016720)
Camille & Henry Dreyfus Foundation (TC-17-011)
Damon Runyon Cancer Research Foundation (DRR-39-16)
Yale Comprehensive Cancer Center

FURTHER READING

A Healy, H Nikolayevskiy, J Patel, J Crawford and S Herzon, A Mechanistic Model for Colibactin-Induced Genotoxicity, Journal of the American Chemical Society, 2016, 138, 15563–15570.

E Trautman, A Healy, E Shine, S Herzon and J Crawford, Domain-Targeted Metabolomics Delineates the Heterocycle Assembly Steps of Colibactin Biosynthesis, Journal of the American Chemical Society, 2017, 139, 4195–4201.

M Vizcaino and J Crawford, The colibactin warhead crosslinks DNA, Nature Chemistry, 2015, 7, 411–415.

P Tripathi, E Shine, A Healy, CS Kim, S Herzon, S Bruner and J Crawford, *ClbS* Is a Cyclopropane Hydrolase That Confers Colibactin Resistance, Journal of the American Chemical Society, 2017, 139, 17719–17722.

CAN TISSUE DAMAGE CAUSED BY RADIATION TREATMENT BE REDUCED?

Radiation therapy is a common treatment for cancerous tumours. However, radiation-induced tissue injury can be a serious side effect of treatment. **Dr Jae Ho Kim** and **Dr Stephen Brown** of Henry Ford Hospital, Detroit, USA, have identified molecular processes associated with radiation injury and have developed anti-inflammatory strategies that can prevent, mitigate, and treat tissue damage. By reducing the amount of tissue damage resulting from radiation therapy, physicians may be able to increase the amount of radiation applied to a tumour, allowing for more effective eradication.

In recent years, advances in technology have allowed for the increased effectiveness of radiation therapy for the treatment of cancer. Examples include greater penetration of the radiation dose using high energy x-rays and newer techniques that allow for precision delivery such as intensity modulated radiation therapy and stereotactic radiosurgery and image guided radiotherapy. Despite improvements in tumour targeting, radiation traverses normal tissues and often inadvertently damages healthy tissue surrounding the tumour, fundamentally limiting the radiation dose that can be safely used.

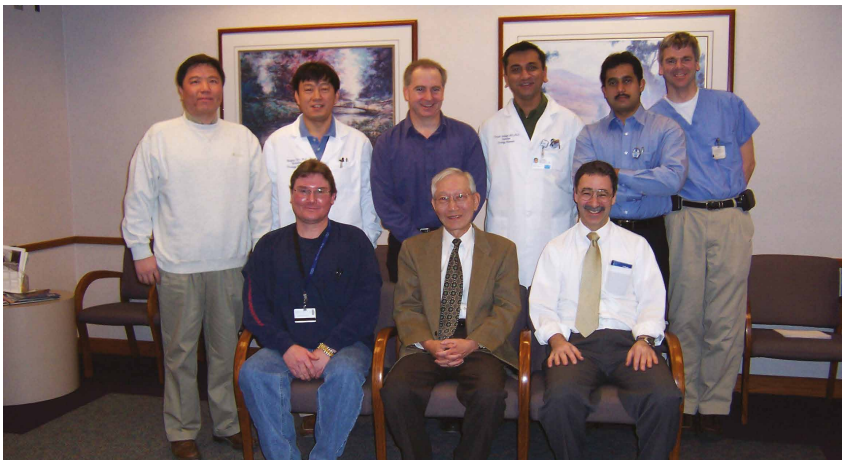
Complementary to technological improvements, medications to alleviate side effects resulting from the treatment have advanced. The working hypothesis of investigators in this field is that if damage to healthy tissue could be prevented or minimised, radiation doses could be increased and allow for more aggressive and effective treatment of tumours. Many classes of drugs have been studied which have potential to enhance the effectiveness of radiation therapy by decreasing normal tissue injury. Dr Jae Ho Kim and Dr Stephen Brown, both of Henry Ford Hospital, Detroit, USA, have shown anti-inflammatory agents are an attractive approach. They have demonstrated that anti-inflammatories both markedly reduce normal tissue injury after radiation exposure and, in addition, sensitize tumours to the damaging effects of radiation. Timing is important. When optimum, anti-inflammatories increase radiation damage to tumours and, at the same time, reduce radiation damage to normal tissues.

Causes of Tissue Damage Following Radiation Therapy

Radiation cell death has been studied for sixty years and was thought to be well understood. The conventional wisdom is that radiation breaks apart, or ionizes, cellular molecules indiscriminately. Most of the cell is composed of water and most ionized water molecules recombine without consequence. Some affected water molecules, although short lived (much less than a second), can travel small distances within cells to react with other molecules.

However, cell survival is relatively unaffected by the radiation damage to most molecules in the cell; like water, radiation has little consequence on most other cell components such as proteins, lipids, and carbohydrates because of adequate redundancy. In stark contrast, there is only one double stranded copy of DNA in the cell and damage to DNA by radiation can be catastrophic to cell survival. Single strand breaks to DNA are common

‘Building on the results of pioneers in anti-inflammatory therapies, we were the first to demonstrate that pan-cytokine inhibition using a small molecule mitigates radiation injury in both acutely responding tissue (i.e. skin) and late responding tissue (i.e. brain).’



Radiobiology Group in the Department of Radiation Oncology at Henry Ford Hospital

and repairable because another complementary template exists. Double strand breaks, although rare, result in the death of a cell. Textbooks on radiation biology focus on DNA damage by radiation as the cause of eventual radiation injury. Experimental studies suggest that a number of secondary, less obvious processes can also lead to much greater cell loss and tissue damage than was sustained from the initial damage to DNA.

Most early damage from radiation injury is treatable. Recently, it has become clear that a main contributor to late radiation tissue damage is inflammation. Shortly after radiation exposure to the tissue and organs, large amounts of proteins known as cytokines and chemokines are released from the irradiated cells, signalling to other inflammatory cells in the body to travel to the site of tissue damage for clean-up and repair. The inflammatory cells produce additional cytokines and chemokines. Problems arise when the inflammatory response stays elevated for long periods of time leading to chronic inflammation and tissue injury.

Irradiation, even at low doses, is one of the few factors known to activate the cytokine called TGF-B. TGF-B activates another protein known as the Smad protein, which, when stopped from being synthesised in mice, reduces skin damage after radiation treatment and prevents inflammatory cells from targeting damaged tissue.

One of the key inflammatory cell types are macrophage, cells that are responsible for detecting, engulfing, and destroying dying or dead cells. Macrophages can be beneficial in the early stages of inflammation. They can also cause chronic inflammation and tissue injury, and have been linked to tumour resistance and recurrence following chemo-radiation therapy.

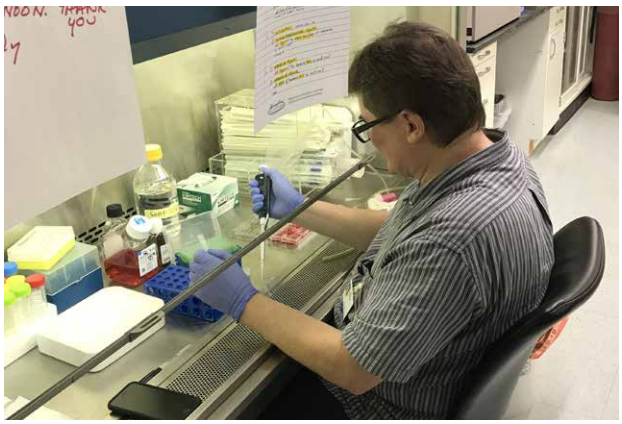
Activated macrophage cause tissue injury after radiation by producing oxidative damage. Under normal conditions, most tissues and organs guard themselves against this type of damage. However, during times of environmental stress, levels of reactive oxygen species (ROS) increase dramatically, overwhelming the cell’s defence systems and resulting in significant damage to cell structures. The generation of these reactive molecules is part of the body’s immune system and functions to rapidly clean wounds from injury. However, the excessive production of ROS can lead to severe tissue damage. Damaging ROS might arise from several sources including infiltrating macrophages. Other cells, such as skin cells, can be stimulated by pro-inflammatory cytokines to produce ROS. Tissue hypoxia (lack of oxygen) resulting from damage to blood vessels is another source of ROS generation.

The roles of the various pro-inflammatory cells and other proteins in the tissue damage processes following radiation treatment remains an area of active study. Researchers Drs Jae Ho Kim and Stephen Brown have devised two approaches to deduce their functions. The first is to suppress cytokine mediated inflammation using small molecule inhibitors to ascertain whether specific macrophages are the major source of the inflammatory cytokines leading to tissue damage.

The second approach of Drs Kim and Brown is to find and eliminate senescent cells (in other words, cells that have ceased to divide) and to determine if their elimination prevents normal tissue injury and if this enhances the efficacy of tumour radiotherapy. This approach is based on their hypothesis that the radiation-induced senescent cells could be the source of release of pro-inflammatory cytokines and chemokines or tissue-damaging proteins. Multiple pharmacological studies are under investigation to remove senescent cells, an area of medicine known as senolytics. The researchers’ preliminary data generated using both these two approaches are highly encouraging, suggesting these are fruitful avenues for future exploration.

Tissue-specific Radiation Injury

Different tissues can react differently to the effects of radiation. In addition, radiation damage may be classified as acute or chronic, with different molecular mechanisms and cells responses which may be held to blame in each case. The research of Drs Kim and Brown has demonstrated that



Drs Kim and Brown's laboratory and Mr Andrew Kolozsvary (Radiobiology researcher).

various tissues targeted by radiation therapy can be affected at the molecular level, and how these different types of damage can best be treated or prevented.

Injuries to the skin as a result of radiation may cover small areas but extend deeply into the soft tissue, even reaching underlying muscle and bone. Symptoms of acute radiation damage to the skin include skin redness and peeling as well as DNA damage. Tissue remodelling due in part to excessive inflammation can occur after radiation exposure. Although the cellular mechanisms remain under investigation, radiation damage to the skin has been attributed to damage to supplying blood vessels and reduced functioning of stem cells that can no longer replace functioning cells.

The research team of Drs Kim and Brown investigated a small molecule known as MW-151, which is a cytokine inhibitor originating from the drug discovery group at Northwestern University. MW-151 was shown to have potential anti-inflammatory properties and promise against brain diseases such as Parkinsons Disease. Drs Kim and Brown found that following skin radiation therapy in mice, MW-151 reduced the damage to tissue, reducing the infiltration of macrophages and the deposition of collagen, which leads to fibrosis (that is, scarring). The effects were highly dependent on the timing of the treatment, with the best results seen on mice given MW-151 on day 7 and day 9 after radiation.

Another organ that can be highly sensitive to radiation treatment-induced injury is the brain. Acute effects occur during and/or shortly after the radiation exposure and are characterised by fatigue and dizziness. From 6 to 12 weeks afterward irradiation, generalised weakness and drowsiness ensue. Although the acute effects are usually temporary, the late effects of brain irradiation may lead to severe irreversible neurological consequences, such as cognitive deficits or death of brain tissue. In the worst cases, seizures and dysfunction of the cranial nerves result from increased intracranial pressure due to fluid build-up in the brain. Of considerable concern is that these effects are usually more pronounced in young children.

The brain has traditionally been regarded as highly resistant to radiation. However, there exist progenitor cell populations in an area of the brain known as the limbic system including the hippocampus, and these are known to be extremely radiosensitive. These cells represent unique sources of new neural cells in the adult brain and are thought to be involved in learning and memory function. When radiation levels are increased in the brain, glial cells known as microglia (similar in function to macrophage in tissues other than brain) become activated and promote the development of chronic neuroinflammation. Anti-inflammatory drugs can reduce microglial activation and chronic neuroinflammation within the brain, partially restoring neurogenesis, or formation of new neurons in the hippocampus. Investigators at Henry Ford Hospital have also used MW-151 to prevent neuroinflammation in rats subjected to brain irradiation and found that this cytokine inhibitor successfully prevented the death of neural progenitor cells and tissue damage when administered to the rats for a period of 28 days.

From the Past to the Future

In summarising their work so far, the researchers explain, 'Building on the results of pioneers in anti-inflammatory therapies, we were the first to demonstrate that pan-cytokine inhibition using a small molecule mitigates radiation injury in both acutely responding tissue (i.e., skin) and late responding tissue (i.e., brain).'

Obviously, the radiation dose to tumours is critically important, but it is the normal tissue damage which limits the radiation dose that can be applied to tumours. What makes the approach taken by investigators at Henry Ford Hospital unique and different from the previous ones is that their pharmacological approach mitigates radiation injury and damage to the tissues and organs without compromising the effect of radiation on the tumour tissue. In this way, the therapeutic ratio is enhanced. Henry Ford investigators have already identified several drugs which block pro-inflammatory cytokines and ROS that are currently available for other indications and therefore in widespread clinical use.

In conclusion, Drs Kim and Brown have shown that there is value in reducing inflammation, both to normal tissues and tumours after radiation therapy. They continue to seek more effective therapeutic targets that may be used for individuals who are not responding to currently available drugs on the market. Drs Kim and Brown plan to carry out additional investigations to study the cellular- and cytokine-mediated processes underlying radiation-caused tissue damage, particularly in regard to macrophage behaviour. In this way, they hope to discover new molecular pathways that could be targeted for new drugs to prevent or heal radiation-induced tissue damage without compromising tumour control.

Meet the researchers

Jae Ho Kim, MD, PhD, FACR
Department of Radiation Oncology
Henry Ford Hospital
Detroit, MI
USA

Dr Jae Ho Kim is a practising radiation oncologist at the Henry Ford Hospital in Detroit, Michigan, and a Professor of Radiation Oncology at Wayne State University in Michigan. He completed his MD in South Korea at the Kyungpook University School of Medicine in Daegu before completing a PhD in Radiobiology at the University of Iowa in 1963. He then undertook postdoctoral work in Biophysics at the Memorial Sloan-Kettering Cancer Center in New York. Board certified in Radiation Oncology, Dr Kim served as department chair at Henry Ford Hospital from 1989 to 2005. His career has been dedicated to improving radiation therapy for cancer treatment by increasing tumour response as well as by decreasing tissue damage caused by radiation.

CONTACT

E: jkim1@hfhs.org

Stephen Brown, PhD
Department of Radiation Oncology
Henry Ford Hospital
Detroit, MI
USA

Dr Stephen Brown has been a Senior Staff Scientist in the Department of Radiation Oncology at Henry Ford Hospital in Detroit since 1994, where he specialises in characterising the effects of radiation therapy on human tissue. He completed his PhD in Medical Physics in 1991 at the University of Toronto before moving to Detroit to complete postdoctoral work in the laboratory of Dr Jae Ho Kim at Henry Ford Hospital. His career goal has been to improve the effectiveness of radiation therapy. Since 2009, he has also been a Professor of Radiation Oncology at Wayne State University Medical School in Detroit, and Adjunct Professor of Physics at the University of Windsor in Canada. Since 2014, he has served as Leader of the Translational Oncology Group at the Henry Ford Cancer Institute in Detroit.

CONTACT

W: https://www.henryford.com/physician-directory/b/brown-stephen

KEY COLLABORATORS

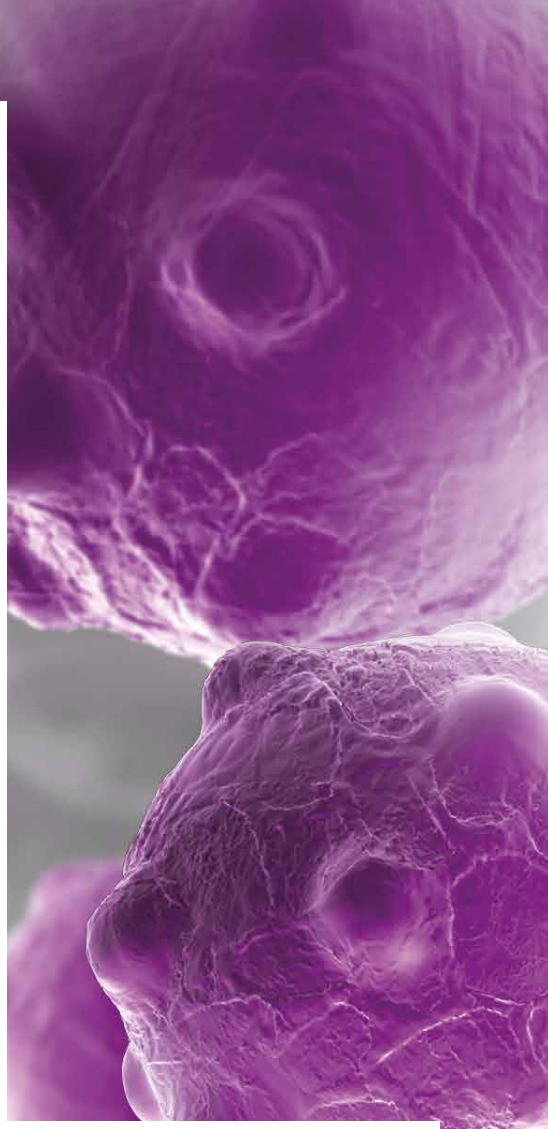
Kenneth Jenrow, PhD, Central Michigan University
Andrew Kolozsvary, BS, Henry Ford Hospital
Linda Van Eldik, PhD, University of Kentucky
D. Martin Watterson, PhD, Northwestern University

FUNDING

NIH R21 CA205660, PI: Jae Ho Kim
NIH R01 CA218596, co-PIs: James Ewing and Stephen Brown

SIMPHOTEK: SHEDDING LIGHT ON NEW CANCER TREATMENTS

The use of radiation therapy to treat cancer has improved the long-term outcome of thousands of patients but is associated with serious side effects. Photodynamic therapy (PDT), a targeted light-based technique, has been approved as an effective treatment for some forms of cancer with fewer side effects than radiotherapy. However, the effectiveness of this technique depends on fine-tuning its application to the patient. Simphotek, a US-based company founded by world leaders in biophysics and computer modelling, together with its collaborative partners at Roswell Park Comprehensive Cancer Center and the Hospital of the University of Pennsylvania, is focused on expanding novel technologies of PDT as a cancer treatment for solid tumours.



Novel Cancer Treatments

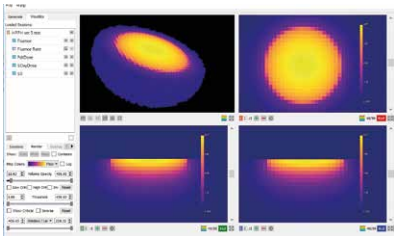
Cancer is one of the leading causes of death worldwide. In the UK alone, around 164,000 people die from cancer each year, accounting for more than a quarter of deaths nationwide. Traditional treatments, such as surgery, radiotherapy, and chemotherapy, have demonstrated success in reducing cancer mortality but are associated with major side effects as well as the recurrence of cancer.

In order to circumvent these difficulties, novel cancer therapies are being investigated that might be able to target tumours directly without causing serious side effects or damage to the surrounding tissue. A promising recent development for solid tumours is the use of photodynamic therapy, or PDT. PDT uses a drug called a photosensitiser and light to kill tumour cells.

During the procedure, the photosensitiser is injected into the bloodstream and absorbed by cancer cells in the body. The photosensitiser is completely non-toxic until exposed to certain frequencies of light, at which point it generates toxic reactive oxygen species that can kill cells. To allow time for the photosensitiser to reach the tumour, the treatment is started a few hours after injection. Exposing the tumour to light causes the photosensitiser to produce toxic reactive oxygen species that kill the tumour cells, while the healthy cells that are not exposed to light are not affected. Unlike x-ray radiation, the therapy can be repeated as many times as needed to treat the tumour.

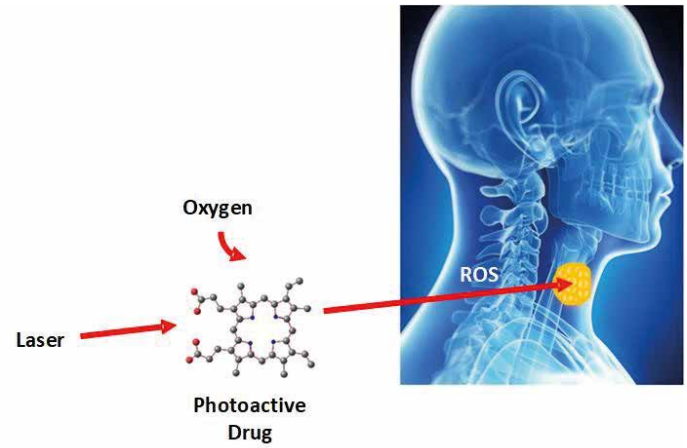
Although PDT has been shown as an effective and targeted therapy for some superficial cancers and has been approved by the FDA for the treatment of some forms of oesophageal and bronchial cancer, it is not approved for solid cancers such as those of the brain, pancreas, head and neck, lung, and prostate. Moreover, the effectiveness

Dosie™ for the application of photodynamic therapy. Credit Simphotek Medical Devices, Inc.



Dosie™ output. Credit Simphotek Medical Devices, Inc.

of PDT depends on several factors including light power, photosensitiser concentration, and the duration of the treatment. The magnitudes of these factors are all interdependent and cannot be determined solely by clinical



In photodynamic therapy, light interacts with a photo-active drug and oxygen to generate highly reactive oxygen species (ROS) that locally kill cancer cells in solid tumours. Credit Simphotek Medical Devices, Inc.

PDT in the Operating Room. Credit Simphotek Medical Devices, Inc.

trials. In fact, advanced mathematical calculations and novel computing devices are required.

Perfecting PDT

Simphotek is an organisation focused on developing novel products to aid researchers and clinicians in the study and practice of light-based therapies including cancer. Led by Dr Mary Potasek, Dr Gene Parilov, and Dr Karl Beeson, they have developed innovative medical computational devices for light-based cancer treatments with the aim of improving patient outcomes following PDT by applying accurate, real-time modelling and monitoring techniques. Together with their world-leading medical collaborators who focus on light delivery systems, their innovations allow clinicians to better plan, deliver, and monitor PDT. The team is also working on optimising the PDT process using computer software that can process information from the procedure and use it to fine-tune the operation in real-time.

High Definition Dosimetry

One of the major advances in the application of PDT made by Simphotek is a device called Dosie™, which is composed of Trade-Secret protected software, tuned to specific high-performance computational hardware. Simphotek is licensing patent-pending dosimetry hardware from its partners to integrate it with its software to create complete operating room-ready cancer treatment systems. Dosie uses simulations of light distribution and photosensitiser concentration to inform oncologists in their planning of PDT. Dosie's dosimetry software allows the calculation of the effective treatment dose. A similar technique is used during radiotherapy, where dose monitoring is used to limit the patient's exposure to harmful ionising radiation while ensuring the tumour is adequately irradiated.

The current approach to determining the treatment dose for PDT is through monitoring the total dose of light given to the patient only. However, this does not necessarily correlate with the efficacy of the treatment. The outcome of PDT also depends on the concentration of the photosensitiser in the tumour, which may not be uniformly distributed within the patient and can vary from patient to patient and even between different types of tumour, and the local concentration of reactive oxygen species in the tumour produced during therapy.

To more accurately determine the effectiveness of the planned therapy, Dosie takes into account the tumour shape with its optical properties and photosensitiser concentration and predicts the required amounts of the light power and treatment time. Using existing data on how well the photosensitiser can produce reactive oxygen species from the local oxygen concentration, Dosie can predict the final dose of toxic reactive oxygen species produced within the tumour to give a much better idea of how effective the treatment will be. These measures are better than simply using the dose of light administered to the tumour because they account for the availability and unevenness of the photosensitiser and oxygen across the tumour.

The data input to Dosie includes parameters specific to the patient, including the shape of the tumour, data about how well light can travel through it, local oxygen concentrations, and the concentration of photosensitiser across the tumour and surrounding tissue. Therefore, it effectively allows the treatment to be personalised to the patient. Currently, the same treatment is given to all patients with a certain indication resulting in less than optimal treatment.

As well as aiding planning, these simulations can be run in near real-time and aid the surgeon in the operating room while actually performing the treatment. Using three-dimensional dose maps defined by Dosie of the tumour, the surgeon can spot areas that are receiving an insufficient or excessive dose and adjust the light intensity or treatment time accordingly. This can reduce damage to surrounding tissue while ensuring that the tumour receives an adequate dose for effective treatment. The hardware is uniquely configured by Dosie and uses multi-core CPU architecture and massive parallel GPU architecture to enable simulation and modification to be carried out in the clinic as the patient is undergoing treatment. This rapid analysis is unique to Dosie.

Drs Beeson, Parilov, and Potasek published a proof-of-concept study in the *Journal of Biomedical Optics* showing that Dosie predictions of reactive oxygen species correlated closely with measurements and PDT outcomes. They showed that these measurements were more reliable in predicting the outcome of PDT than existing commonly used techniques that use the dose of light administered to the tumour.

In-depth, Real-time

For tumours on the skin, an external laser can illuminate the tumour. For internal, hard to reach cancers, one or more optical fibres may be needed to deliver light to the tumour through a process known as interstitial PDT, or I-PDT, a light delivery method used at, for example, Roswell Park Comprehensive Cancer Center. Although the FDA has approved PDT for the treatment of some superficial cancers, I-PDT has not yet been approved for use in internal solid tumours, although clinical trials have shown it is effective at treating some types of cancer.

In order to advance the journey of interstitial PDT into the clinic, Simphotek is licensing patent-pending technology from its collaborators to develop a clinical and operating room cancer treatment system known as INTELLI. The idea behind INTELLI is that it will be able to automatically calculate where laser fibres should be placed in order to deliver adequate treatment throughout the tumour. Additionally, Simphotek is creating new software to monitor treatment during the cancer operation and automatically control the laser light, again using real-time PDT dose calculations in Dosie.

Simphotek hopes that this complete automated bench-top system will be able to assist clinical trials into PDT for deep-seated tumours of more than 10mm thickness. A major advancement of Dosie software is that light simulations can be completed and repeated many times during the treatment, as opposed to the current simulation time of many minutes or longer.

Successful Systems

Through collaborating with the Hospital of the University of Pennsylvania, Simphotek is also focused on delivering a complete dosimetry solution for PDT of tumours located in the body cavities, a technique known as intracavitary PDT. By combining licenced patent-pending cutting-edge optical hardware from the University of Pennsylvania with Dosie software, Simphotek is producing a novel technology named PEDSy – the PDT Explicit Dosimetry System.

The major innovation of PEDSy is that it will be able to monitor both light dose and photosensitiser concentration in real-time from eight places, combining these measures to interpret the 'PDT dose' across the tumour. The PEDSy system uses eight light detectors and eight spectrometers attached to the tumour that can measure the light dose and the dose of the photosensitiser across the tumour, respectively. The latter monitors photosensitiser concentration and photobleaching, based on the measurements of the fluorescent light.

Early clinical work with a prototype of the system is focused on monitoring treatment in patients undergoing PDT for mesothelioma, a lung cancer that is difficult to treat using other methods. Currently, mesothelioma has a very poor survival rate, with most patients only surviving for 12–18 months. However, recent studies by the University of Pennsylvania have shown that even an early prototype of PEDSy can increase the median survival by at least a factor of two. Animal studies have shown that the efficacy of PDT can be improved by a factor of 2–4 when using the experiment 'PDT dose' to monitor the treatment. This could translate to an even better prognosis for mesothelioma patients treated with PDT when the PEDSy system is fully developed and used as a guiding medical device and during PDT treatment sessions.

Photodynamic Futures

In addition to assisting with existing clinical applications of PDT, the group is also hoping to explore novel combination treatments using PDT technology. PDT leads to necrosis and apoptosis resulting in cell death. The necrotic cells swell and disrupt the plasma membrane resulting in the release of intracellular molecules. The tumour cell develops acute local inflammation leading to the influx of neutrophils and dendritic cells, which activate the adaptive immune response. Thus, PDT engages both the innate and adaptive immune responses.

Major advances in immuno oncology have seen the use of therapeutic monoclonal antibodies such as checkpoint inhibitors that can specifically target cancer cells and label them for killing by the body's immune system. Bioconjugates of photosensitisers with antibodies such as anti-cetuximab have been used with PDT. Recent studies have shown that this combination can be even more effective in clearing cancer cells than either technique alone. In addition, nanoparticle-based PDT has been used with checkpoint blockade immunotherapy and shown positive results.

Through creating novel dosimetry and treatment monitoring software for PDT, Simphotek is working to make PDT as commonplace as x-ray radiation therapy is today. Together with their academic partners, they are aiming to develop more accurate dosimetry hardware and software capable of making PDT a routine procedure, avoiding the complications of chemotherapy, radiotherapy, and surgery.

Meet the researchers

Simphotek Medical Devices, Inc

Newark, NJ

USA

Dr Mary Potasek, PhD

Co-founder and Chief Scientific Officer

Dr Mary Potasek received her PhD in Physics from the University of Illinois before going on to fellowships at Princeton University and the Max Planck Institute. She has held research professorships at both Columbia University and New York University, with work focusing on molecular models, nanotechnology, biophotonics, and photomedicine. In addition to her renowned research work, she has extensive experience in finance and entrepreneurship, and in 2016 was selected as one of NJBiz's Best Fifty Women in Business.

CONTACT

E: mpotasek@simphotek.com
W: https://www.linkedin.com/in/mpotasek/
http://twitter.com/simphotek

Dr Gene Parilov, PhD

Co-founder and Chief Technology Officer

Dr Gene (Evgueni) Parilov received his PhD in computer science from Courant Institute, New York Univeristy and has over 20 years of experience in mathematical modelling and optimisation, advanced real-time computer graphics, computational dosimetry and developing academic and industrial simulation software. In addition to his academic success, his career has included the role of marketing and sales manager for L'Oreal (Paris). He has over 25 technical publications in photonics and image processing, and has two issued patents. Dr Pariov also holds a black belt in Taekwondo.

CONTACT

E: gene@simphotek.com
W: https://www.linkedin.com/in/gparilov/
http://twitter.com/simphotek

Dr Karl Beeson, PhD

Co-founder and Vice President

Dr Karl Beeson received his PhD in Physics and has over 20 years of product development experience, with stints in industry at Allied/Honeywell and Corning, leading product design and device development. Dr Beeson has prior experience in entrepreneurship, co-founding Goldeneye Inc, a company specialising in LED sources. He is listed on 67 US patents and has published over 40 technical papers.

CONTACT

E: beesonk@simphotek.com
W: https://www.linkedin.com/in/karl-beeson-133b7850/
http://twitter.com/simphotek

CONSULTANTS

Dr Christopher Schaber, PhD
Dr Susan Alpert, MD, PhD

KEY COLLABORATORS

Dr Tim Zhu, Keith Cengel, and Theresa Busch, University of Pennsylvania, Philadelphia, PA
Dr Gal Shafirstein, Roswell Park Comprehensive Cancer Center, Buffalo, NY

simphotek
Light Interactions Matter

INNOVATIVE STRATEGIES FOR IMAGING CANCERS

Dr Frank Wuest is the Director of the Division of Oncologic Imaging at the University of Alberta, Canada, where he holds the Dianne and Irving Kipnes Chair in Radiopharmaceutical Sciences. His main research interests revolve around the multidisciplinary field of cancer imaging but he is particularly interested in the possibilities of translating techniques developed in the laboratory to clinical settings to enhance patient care. In particular, he focuses on the design, synthesis, and validation of novel molecular probes to optimise the current diagnosis and treatment of cancer.

The aim of Dr Frank Wuest's research at the University of Alberta, Canada, is to dig deeper into the use of molecular imaging techniques to detect cancer. His ultimate goal is to facilitate the translation of novel biomarkers, which have diagnostic and therapeutic potential, from the laboratory to clinic. Biomarkers are defined as characteristics that can be measured from imaging data as indicators of biological processes, including diseases. In addition to his work on cancer imaging, Dr Wuest has also been involved in investigating how cancer cells change to adapt to different conditions, such as low oxygen.

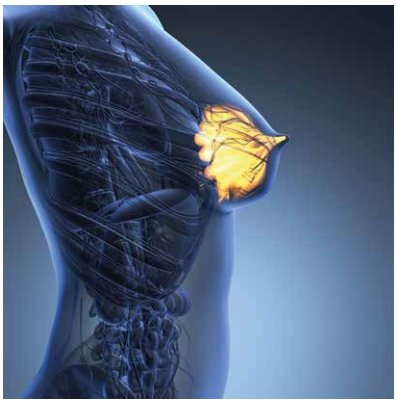
Particular attention is given to the use of novel positron emission tomography (PET) radiopharmaceuticals and pre-clinical PET imaging technology. PET imaging is a technique that can be used to observe metabolic and molecular processes in the body and is often used for diagnosing diseases.

The process works by detecting pairs of gamma rays, which originate from the collision of a positron, emitted by positron emitters, with an electron.

The most common positron-emitting radionuclide is fluorine-18 (^{18}F). Radionuclides like ^{18}F are introduced into the body attached to biologically active molecules called radiotracers, and it is the accumulation of these radiotracers in certain tissues and organs which can be turned into a 3D image to reflect their spatial and temporal biodistribution *in vivo*.

The radiotracer is normally associated with a specific biological process, such as glucose uptake by cells, and can, therefore, be used to infer information about tissue metabolic activity. As Dr Wuest explains, 'The availability of innovative PET radiotracers to visualise and study biochemical processes in living organisms is an important driving force and a key element for the successful application of PET imaging technology in biomedical and clinical research.'

In the 15 years since becoming an independent researcher, Dr Wuest and his group have pioneered the development of novel chemistry techniques for molecular imaging and the validation of novel PET



radiotracers and PET imaging assays that are associated with human pathologies. These assays include the molecular imaging of cancer, metabolic diseases, and neuroreceptors (which are receptor molecules activated by neurotransmitters).

Click Chemistry and COX-2

Part of Dr Wuest's current research is dedicated to the design and validation of probes for molecular imaging of COX-2, which is an important biomarker in cancer and inflammation. His efforts were rewarded by the development of the first successful PET imaging probe ([^{18}F]pyricoxib) for targeting COX-2 in cancer.

'The availability of innovative PET radiotracers to visualise and study biochemical processes in living organisms is an important driving force and a key element for the successful application of PET imaging technology in biomedical and clinical research.'

Leading on from this, Dr Wuest's group has also pioneered the development of fluorescent-labelled probes for the molecular imaging of COX-2 and were the first to use click chemistry for the design of highly specific and potent COX-2 inhibitors. Click chemistry is a class of biocompatible small molecule reactions to generate new compounds. This is often done between a biomolecule and a molecule containing a radionuclide (as for PET imaging). These reactions normally produce rapidly the new molecule in high yield with high specificity. This makes click chemistry ideal for targeting molecules in complicated biological environments, including in living organisms. Dr Wuest's team was among the first to apply click chemistry to the radiolabelling of peptides with ^{18}F.

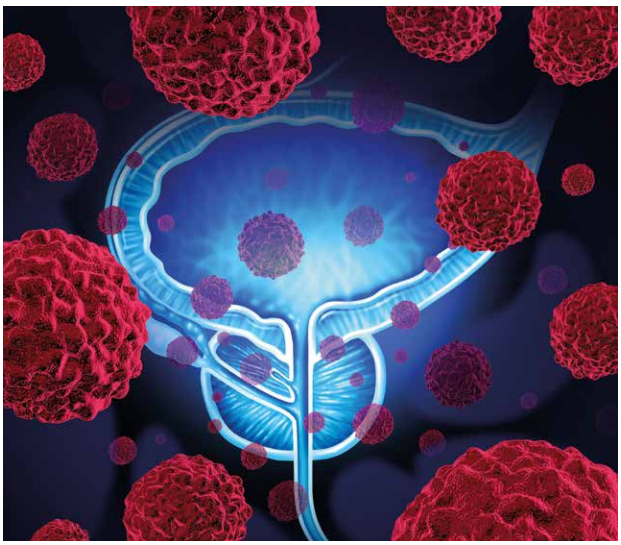
Even more excitingly, the team used *in situ* click chemistry for the preparation of highly potent and selective COX-2 inhibitors, where the COX-2 binding site was used as a molecular template to generate its own inhibitors. This removed the need for laborious synthesis and screening of a range of compounds, which is the usual approach for drug discovery. The methods used in the study described above have the potential to be extrapolated for economical and fast screening of other possible drug targets. Dr Wuest's team is currently investigating how the technique could be expanded to detect COX-2 expression at the cellular and whole-body level.

Various ^{18}F-labelled peptides have been designed by Dr Wuest and his collaborators and these molecules can be used as radiotracers to target a wide range of biomarkers associated with cancer and inflammation. The group has also worked with a different short-lived positron emitter, ^{11}C. Together with ^{18}F, the expansion of ^{11}C- and ^{18}F-labeled radiotracers has enabled Dr Wuest's research group to investigate site-specific labelling techniques to incorporate the radionuclide into a specific position of a given molecule. Other, non-standard PET radionuclides have also been investigated in order to determine whether they may be useful for the molecular imaging and therapy of cancer.

Breast Cancer

Breast cancer is the most common cancer affecting women worldwide. As cancer cells proliferate rapidly, there is often limited oxygen available to the cells resulting in changes in molecular processes linked to low oxygen and hexose transport. These hexose transport molecules can be targeted by radiotracers and it has been suggested that low oxygen conditions indicate a poor prognosis for therapy outcomes and survival in patients with breast cancer.

Dr Wuest and his team investigated the expression of these hexose transport molecules in different cancer cells grown in the laboratory under low oxygen conditions. Identification of specific metabolic markers on tumour cells will allow a 'molecular fingerprint' to be established which can subsequently guide the choice of imaging probe used for diagnosing and treating the different forms of breast cancer.



Taking a slightly different approach, Dr Wuest and his team have also recently started to develop a molecular imaging assay for autotaxin, an enzyme also thought to be associated with chronic inflammation, including cancer. This may also have a particular impact on the treatment of breast cancer patients undergoing radiation therapy.

Prostate Cancer

Prostate cancer is the fifth leading cause of cancer-related death in men in Western countries. A molecule called prostate-specific membrane antigen (PSMA) is often increased in prostate cancer. Previously, PET imaging of PSMA with ^{18}F-labelled radiotracers had several limitations, including the amount of time and effort required in producing ^{18}F-labelled PSMA inhibitors.

Using an automated synthesis would remove this limitation – and this is what Dr Wuest and his team set out to do. They were able to simplify the synthesis of clinically important radiotracer [^{18}F]DCFPyL, using a single step radiofluorination procedure in an automated synthesis unit. In addition, [^{18}F]DCFPyL is taken up by tumours and effectively cleared from the body, preventing unnecessary accumulation of the radiotracer in most organs and tissues of the human body. Pre-clinical results confirmed that this molecule is a promising option for targeted molecular imaging of PSMA in prostate cancer.

In addition, work by Dr Wuest and others at the Washington University School of Medicine tested the 'serve and protect strategy', which suggests that administering enzymes at the same time as radiolabelled peptides can increase the survival of the peptides, may not be valid for peptides which naturally show metabolic stability in animal models. Other research projects undertaken by Dr Wuest include the development and clinical translation of radiolabelled and metabolically stabilised peptides for molecular imaging and therapy of breast and prostate cancer. In particular, he has already developed a molecule called BBN2 which, when linked to ^{18}F, may be important for looking at certain types of tumour in breast and prostate cancers.

Ovarian Cancer

The lack of clear symptoms, combined with the high incidence of recurrence, means that ovarian cancer has only a 44.6% five-year survival rate. Cancer antigen 125 (CA125) is a glycoprotein that is overexpressed on the membrane of ovarian cancer cells and may act as a biomarker for ovarian cancer. However, normal levels of the protein can still be associated with the presence of disease. Current assays may be limited in their ability to detect early-stage cancers.

A process called immuno-PET, a version of PET imaging which uses an antibody linked to a radionuclide has shown promise as a strategy that unifies the specificity of antibody binding to a target on the tumour cell with the sensitivity of detection via positron-emitting radionuclides. Dr Wuest and colleagues at MSKCC produced a radiotracer based on an antibody for CA125, which could be used for molecular imaging of the glycoprotein in the body. Furthermore, using a radionuclide to label the antibody for immuno-PET imaging did not compromise the ability of the antibody to bind to its target.

Future Directions

The novel imaging assays that Dr Wuest is involved in designing are vital contributions to cancer care. Specifically, the 'precision oncology' concept aims to enhance patient care and outcome through early, more accurate diagnosis and better treatment regimens specifically tailored to each individual patient's requirements.

Ultimately, the more improvements that can be made to cancer imaging, the better the outcome for the patient. It is this seemingly simple statement that makes the research of Dr Wuest and his colleagues so important for the future of cancer diagnosis and treatment.

Meet the researcher

Dr Frank Wuest
Department of Oncology
University of Alberta
Edmonton, Alberta
Canada

Dr Frank Wuest is the Director of the Division of Oncologic Imaging at the University of Alberta, where his research focusses on the use of molecular imaging techniques to assess the potential of novel molecules in the treatment of cancer. He obtained his PhD in Chemistry at the prestigious University of Technology (TUD) Dresden in Germany in 1999, where he also obtained his habilitation in biochemistry in 2006. Between 1999 and 2001, he worked as a postdoctoral fellow with Dr Michael Welch, one of the fathers of radiopharmaceutical sciences, Washington University, School of Medicine (St Louis). Dr Wuest has published over 140 articles to date and he is a Guest Professor at Beijing Normal University in China. He holds the Dianne and Irving Kipnes Chair in Radiopharmaceutical Sciences and is a Senior Scholar of Alberta Innovates – Health Solutions. His research has pioneered the use of novel chemistry for use in molecular imaging and has contributed greatly to the field of oncologic imaging.

CONTACT

E: wuest@ualberta.ca
W: https://www.ualberta.ca/medicine/about/people/frank-wuest
T: +1 780-989-8150

KEY COLLABORATORS

Dr David Brindley, Department of Biochemistry, University of Alberta
Dr Frederick West, Department of Chemistry, University of Alberta
Dr Jason Lewis, Memorial Sloan Kettering Cancer Center (New York)
Dr Buck Rogers, Washington University, School of Medicine (St Louis)
Dr Francois Benard, BC Cancer Agency (Vancouver)

FUNDING

Natural Sciences and Engineering Research Council of Canada
Canadian Foundation for Innovation
Canadian Institutes of Health Research
Alberta Cancer Foundation

FURTHER READING

S Kiran Sharma, M Wuest, M Wang, D Glubrecht, B Andrais, SE Lapi, F Wuest, Immuno-PET of epithelial ovarian cancer: harnessing the potential of CA125 for non-invasive imaging, 2014, EJNMMI Research, 4(1), 60–73.

V Bouvet, M Wuest, HS Jans, N Janzen, AR Genady, JF Vaillant, F Benard, F Wuest, Automated synthesis of [^{18}F]DCFPyL via direct radiofluorination and validation in preclinical prostate cancer models, 2016, EJNMMI Research, 6, 40–55.

S Richter, M Wuest, CN Bergman, S Krieger, BE Rogers, F Wuest, Metabolically stabilized 68Ga-NOTA-Bombesin for PET imaging of prostate cancer and influence of protease inhibitor phosphoramidon, 2016, Molecular Pharmaceutics, 13, 1347–1357.

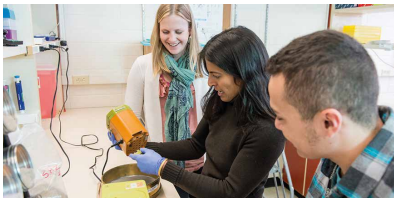
A Bhardwaj, J Kaur, M Wuest, F Wuest, In situ click chemistry generation of cyclooxygenase-2 inhibitors, 2017, Nature Communications, 8(1), doi: 10.1038/s41467-016-0009-6.

I Hamann, D Krys, D Glubrecht, V Bouvet, A Marshall, L Vos, JR Mackey, M Wuest, F Wuest, Expression and function of hexose transporters GLUT1, GLUT2, and GLUT5 in breast cancer – effects of hypoxia, 2018, The FASEB Journal, 32(9), 5104–5118.

UNIVERSITY OF ALBERTA

EFFICACY OF WHOLEFOODS FOR HEALTH AND CANCER PREVENTION

Cancer is a leading cause of death worldwide. With up to 70% of new cases predicted to occur in the developing world, finding affordable and effective cancer preventive agents for global use is critical. **Dr Elizabeth Ryan**, from the Department of Environmental and Radiological Health Sciences in the College of Veterinary Medicine and Biomedical Sciences at Colorado State University, has been working on precisely this. Her team's goal is to prove the disease-prevention efficacy and mechanisms for whole foods in people that have supporting evidence for protection against colon cancer in animals.



Erica Borresen, MPH, Dr Elizabeth Ryan, and Dustin Brown (graduate student) polishing rice to produce rice bran in the laboratory

From left to right: Mel Beale (graduate student), Dr Elizabeth Ryan, Bridget Baxter (Research associate/clinical coordinator), and Dr Heather Leach (collaborator and Assistant Professor of Health and Exercise Science at Colorado State University)

Why Foods for Cancer Prevention?

In rich countries, groups of patients who are deemed to be at high risk of cancer are treated with expensive and toxic drugs, including non-steroidal anti-inflammatories and tamoxifen. However, these drugs are not viable options for use in developing countries and there is a clear and urgent need for affordable and effective alternatives. There is wide recognition that consuming some types of food, more specifically including fibre-rich whole grains and legumes, is associated with a reduced risk of cancer. The American Association of Cancer Research and the World Cancer Research Fund estimate that up to an astonishing 40% of cancers are preventable by eating an appropriate diet and controlling body weight.

For global use, these foodstuffs are both safe and low cost in comparison to developing conventional cancer preventive agents, and as such, are attractive options. Dr Ryan's team have focused their attention on rice bran and navy beans to determine how effective such foodstuffs are in the prevention of cancer, and to define the mechanisms by which such effects may be induced.

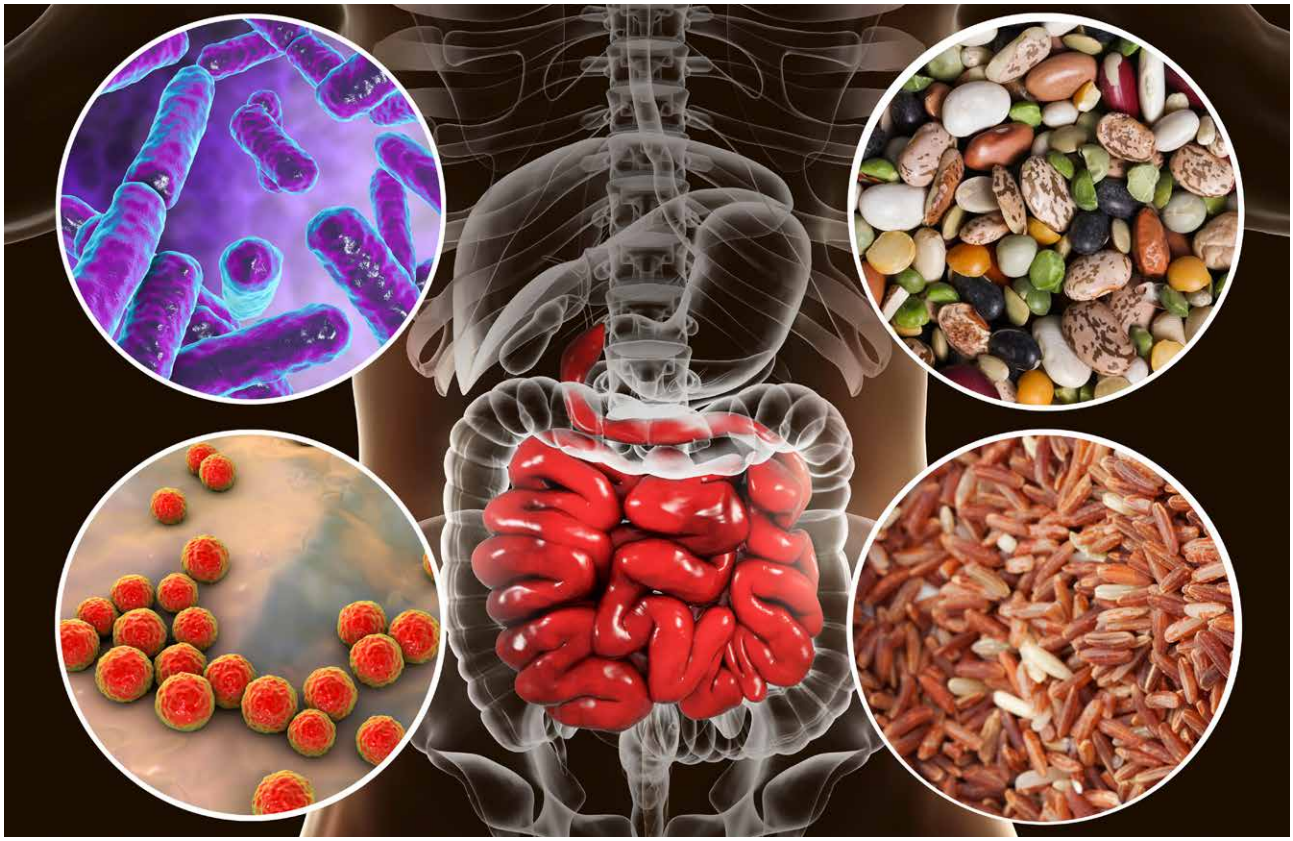
Understanding the Colon Cancer Preventive Properties of Brown Rice and Rice Bran

There are many scientific studies demonstrating that fibre from whole grains is associated with a lower risk of colorectal cancer. While brown rice is the whole grain, much of the world consumes processed white rice. This is unfortunate, as the cancer-preventing compounds have been identified in the rice bran fraction of brown rice. Dr Ryan and her team reported in their 2012 review paper that the vast majority of compounds found in rice bran have at least some published evidence supporting a cancer protective mechanism of action.

Importantly, the team's 2012 review looked at many types of cancer, including colon, gastric, breast, bladder, and liver cancer, and others. The studies were primarily based on animal models or cell cultures, rather than humans. Notably, testing in cell lines has been found to be especially useful in identifying the specific components of rice bran that produce an anti-cancer effect as well as in exploring the precise mechanisms of action.

Mechanisms of Cancer Prevention in Rice Bran Compounds

The number of potential anti-cancer compounds present in rice bran



Rice bran and beans interact with microbes to reduce cancer growth in the colon and lower the progression of disease

enhances its value for cancer control and prevention. Cancer preventive compounds can inhibit malignancy at many stages of cell proliferation and stop progression towards cancer directly or via activation of the immune system. For example, rice bran compounds were shown to stop uncontrolled growth of cells and correspondingly increase the rate of death of fast growing, preneoplastic cells. In the environment surrounding the cells, the rice bran components can also reduce the damage caused by oxidation and protect against cellular damage.

These compounds have also been shown to directly manipulate the immune system, reducing damaging inflammation and boosting anti-cancer immune cells. Furthermore, they act directly on the gut, to improve production of a protective layer, enhance beneficial bacteria growth and bacteria metabolism, and minimise harmful enzyme activities.

The number of individual components of rice bran that have been shown to have an effect on cancer development and growth is considerable and using the full fraction (rice bran) that contains a multitude of diverse phytochemical agents is likely to be more effective than extracting single compounds from the whole grain.

Cholesterol-reducing Effects of Wholefoods

Both navy beans and rice bran are known to reduce cholesterol in adults, and subsequently reduce the associated risk of cardiovascular disease. However, Dr Ryan and her team investigated the cholesterol-reducing effects of these wholefoods in children and reported on the outcomes in 2016. Children who had previously shown abnormal lipid profiles were later found to show improvements in plasma lipids following increased fibre intake with rice bran or navy beans.

The results of this pilot study showed that children tolerated the increased fibre well. While the results of blood cholesterol tests were not conclusive over the 4-week time period alone, there was an indication of improvement with regards to an increase in high-density lipoprotein cholesterol – considered the 'good' type of cholesterol in children consuming a cooked navy bean powder daily (equivalent to ~1/4 cup cooked navy beans/day). The researchers point out that longer-term work is now needed to accurately determine the effects of navy beans and navy beans combined with rice bran on cholesterol levels in children at risk for cardiovascular disease.

Post-cancer Implications of Fibre-enriched Diet

It is widely accepted that the diets of colorectal cancer patients are low in fibre, and a number of studies have demonstrated protective functions for both rice bran and navy beans. Dr Ryan and colleagues conducted a clinical trial in 2016 that identified the effects of these compounds in patients who had previously suffered from colon cancer, hoping to determine the tolerance and effect of consuming cooked navy bean power and a heat stable form of rice bran.

Over the 4-week pilot study, the team found that the participants were able to tolerate levels of fibre from both the navy bean powder and the rice bran that have been shown to prevent colorectal cancer formation. In addition, they found significant changes in levels of a number of vitamins and minerals, including iron, zinc, thiamine, vitamin B6, folate, and vitamin D.

The researchers note that the American Institute for Cancer Research does not recommend increasing intake of vitamins and minerals for cancer treatment; however, where the increase occurs as a result of improved fibre intake, this could work to enhance the benefits for both colorectal cancer patients and the general population.

While this study was fairly short in duration, the researchers found significant changes in a number of key biomarkers, indicating improvements in colon health. Finally, they concluded that a longer-term study was needed to provide definitive evidence of the benefits of consuming these whole foods to prevent reoccurrence of cancer.

Personalised Medicine – Specific Metabolite Biomarkers for Colorectal Cancer

An emerging and important area of science is personalised medicine, the ability to tailor a treatment for a patient-specific disease or condition. Scientists know that the 'same' cancer can demonstrate quite different profiles in individual patients. Dr Ryan and colleagues conducted a study in 2016 to define the biomarkers that are associated with colorectal cancer in a group of patients.

Using the emerging science of metabolomics – the study of metabolic products and chemical processes involving metabolites – they sought to define the small molecules present within samples extracted from colorectal cancer patients, from both the cancerous and nearby healthy tissue. Defining these metabolic pathway changes in colorectal cancers is vital to develop a quick, accurate, and informative diagnostic assay. In addition, this information may also help with treatment decisions.

The team identified five metabolites that are significantly increased and fourteen that are significantly lower in colorectal cancers compared to the healthy mucosal tissue. Further study of the metabolite profiles indicated a number of pathways associated with these changes. Investigating stool samples from the same patients, the team found an overlap of only seven metabolites across cancer, stool, and mucosal samples. Abberrant regulation of metabolic pathways involving amino acids and lipids were also identified in larger cohort studies in association with gut microbiome analyses.

Effect of Rice Bran on Specific Metabolites

Rice bran and navy beans were found by this team to alter the microbes and the metabolism by the microbes found in the stool, and the team proposed that consumption of rice bran or navy beans leads to a metabolite profile in the stool samples that reflects good gut health and reduced likelihood of colorectal cancer progression. At the end of a 4-week study, the team found 93 metabolites that were significantly different in patients who consumed rice bran compared to their own baseline measures and 39 metabolites which were significantly different compared to the control group at the end of the study. When navy bean powder was consumed daily, there were 30 metabolites that changed from baseline and 26 metabolites statistically significant for differences from a control group. In this group of colorectal cancer survivors, consumption of rice bran or navy beans favourably altered the stool metabolite content, indicating the benefits of reduced likelihood of cancer recurrence in these patients.

Different Types of Rice

The differences in the metabolite profiles in patients who consume rice bran or navy beans are substantial and distinct. However, given the known variation in levels of different compounds in different types of rice bran, Dr Ryan and colleagues recently conducted a series of tests to determine the metabolites present across seventeen different strains of rice that had been sourced globally. They found variation in 71 compounds across the 17 types of rice bran, including amino-acids, carbohydrates, co-factors, vitamins, lipids, nucleotides, and secondary metabolites. Of the 71 detected differences, the team was able to link 34 of them to specific rice genes.

The various metabolites found in rice bran include compounds that are anti-inflammatory, anti-oxidant, nutrient enhancing, cancer-preventing, anti-diabetic and have cardiovascular-health promoting actions. The researchers believe that understanding the genetic-basis for metabolite production across the different types of rice will inform the cultivation of plants that are optimised for specific health-giving benefits, both from a nutritional and medicinal perspective.

Further Work

Dr Ryan and colleagues ascertain that a greater focus on improving public health messages and public awareness of the benefits of wholefoods that are widely available and low cost is necessary. They also propose that continued advancement in the field of metabolomics to identify key compounds, pathways, and related genes in wholefoods, and in the cancerous and normal tissue that impact on (or change) a disease state, are critical to improve clinical outcomes for a wide range of health conditions. This team is committed to providing scientific evidence for whole foods with respect to disease prevention as the primary outcome.

Meet the researcher

Dr Elizabeth Ryan
Department of Environmental and Radiological Health Sciences
Colorado State University
Colorado, CO
USA

Dr Elizabeth Ryan received her PhD in Molecular Toxicology and Environmental Medicine from the University of Rochester in NY, USA, in 2006. After several positions at Colorado State University, she secured an associate professorship there in 2016, where she now collaborates across disciplines to fulfil her research aims focussing on food component interactions with gut microbiota and the immune system, specifically for cancer detection and prevention. Dr Ryan's research embraces a multiplatform approach, incorporating molecular testing, animal models, and human clinical trials. Dr. Ryan holds concurrent placements at the Colorado School of Public Health and the University of Colorado Cancer Center. Among many other accolades and achievements, in 2015 she won the Colorado State University Emerging Individual Interdisciplinary Scholarship Award.

CONTACT

E: e.p.ryan@colostate.edu
W: http://csu-cvmbs.colostate.edu/academics/erhs/Pages/elizabeth-ryan.aspx

FUNDING

Rice Bran Microbial Metabolism for Colon Chemoprevention 2016–2021

FURTHER READING

BA Baxter, RC Oppel, EP Ryan, Navy beans impact the stool metabolome and metabolic pathways for colon health in cancer survivors, Nutrients, 2018, 11, pii: E28.

I Zarei, et al, Comparative rice bran metabolomics across diverse cultivars and functional rice gene–bran metabolite relationships, Metabolites, 2018, 8, pii:E63.

G Dustin, ECB Brown, RJ Brown, EP Ryan, Heat-stabilized rice bran consumption by colorectal cancer survivors modulates stool metabolite profiles and metabolic networks: a randomized controlled trial, British Journal of Nutrition, 2017, 117, 1244–1256.

EC Borresen, et al, A pilot randomized controlled clinical trial to assess tolerance and efficacy of navy bean and rice bran supplementation for lowering cholesterol in children, Global Pediatric Health, 2017, 4, 2333794X17694231.

EC Borresen, et al, A Randomized controlled trial to increase navy bean or rice bran consumption in colorectal cancer survivors, Nutrition and Cancer, 2016, 68, 1269–1280.

DG Brown, et al, Metabolomics and metabolic pathway networks from human colorectal cancers, adjacent mucosa, and stool, Cancer & Metabolism, 2016, 4, 11.

AJ Henderson, et al, Chemopreventive properties of dietary rice bran: current status and future prospects. Advances in Nutrition, 2012

UNDERSTANDING CANCER DEVELOPMENT IN HUMANS AND THEIR COMPANION ANIMALS

Dogs are renowned for their status as man's best friend. Based first at the University of Colorado and now at the University of Minnesota in the Twin Cities, **Dr Jaime Modiano** and his team have spent the last 25 years trying to understand how cancer develops at a basic level, aiming to use this knowledge to improve the health and wellbeing of both humans and their companion animals.

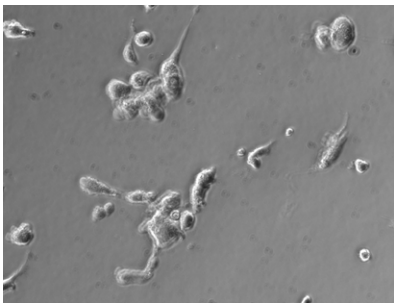
Dogs as Models for Cancer Research

Throughout human history, dogs have helped us to hunt and care for our livestock. Not only are dogs widely accepted as being the first domesticated animals but they also quickly became our most favoured companions. Today, many dog breeds are the result of breeding for desirable characteristics, such as a particular colouring or size, in addition to their historical roles of hunting, herding, and protection. As a result, many breeds have been developed under intense artificial selection, often drawing from a small pool animals.

Dog breeders are starting to recognise problems with this approach: while the process has produced specific breeds with particular characteristics, it has also led to serious health concerns, such as increased susceptibility to many genetic diseases and possibly certain cancers. Curiously, some types of cancer seem to be more prevalent in a few breeds.

Dr Jaime Modiano and his team at the University of Minnesota in the Twin Cities, USA, focus on trying to decipher the mechanisms associated with cancer development by comparing several malignant diseases in humans, dogs, and laboratory animals. 'Recognising that the full spectrum of malignancies seen in humans also occurs in dogs sets an ideal stage for dual-purpose research that can accelerate progress against these diseases in both species,' explains Dr Modiano.

Findings from Dr Modiano and his team converge with those from other researchers to suggest that it is now time to take things a step further by looking at dogs to identify new and perhaps better ways to treat humans. 'We are faced with a unique opportunity to advance our understanding of similar diseases in unrelated species, and specifically those that might have a link to the evolutionary origin of these species, by using comparative genomics,' proposes Dr Modiano. 'The answers we obtain by studying cancers in dogs will contribute to our ultimate goal of designing strategies to prevent and treat cancer in both dogs and people.'

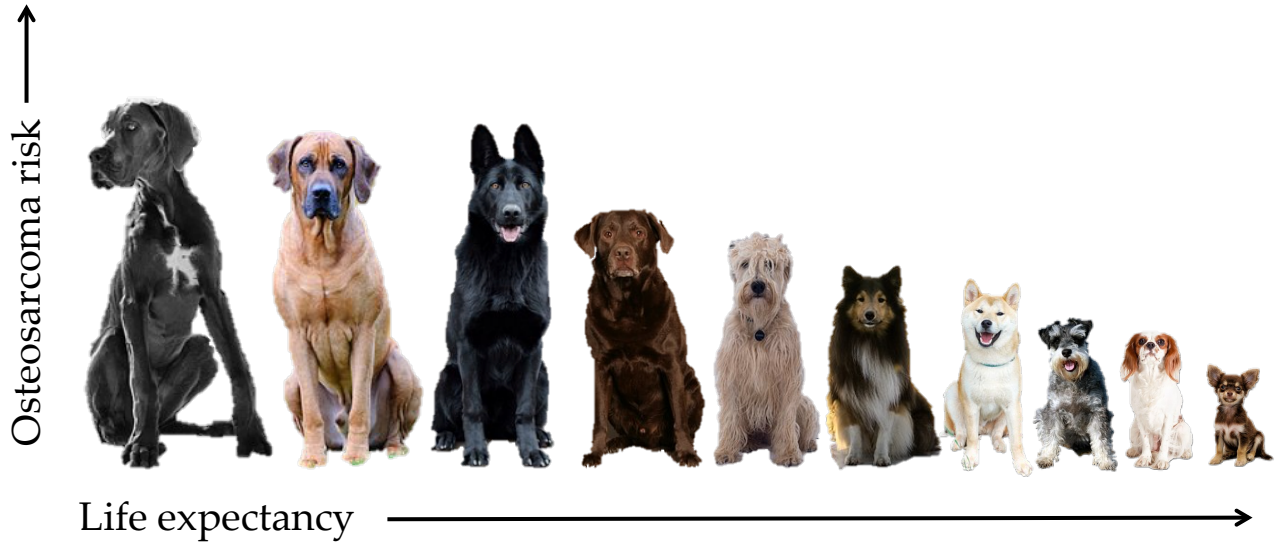


Cancer cells in culture – phase contrast image, credit Jaime Modiano

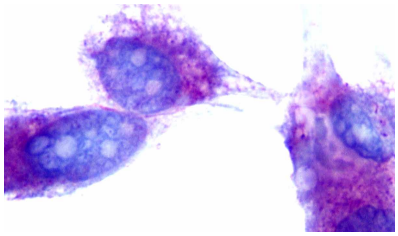
Similarities in Dog and Human Cancers

At first, dogs seemed to provide the perfect model by which to study human cancer for Dr Modiano and his team. As animals that share our homes, dogs make it possible for us to examine the intrinsic risk factors for the development of cancer within two species sharing a common environment. In addition, dogs have shorter life spans, and, as up to four or five generations can exist at the same time, they provide unique opportunities to address critical questions about the origins and behaviours of cancer. 'Our original premise was that there would be highly conserved or even identical genetic factors that could cause cancer in humans and dogs, but we later found that this was only rarely the case,' notes Dr Modiano.

‘Our original premise was that there would be highly conserved or even identical genetic factors that could cause cancer in humans and dogs, but we later found that this was only rarely the case.’



Risk of osteosarcoma and life expectancy are associated with body size in dogs. Large and giant breed dogs generally have shorter lifespans and an increased risk of osteosarcoma compared with smaller breed dogs.
Credit Kelly Makielski and Aaron Sarver



Cancer cells in culture – protein stain image, credit Jaime Modiano

One of their initial approaches, conducted in close collaboration with Dr Matthew Breen at North Carolina State University in Raleigh, USA, involved looking at certain regions in the canine genome that were related to regions in the human genome and which, when scrambled from their original order and rearranged to come together into new, abnormal conformations, are known to cause lymphoma and leukaemia (blood cancers) in humans and mice.

Drs Breen and Modiano identified peculiar similarities in certain lymphomas and leukaemia between dogs and humans. These findings led them to suggest that some cancer risk might trace back to primordial similarities that preceded the evolutionary separation of both species.

However, it also was possible that risk was attributable to random errors in the process of cell division. In this case, cancer would happen because the alteration in the order of critical genes would provide cells a strong survival advantage over its neighbours, setting the stage for more and faster cell divisions, additional mutations, and eventually, uncontrolled proliferation and cancer. Either of these mechanisms could explain the mutations shared between certain types of cancer in dogs and humans.

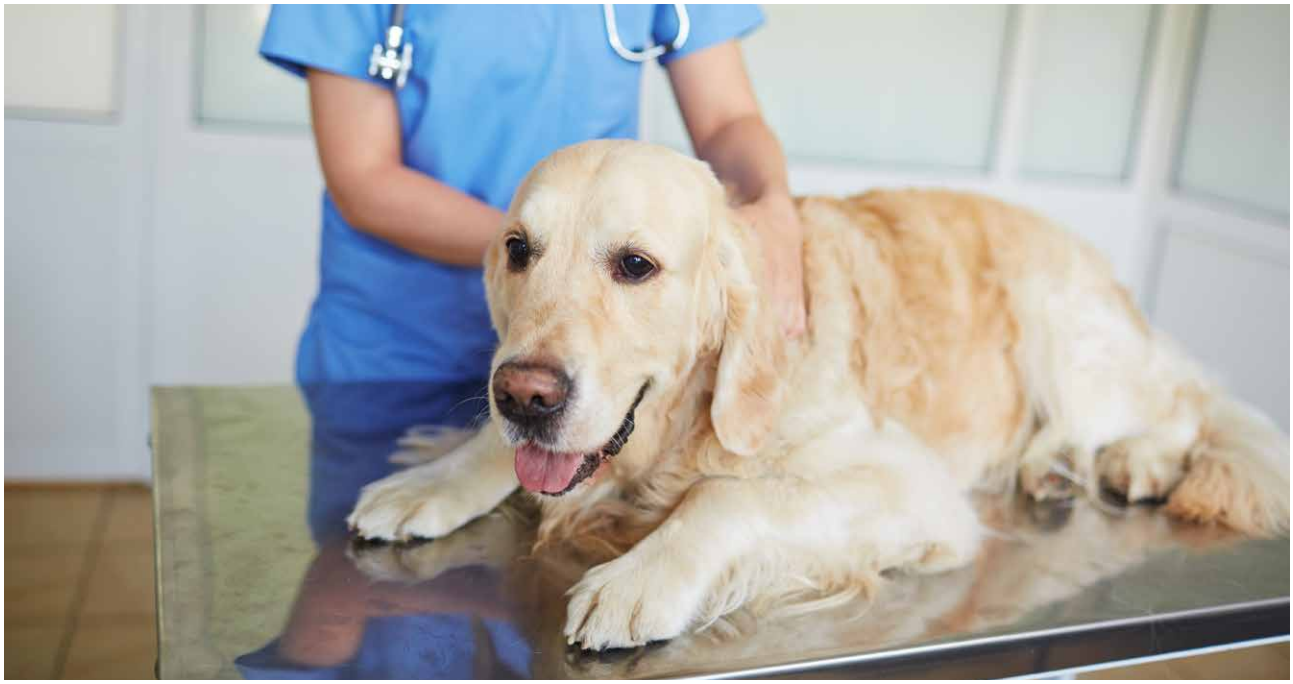
An Unexpected Twist

The trend for finding similarities between dog and human cancers was growing stronger with every study, until early 2011, when Dr Modiano and his colleagues happened upon some unexpected results. The original plan was to categorise dog lymphomas using the same parameters used for human cancers. The research team identified three groups based on prognosis, ranging from slow progression, typically treated with low-intensity chemotherapy, to aggressive and rapidly progressing tumours with a poor response to treatment.

However, some odd results emerged in this work that did not match the patterns of gene expression characteristic of human lymphomas. ‘Despite other groups publishing data that they interpreted as supportive, our own data did not agree,’ emphasises Dr Modiano. The exact reasons to explain this disparity were difficult to pinpoint. One explanation could be the large natural variability in this type of cancer, or perhaps the canine version refers to a single subtype of the cancers found in humans, therefore masking the distinction between subtypes. Another explanation could be that dog and human variants of the cancer are convergent forms of similar diseases; that is, being of different cellular origin but having other shared features.

Findings arising from Dr Modiano’s long-standing collaboration with Dr Kerstin Lindblad-Toh (at the Broad Institute of the Massachusetts Institute of Technology and Harvard University in Cambridge, USA), and with Dr Breen, posed further critical questions. The researchers observed mutations in three breeds with a high risk of developing lymphomas – boxers, cocker spaniels, and golden retrievers. As Dr Modiano

‘The data suggested that heritable causes were not the singular explanation for the similarities and differences in the cancers observed among different breeds of dog.’



explains, ‘The data suggested that heritable causes were not the singular explanation for the similarities and differences in the cancers observed among different breeds of dog.’ Moreover, with very few exceptions, the recurrent mutations found in canine lymphomas did not match mutations present in human cases of the same cancer.

Dr Modiano elaborates that, ‘We now know that a major similarity between dogs and humans is that they have both broken the evolutionary constraint on longevity – or life-expectancy – and so the risk of cancer that is attributable to chance (which accounts for the vast majority of tumours) is comparable.’ Simply stated, people and dogs are living longer, so both are at greater risk of getting tumours. In both species, mutations are introduced during the normal processes of DNA replication and DNA repair, but the actual mutations that provide a selective advantage for the tumours to grow are quite different.

A Different Perspective

On the basis of this work, Dr Modiano and his colleagues arrived at a critical, new perspective. ‘Looking at the data more broadly allowed us to become a bit more realistic about the role of the dog as a model,’ explains Dr Modiano, ‘and we started seeing cancer in both humans and dogs as the consequence of events associated with longevity.’

By leading to higher levels of cell division, more DNA damage, and a higher probability to accrue the mutations that result in cancer, the longevity that we may justifiably consider a blessing comes at a cost, according to Dr Modiano. Animals that have a naturally long life, such as elephants, whales, and mole rats, for example, have developed ways to protect themselves against cancer. Humans and their pets, however, have expanded their evolutionary life spans several-fold in only a relatively short space of time. This may well explain the higher rates of cancer as we get older, as such a rapid increase in relative longevity is uncommon in the animal kingdom. For cats and dogs, the usual process of slow natural selection has been replaced by an accelerated artificial selection.

The problem with this artificial selection is that it does not allow natural processes to select for animals adapted to the new longevity. Natural selection is driven by interactions with the whole social structure of the species and the environment, and this has happened for every species independently. Our dogs and cats evolved to live to about four and eight years old, respectively, but now live to around 10 and 20 years old.

‘We have underestimated the significant role evolution has played in selecting for cancer-protective mechanisms across the animal kingdom,’ concludes Dr Modiano, ‘and we have significantly under-appreciated the importance of how our success in outliving our evolutionarily determined lifespan has greatly influenced the risk for cancer in our species.’ Nevertheless, in a positive vein, Dr Modiano proposes that the scientific community should not see cancer as inevitable but should now focus on developing ways to delay or even prevent its development both in humans and in our companion animals.

Meet the researcher

Dr Jaime Modiano
College of Veterinary Medicine and Masonic Cancer Center
University of Minnesota – Twin Cities
Minneapolis, MN
USA

Dr Jaime Modiano completed both clinical veterinary training and a PhD in Immunology at the University of Pennsylvania, followed by a residency in Veterinary Clinical Pathology at Colorado State University and a post-doctoral fellowship at the National Jewish Center for Immunology and Respiratory Medicine. After a brief period at the Texas A&M College of Veterinary Medicine and eight years at the AMC Cancer Research Center and the University of Colorado Health Sciences Center, Dr Modiano joined the University of Minnesota in 2007. He currently holds the prestigious Alvin and June Perlman Endowed Chair of Animal Oncology and is the Director of the Animal Cancer Care and Research Program of the College of Veterinary Medicine and the Masonic Cancer Center at the University of Minnesota.

CONTACT

E: modiano@umn.edu
W: http://www.modianolab.org/

KEY COLLABORATORS

Dr Aaron Sarver, University of Minnesota (USA)
Dr Kerstin Lindblad-Toh, Broad Institute of MIT and Harvard (USA) and Uppsala University (Sweden)
Dr Matthew Breen, North Carolina State University (USA)

FUNDING

National Cancer Institute/National Institutes of Health (USA)
United States Army Medical Research and Materiel Command/ Peer Reviewed Cancer Research Program
Morris Animal Foundation
AKC Canine Health Foundation
The Zach Sobiech Fund of the Children's Cancer Research Fund
Animal Cancer Care and Research Program, University of Minnesota
The V Foundation for Cancer Research

FURTHER READING

K Megquier, J Turner-Maier, R Swofford, et al, Genomic analysis reveals shared genes and pathways in human and canine angiosarcoma. bioRxiv, 570879, doi:

KM Makielski, LJ Mills, AL Sarver, et al, Risk factors for development of canine and human osteosarcoma: A comparative review, Veterinary Sciences, 2019, 6, E48.

MC Scott, NA Temiz, AE Sarver, et al, Comparative transcriptome analysis quantifies immune cell transcript levels, metastatic progression, and survival in osteosarcoma, Cancer Research, 2018, 78, 326–337.

I Elvers, J Turner-Maier, R Swofford, et al, Exome sequencing of lymphomas from three dog breeds reveals somatic mutation patterns reflecting genetic background, Genome Research, 2015, 25, 1634–45.

D Ito, AM Frantz, JF Modiano, Canine lymphoma as a comparative model for human non-Hodgkin lymphoma: recent progress and applications, Veterinary Immunology and Immunopathology, 2014, 159, 192–201.

BH Gorden, JH Kim, AL Sarver, et al, Identification of three molecular and functional subtypes in canine hemangiosarcoma through gene expression profiling and progenitor cell characterization. American Journal of Pathology, 2014, 184, 985–995

AM Frantz, LA Sarver, D Ito, et al, Molecular profiling reveals prognostically significant subtypes of canine lymphoma, Veterinary Pathology, 2013, 50, 693–703.

M Breen, JF Modiano, Evolutionarily conserved cytogenetic changes in hematological malignancies of dogs and humans--man and his best friend share more than companionship. Chromosome Research, 2008, 16, 145–54.

UNIVERSITY OF MINNESOTA

HEALTHCARE, SCIENCE AND SOCIETY

HEALTH AND SCIENCE: STEPPING BEYOND THE LABORATORY

Our health is intrinsically linked to our environment, lifestyle, and behaviours. In this final section, we showcase research highlighting the importance of understanding our physical, psychological and social well-being outside the tightly controlled constraints of the laboratory. From informing policy to understanding how our diet, lifestyle choices, and even the air that we breathe affects our health, we now meet the researchers whose research is very firmly rooted in the real world.

The air that we breathe is, of course, critical for our survival. In addition to oxygen and other chemicals, we also inhale a complex mixture of volatile organic compounds (VOCs), the impact of which is poorly understood. We open this section by meeting Dr Johanna Gostner at the Medical University Innsbruck in Austria and read how she is driving forward our understanding of the complex molecular impact of VOCs on our lungs and health.

The impact of the external environment on our health is also the focus of Dr Lei Cao's research at Ohio State University, USA. Dr Cao is demonstrating how critical the complicated interactions between our environment and our internal biochemistry are for our wellbeing. We read how an enriched, stimulating environment can bring a surprising number of benefits, not only for our activity levels and weight loss, but may even help reduce the risk of cancer.

Obesity and the associated onset of chronic diseases such as Type 2 diabetes and heart disease is the major public health problem that Professor Douglas Goff from the University of Guelph in Canada is seeking to address. In addition to lifestyle factors such as exercise, the supplementation of food with fibre may help reduce the risk of individuals developing such conditions. We read how Professor Goff is working to identify the underlying beneficial mechanisms of dietary fibre when added to food, and how best to achieve this supplementation for greatest benefit.

Taking an alternative perspective, we then turn to the work of Dr Mark D. Hayward at the University of Texas in Austin

and Dr Jennifer Karas Montez at Syracuse University, who are investigating health-related differences across America's states. We read how Dr Hayward and Dr Karas Montez are working to achieve better health for all American citizens. Their proposed policies include ensuring equal access to quality education and initiatives aiming to overcome problems associated with tobacco and substance use.

Substance use is also the focus of research by Professor Patricia Erickson at the University of Toronto and Professor Andrew Hathaway of the University of Guelph. Cannabis is by far the most widely used illicit drug across the globe, and in recent years, several countries have moved towards decriminalisation. Through exploring the attitudes and practices of users and non-users in this changing context, we read how Professor Erickson and Professor Hathaway are providing much-needed insight into the risks and benefits of decriminalisation for both individuals and society more broadly.

We conclude this final section by meeting Dr Stephen Lane at Stargates Inc, USA. A trip to the hospital is for many patients, an unwanted necessity but in addition to the required medical treatment, the cross-contamination of infections between healthcare workers, the environment, and patients is a growing and global concern to public health. We read how potentially fatal hospital-acquired infections are being reduced by Dr Lane's novel computer-based system involving voice reminders to increase patient hand hygiene in hospitals.

SOMETHING IN THE AIR TONIGHT

The air around us contains a complex mixture of volatile compounds, to which we are inevitably exposed with largely unknown effects on our health. Leading the way in identifying the molecular consequences of such exposures is **Dr Johanna Gostner** of the Medical University Innsbruck in Austria.

Volatile Organic Compounds

Take a deep breath. Now another. Now through your nose. What have you just inhaled? Oxygen, obviously, and a lot more nitrogen – carbon dioxide too. But you've also inhaled an endless number of other chemicals, such as volatile organic compounds (VOCs), nitric and sulphur compounds, polycyclic aromatic hydrocarbons (PAHs), and numerous particles too. Some of these chemicals have short- and long-term effects on human health, and some even exacerbate climate change.

But what exactly are volatile organic compounds? 'Organic' simply refers to the fact that they are carbon based, 'volatile' indicates that such liquids have a lower boiling point than non-volatile substances – meaning they will more readily evaporate into the air and float around as a gas.

Volatile organic compounds, or VOCs, are extremely varied in their sources and attributes – perfumes rely upon VOCs to provide their scents, plastics release VOCs into the air (think of that 'new car smell'), cigarette smoke contains many different VOCs. Many VOCs inside the house are released from paints, carpeting, building materials or furnishings. While for some VOCs there are regulatory threshold or guideline values, many others are not regulated. However, many volatile substances also come from natural sources such as microbes, plants and animals – where VOCs play important roles in facilitating communication between and among species.

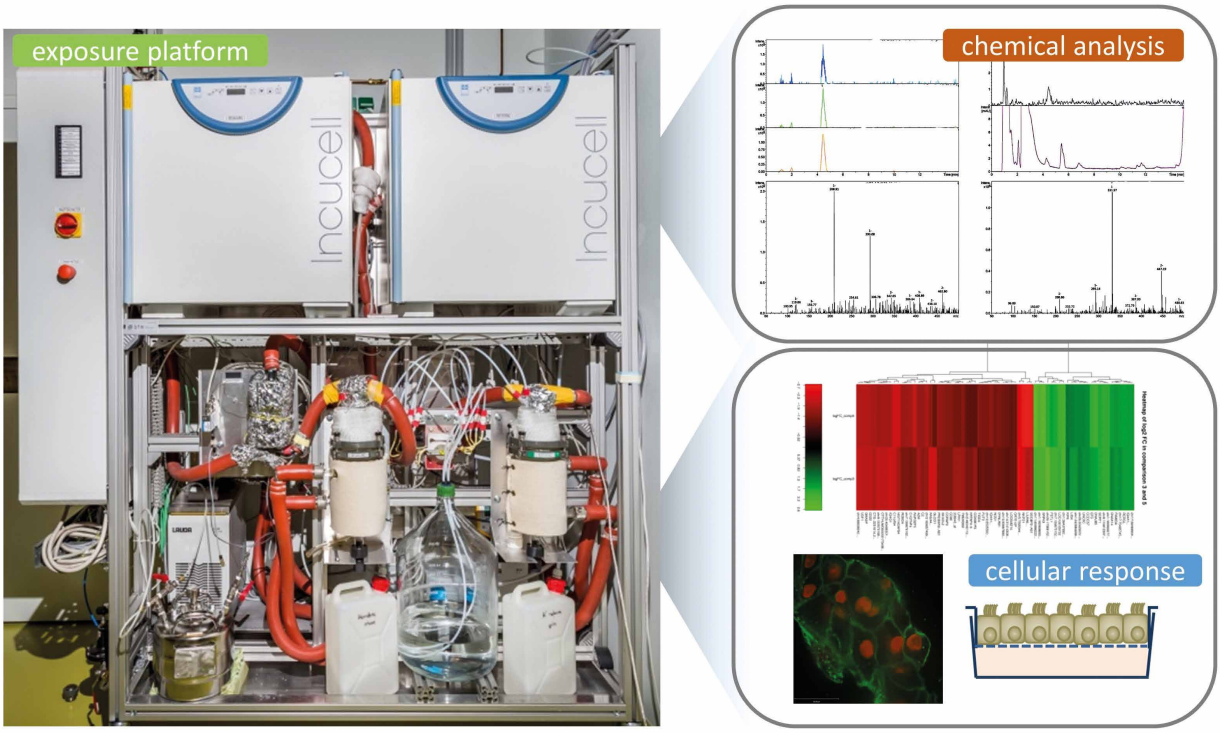
The mean surface of the adult human lung is approximately 140 square metres in size – and compounds that pass the 'alveolar barrier' in the gas-exchange region of the lung have direct access to the circulatory system. Important questions are whether inhaled VOCs are biologically active, whether exposure can lead to stress or adaptation, and whether some might even promote healing and reduce inflammation. Indeed, not all VOCs are harmful if present under controlled conditions, and many are actually used for therapeutic purposes – just think about anaesthetics or aromatherapy.

One difficulty in assessing the health effects of indoor VOCs is that often the exposure occurs at very low concentrations, but over longer periods. Currently, most chemical testing focuses on immediate effects and acute toxicity, allowing the effects of chronic exposure to slip through the cracks. For example, one may become sensitised by VOCs long before any obvious signs of allergy arise. There is thus an urgent need to better understand the biological processes activated by VOCs and to depict the molecular pathways that are associated with potential health consequences.

Breathing Without Lungs

This is where Dr Johanna Gostner of the Medical University of Innsbruck, Austria comes in. Her work revolves around cellular signalling pathways, and the manner in which they can be used to study the biological effects of VOCs. Their most recent focus is on, as she explains, 'assessing the mode of action of VOCs *in vitro*, which means at the molecular level in cell culture models.

‘With our exposure platform, we are the first to provide a stable atmosphere for lung cell exposure to low VOCs concentrations over long time. It is great that we can do this for days without impairing cell viability.’



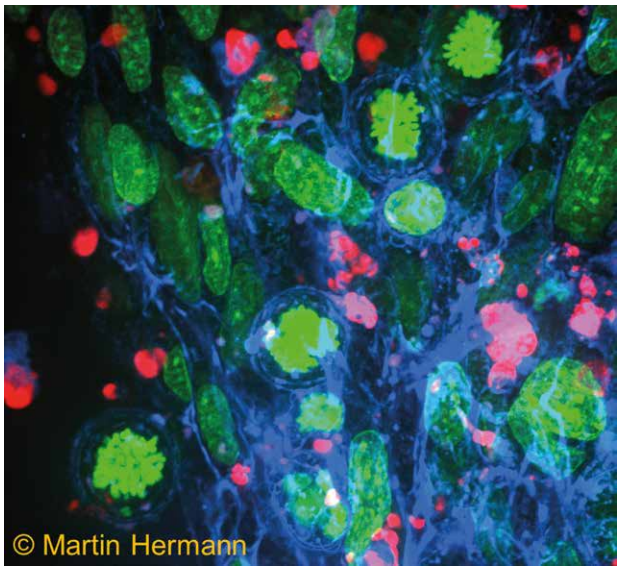
We are using air–liquid interface cultures of lung cells, and a culture system that enables airborne exposure to defined concentrations of volatiles.’

So, what does this mean? Dr Gostner’s team makes use of a method whereby VOCs can be brought into contact with cultured human lung cells. The lung cells are cultivated in a way that they have their upper-side exposed to the atmosphere, while nutrients can be taken up from the bottom side, as the cells growth on a porous membrane (this is called ‘air–liquid interface (ALI) culture’). Thus, in comparison to the usual way of culturing cells (so-called ‘submerged cultures’), where cells grow attached to a plastic surface and are covered by the feeding medium, there is no interfering liquid at the gas contact site. ‘This is important, as any liquid may act as a barrier that changes the reactivity of the compounds, or compounds that are insoluble in water would not be able to access the cells,’ Dr Gostner says.

Her team’s initial work investigated the common indoor air pollutant formaldehyde, a compound often released from wood-based materials, carpets or tobacco smoke, for example. Formaldehyde is a regulated and monitored airborne pollutant, due to its wide usage, and a wealth of literature is available on its health-related effects in different tissues, organs and organisms. However, until now, it had not been possible to understand what molecular changes (if any) occur in cells when formaldehyde is present at very low concentrations.

The first problem Dr Gostner’s team needed to solve was a technical one, and it involved finding a way to actually bring airborne formaldehyde into contact with the cells. ALI cultured, living lung cells are not protected from dehydration, and passing a VOC-loaded gas stream over the cells will lead to the liquid evaporating (and thus the cells being stressed or even dying), which has limited previous attempts to a few hours of exposure at most. Evaporation can be minimised by increasing the humidity of the gas stream, as is done with inhaled air in the lung. However, this technique can often lead to condensation, which is particularly problematic when the substances of interest are readily soluble in water. The team dedicated a significant amount of time to develop a solution to this problem. In the end, they managed to construct a system that ensured the cells would be exposed to a constant level of VOC constant humidity, and would stay alive for at least three days of testing – an impressive improvement over previous systems.

The second problem was to determine the biological endpoint – a variable



that would indicate if the VOC had any effect on the cells. Compounds such as formaldehyde are known to have both specific activities (affecting biological signalling pathways) and non-specific activities (binding to any protein it comes across – this is the source of its use as a tissue fixative in histology). This range of effects means that testing individual biological variables may miss important information provided by others. To solve this problem, the group decided to perform whole-genome transcriptional analysis – this examines the RNA that provides the blueprint for protein production and thus shows the cellular reaction to its environment. Being a broad, non-biased approach, transcriptional analysis provides large amounts of data that can be used to chase down even peripheral effects.

Using this screening method, Dr Gostner and her group attempted to answer the question of whether it is possible to detect relevant responses and whether potential biomarkers could be identified. 'With our strategy, we can now measure not only acute but also chronic responses – which is important when trying to understand the relevance of molecular changes associated with the development of disease symptoms, especially those correlated with poor air quality such as asthma, bronchitis or sick building syndrome,' says Dr Gostner.

Exposure to very low concentrations of formaldehyde (0.1 parts per million – comparable to two glasses of wine dissolved in an Olympic swimming pool) led to over 300 changes in gene expression in the cells. Not just one, but a multitude of pathways were affected – the studies showed that VOC effects are more varied and complicated than was previously suspected. Though any health-related interpretation of the obtained results of this first proof-of-principle study remains a challenge, the team clearly showed that concentration did indeed have biological effects. In further experiments, they could even show that the cellular responses were dose-dependent.

A major advantage of the approach developed by Dr Gostner is the ability to monitor the activity of low doses of VOCs over long time periods, something that was previously very difficult to do. Using the team's approach, the mechanisms of action of many therapeutic VOCs can also be analysed. It would also be interesting to investigate what cellular changes are induced by VOCs contained in beauty products and perfumes. This method is ideal for mimicking real life situations, and thus provides a better understanding of what is harmful, or what harmful actually means in terms of cell signalling.

Exhale! Improve!

No testing method is perfect, and no testing method can completely replicate the vast complexity of the human response to any chemical. One limitation is that the technique pioneered by Dr Gostner currently utilises immortalised cell lines – a standard approach in biomolecular and toxicological research. Immortalised cell lines are often less sensitive to damage than normal human cells in the body, and thus, results need to be considered with caution.

Work is currently underway to improve the team's technique, with the aim of using primary cell populations from human donors in order to more accurately replicate actual body conditions. This process is naturally more complex and thus could perhaps be considered a follow-up diagnostic approach to the cell-culture VOC screening technique.

A further challenge is the low level of information available on the pathways involved in VOC toxicity – particularly at low concentrations. As Dr Gostner comments, 'A limitation for investigations of biological functions and processes with low-dose treatments is that molecular interactions recorded in databases were typically characterised in diseased stages or situations of stress. Only a few pathways are also described in a low-level context where more subtle responses become important.'

Where does the team go from here? The biological response to VOCs is a many-faceted one, complicated by the multitude of cells and cellular signalling pathways involved. Preliminary work has identified a number of tantalising possibilities, but many studies remain to be performed before these options can truly be chased down. It is very apparent, however, that Dr Gostner and her colleagues will be at the forefront of this upcoming research.

Meet the researcher

Dr Johanna M. Gostner
Biochemical Immunotoxicology Group
Division of Medical Biochemistry Biocenter
Medical University of Innsbruck
Innsbruck
Austria

Dr Johanna Gostner began her studies in genetics and biotechnology at the University of Salzburg, Austria. She then moved to the renowned Max Planck Institute in Munich, Germany, to carry out her Master's studies and then moved to Innsbruck for her PhD at the Tyrolean Cancer Research Institute. Later on, while expanding her knowledge in chemistry, she joined the Division of Medical Biochemistry at the Medical University of Innsbruck as a postdoctoral fellow. Here, she worked in the field of biochemical and immunotoxicology, with a particular interest in signalling processes that are affected by external factors such as diet and pollution. In 2015, she expanded her expertise through a stint at the research powerhouse of Imperial College London, from where moved back to Innsbruck to build up an independent junior research group, supported by the prestigious Hertha-Finberg fellowship for career development of the Austrian Science Fund. Dr Gostner is author of more than 50 original articles and has been awarded several prizes, including the Blair-Curtius-Pfleiderer-Wachter Award for Pteridine Research and the ISTRY Young Investigator Award. In 2019, she habilitated in biochemistry at the Medical University of Innsbruck.

CONTACT

T: (+43) 512 9003 70122
E: johanna.gostner@i-med.ac.at
W: https://www.i-med.ac.at/imcbc/staff_doc/johanna_gostner.html

ACKNOWLEDGEMENT

The funding support by the Austrian Science Fund (T703), the Medical University (MUI-START 2014-05-023) and the Austrian Promotion Research Agency (FFG 834169) is gratefully acknowledged.
Thank you to all enthusiastic expert scientists who became involved in my research activities throughout the past years, without them none of the described achievements would have been possible.

FURTHER READING

JM Gostner, J Zeisler, MT Alam, P Gruber, D Fuchs, K Becker, K Neubert, M Kleinhappl, S Martini, F Überall, Cellular reactions to long-term volatile organic compound (VOC) exposures, Science Reports, 2016, 6, 37842.

MEDIZINISCHE UNIVERSITÄT
INNSBRUCK

THE IMPACT OF OUR ENVIRONMENT ON OUR WELL-BEING

The human body is a bewildering set of interacting systems, a complex web of signals and pathways which are constantly adjusting to the conditions which we find ourselves in. Ground-breaking research by **Dr Lei Cao**, of Ohio State University, USA, is providing new insights into the impact of our environments on our health. Read on to learning about how an interesting environment leads to fat loss and even protects against cancer.

The Outside World and Our Inner Physiology

Whether it be the outside temperature, our blood sugar levels, or even the time of day, our bodies measure, assess, and interpret these factors in order to provide the optimum response. Yet this complexity goes even further. Recent research has shown that even seemingly unconnected factors such as living conditions and social contact can lead to measurable changes in the activity of our brains and the biochemistry of our bodies – with remarkable impacts on our health.

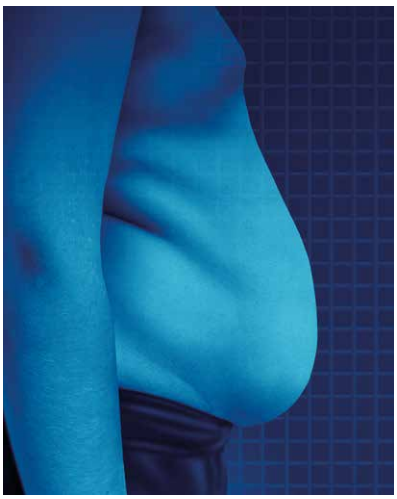
One researcher working in this field is Dr Lei Cao of Ohio State University, USA. As Dr Cao notes, her work has covered a broad range of topics and discoveries that is showing how 'lifestyle can efficiently influence brain activity, and how these changes in the brain interact with other systems both at the molecular level and at the systemic level, influencing metabolism and various diseases including obesity, diabetes, and cancer.' Alongside her network of collaborators, Dr Cao has identified a number of interlocking systems which connect the outside world to our inner physiology.

Many of these systems involve a protein known as BDNF, a conveniently short acronym for the significantly-lengthier term 'brain-derived neurotrophic factor.' BDNF is a protein which is manufactured widely in the brain including the hypothalamus, a small part of the brain which is responsible for secreting a number of hormones and thus (via various intermediary steps) controlling things such as temperature, hunger, and sleep. BDNF itself is involved in several of these pathways, though it is most commonly associated with the control of metabolism.

Fat Mouse, Skinny Mouse

A number of scientific studies have linked BDNF to the control of our metabolism and energy balance. A lack of BDNF leads the body to begin storing energy as sugars and fats, leading to obesity and hyperglycaemia. By contrast, an increase in BDNF levels leads to a reduction in hunger, increased energy use, and thus, weight loss. Interestingly, this occurs even when the protein is injected, making it an interesting target for clinical researchers.

Dr Cao and her research group wanted to test the effectiveness of BDNF in combating obesity. To do this, they used mouse models of the disease, that is, ones that had been genetically



modified to lack a receptor protein, and which as a result gained vast amounts of weight. The gene encoding BDNF was then transferred into the mice, where it then caused the protein to be produced. This was, however, slightly more complicated than it first seemed. Continuously producing the BDNF protein would lead to weight loss but would lead to *continuous* weight loss – eventually overshooting the healthy ideal and leading to muscle wasting and weakness.

‘Given the increased cancer risk associated with obesity and insulin resistance, this patented technology might have therapeutic potential beyond genetic forms of obesity.’

To avoid this problem, the researchers created a dual-effect genetic vector. In Dr Cao’s words, ‘We have developed a built-in auto-regulatory system to control therapeutic gene expression mimicking the body’s natural feedback systems.’ How was this achieved? One part of the vector brought the BDNF gene into the body, raising levels of the protein, and thus driving weight loss. The other part produced a microRNA, a small fragment of nucleic acid matching part of the BDNF gene. These fragments trigger the viral-protection mechanisms within the cells of higher animals. These mechanisms track down and destroy all RNA sharing the same sequence as that fragment. Although complex, this gene silencing process is a reliable way to allow researchers such as Dr Cao to turn a gene ‘off,’ preventing the production of proteins such as BDNF.

It may appear somewhat foolish to continuously express a gene only to destroy it directly afterwards. However, the activity of the microRNA portion can be tuned separately, for example, by placing it under the control of a system which is activated when losing weight. As the researchers showed, this causes BDNF to be produced in high levels by obese mice, but in steadily-decreasing levels as they lost weight. This led to a quick initial drop in body-weight but then a gentle glide down to a sustainable and healthy plateau. In other words, the self-regulatory vector was able to create a change in obese mice which mimicked the normal, healthy weight-loss process.

Importantly, this is not the only use for such a vector. As Dr Cao comments, ‘given the increased cancer risk associated with obesity and insulin resistance, this patented technology might have therapeutic potential beyond genetic forms of obesity.’

Gently Browning the Fat

Another area in which BDNF plays a role is that of environmentally-triggered changes to obesity. It has long been known that an environment full of interesting things to see and interact with (referred to simply as an enriched environment) leads to an increase in mental ability and a corresponding improvement in overall health. This requirement for enrichment is commonly found from mice to humans, and research by Dr Cao and her collaborators had previously shown that mice living in an enriched environment tended to be leaner than those in standard cages, despite being fed identically.

The relationship between weight loss and metabolism is complex and highly-interconnected, with much centring on the actions of two types of fat-containing tissues. Known as white adipose and brown adipose tissue, they have significant differences in their physiological roles. Brown adipose tissue is capable of ‘uncoupling’ the usual cycle involved in producing cellular energy from stored fats. This leads to the production of heat, and acts as an important mechanism in maintaining temperatures in cold conditions – particularly for babies and young children. Uncoupled energy use is particularly interesting for scientists as it burns a lot of energy very quickly – making it a potential target for weight-loss drugs.

The researchers were able to show that mice in enriched environments had higher levels of brown adipose tissue. As well as having pockets of cells within the white tissue which were ‘browning,’ they were starting to exhibit typical features associated with brown fat. This was occurring in response to a complex set of molecular signals, themselves triggered by metabolic-mediating signals from the hypothalamus. One of these signals was BDNF, and the research group was able to show that increased expression of the protein was able to reproduce the effects seen in

‘We have expanded this work to several solid tumour models including colon cancer, breast cancer, pancreatic cancer, lung cancer, melanoma, and glioma. In addition, we have observed a robust anti-leukaemia effect of environmental enrichment.’

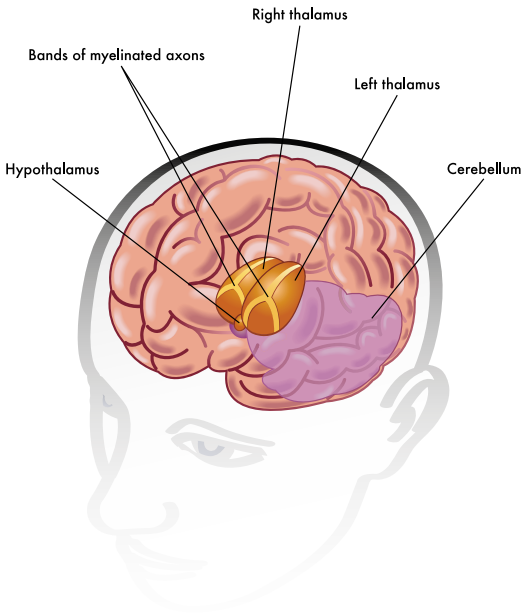


Illustration of the Hypothalamus in the Human Brain

enriched mice – a general shift towards brown adipose tissue and a subsequent loss of weight. Inhibiting this BDNF signalling reversed the effect, showing just how vital the protein was for the process.

In other words, Dr Cao’s research group had successfully demonstrated the existence of a new connection in the metabolic web. Interesting, enriched environments triggered the production of BDNF within the hypothalamus, which in turn led to ‘browning’ of the fat-containing tissue and subsequent weight loss.

Enriching Lifestyles

The role that an enriching environment plays is surprisingly varied, affecting a number of factors related to our overall health. Dr Cao and colleagues have shown that the presence of an enriching environment is not only linked to metabolic activity and weight loss but may also help reduce the risk of cancer.

Cancer is a disease in which the microscopic environment of the tumour cell has incredibly varying effects on the overall progression of the disease. Although many researchers have focused on the cellular factors which drive cancer progression, few have examined the role of the overall environment – the social and living space in which the patient lives.

Dr Cao’s group first looked at the growth of implanted tumours in mice living in enriched or normal environments. They discovered that the mice living in enriched environments had reduced tumour size, growth rates, and progression to further stages. This was rather surprising, and so the group then attempted to determine the molecular mechanisms behind this.

Further experiments using cultured cells grown in bottles showed that blood serum from enriched mice was also able to inhibit cancer cell growth outside the body. At the same time, the serum showed a significantly decreased level of the signalling molecule leptin. This hormone has a number of different roles but one of the major ones is to control metabolism, acting via signalling in the hypothalamus.

You may recall that BDNF is produced in the hypothalamus, and indeed, the researchers were able to show again that BDNF was more abundant in those mice living under enriched conditions. Further genetic experiments were able to show that increasing expression of BDNF protected against tumour growth, with high-BDNF mice having slower tumour growth even when living in standard conditions. Even more convincingly, removing BDNF removed the tumour-reducing effect seen when living under enriched conditions.

Based on this information, the researchers were able to conclude that BDNF plays a crucial role in allowing the outside environment to improve disease outcomes and reduce the progression of cancer.

Striding Forward

Research by groups such as Dr Cao’s has only begun to scratch the surface of the interactions between the environment and our internal biochemistry. The researchers intend to expand on these initial discoveries, notably within the field of tumour prevention. Dr Cao notes, ‘We have expanded this work to several solid tumour models including colon cancer, breast cancer, pancreatic cancer, lung cancer, melanoma, and glioma. In addition, we have observed a robust anti-leukaemia effect of environmental enrichment.’

The outcome of this work remains to be seen. However, if previous efforts are anything to go by, we will soon see a number of novel discoveries flowing from the enriched laboratories of Dr Cao and collaborators.

Meet the researcher

Dr Lei Cao
William C and Joan E Davis Cancer Research Professorship
The Ohio State University Comprehensive Cancer Center
James Cancer Hospital and Solove Research Institute
Ohio, OH
USA

Dr Cao is currently employed at the Comprehensive Cancer Center, Ohio State University. Her research career began at the Chinese Academy of Sciences, followed by research fellowships at the University of Freiburg and Thomas Jefferson University. After this period of travelling and adventure, she settled in her current location at Ohio State University in 2006. Her research focus is the effect of environmental stimuli on the central nervous system and the subsequent effect on biological processes and disease pathology. A highly successful career has led to publications appearing in a range of prestigious journals, including Cell, Nature Medicine, Nature Genetics, and Cell Metabolism.

CONTACT

E: lei.cao@osumc.edu
W: https://cancer.osu.edu/research-and-education/find-a-researcher/search-researcher-directory/lei-cao

FUNDING

National Institutes of Health grants AG041250, CA166590, CA178227, and CA163640

REFERENCES

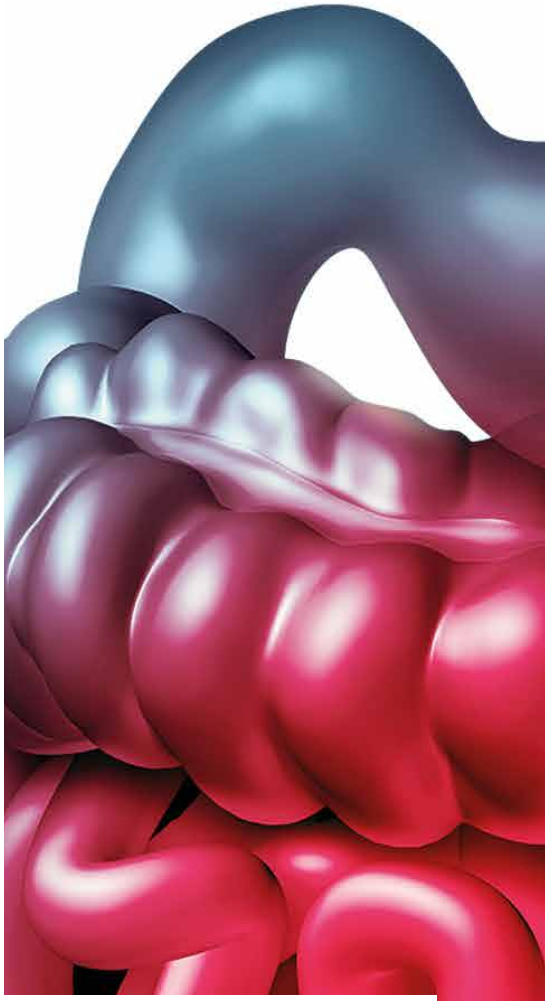
L Cao, E Y Choi, X Liu, A Martin, C Wang, X Xu, MJ During, White to brown fat phenotypic switch induced by genetic and environmental activation of a hypothalamic-adipocyte axis, Cell Metabolism, 2011, 14, 324–38.

L Cao, ED Lin, MC Cahill, C Wang, X Liu, MJ During, Molecular therapy of obesity and diabetes by a physiological autoregulatory approach, Nature Medicine, 15, 2009, 447–454.

L Cao, X Liu, ED Lin, C Wang, EY Choi, V Riban, B Lin, MJ During, Environmental and genetic activation of a brain-adipocyte BDNF/leptin axis causes cancer remission and inhibition, Cell, 2010, 142, 52–64.

THE OHIO STATE UNIVERSITY

SOLUBLE DIETARY FIBRE AND TYPE 2 DIABETES – MECHANISMS OF ACTION AND FOOD



There are numerous health benefits related to eating fibre-containing foods, including lowering the levels of serum glucose and lipids, thus reducing the risk of type 2 diabetes and heart disease. Furthermore, by creating an increased feeling of fullness, eating fibre-rich foods reduces caloric intake and obesity. **Professor Douglas Goff** from the University of Guelph, Ontario, Canada, researches the supplementation of food with fibre and the specific mechanisms of beneficial action, with a focus on blood glucose reductions after eating a carbohydrate-rich meal. Along with his team, his goal is to define the relationship between the molecular structure and physiological functionality of soluble dietary fibres.

Dietary Fibre and Health

The health benefits of dietary fibre consumption are well-documented, including improved colonic health, enhanced gastro-intestinal immunity, and a decrease in blood glucose and lipid levels. Decreased blood glucose levels after eating a carbohydrate-rich meal confer benefits to type 2 diabetic and insulin-resistant individuals, and may also help to reduce the risk of developing these chronic diseases. It is widely accepted that the modulation of specific lifestyle factors, such as diet and exercise, can reduce the risk factors associated with type 2 diabetes. Several meta-analyses of data from previous clinical studies have demonstrated that dietary fibre improves the glycaemic response in patients, that is, their blood sugar levels after food consumption.

Several international health and food safety regulators currently authorise the use of health-related claims for some foodstuffs containing specific dietary fibres, stating the benefits of reduced risk of diabetes via enhanced glucose regulation. Yet, irrespective of the renowned benefits and the public awareness of these, typical consumption of fibre falls well below the recommended levels in many countries.

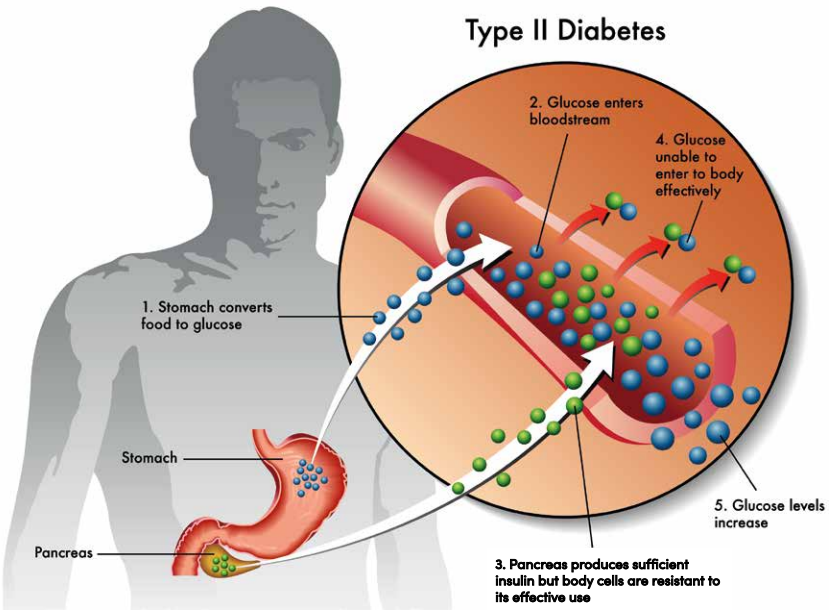
The Cost of Diabetes

The global epidemic of type 2 diabetes comes with significant health and cost implications. The global incidence of diabetes is expected to reach 592 million by 2035, and diabetes is currently responsible for 5.1 million deaths across the world each year. Of particular concern is that over one-quarter of deaths in some groups of south-east Asian women are attributed to diabetes.

The cost to Europe and North America is around 70% of the total annual estimated health costs, somewhere in the range of US$612–1099 billion. Thus, reducing this vast financial burden through lifestyle interventions and effective management and prevention is a major public health priority.

Soluble vs Insoluble Fibre

There are two major classifications of dietary fibre, namely, insoluble or water-soluble. The solubility of fibre is key to what happens to it as it moves through the gut. Insoluble fibre such as plant cellulose and wheat bran support the action of the gut by maintaining faecal bulk, and a portion of insoluble fibre is fermented by gut bacteria.



In contrast, soluble fibres such as gums and pectin produce thick, viscous networks when hydrated in the gut. Soluble fibres are more viscous than insoluble fibres, and differences in viscosity of individual types of soluble fibre in the conditions of the gastrointestinal tract are likely to be a key component in determining their specific biological functions. There are many potential mechanisms by which an increase in the viscosity of gut contents may affect the breakdown of carbohydrates and the uptake of glucose from the intestine.

The viscosity of a soluble dietary fibre depends upon the specific gut conditions. The pH level, water content, and presence of other excreted components (such as bile from the liver), all alter viscosity. A further role is played by the specific structures of the soluble fibres themselves, since their molecular structures can vary widely. Long, linear polymers are able to produce greater viscosities than branched polymers, likely due to the ability of the linear molecules to 'tangle' in solution compared to other structures.

Soluble Fibre and Viscosity

Professor Goff and his colleagues used a two-stage laboratory-based *in vitro* model of digestion to identify the specific gastric and intestinal conditions that alter the viscosity of a range of soluble fibre polymers.

The team found that some of the soluble fibres tested, initially at the same viscosity, resulted in almost no viscosity following simulated digestion in the laboratory, whilst others such as xanthan retained approximately 30–40% of their initial viscosity. Furthermore, the addition of both protein and starch to the test compounds to simulate foodstuffs increased viscosity across the range but did not modify the relative viscosity retention results.

The team concluded from these experiments that viscosity in the conditions of the gastrointestinal tract may be more important in determining the physiological function of soluble fibres and gums rather than the initial viscosity or starting concentration in food or solution.

Plant Starch Digestion in the Presence of Dietary Fibre

Unmodified plant starch, a carbohydrate that is an important energy source, has a granular structure, comprised of polymers of the sugar, D-glucose, linked via one of two chemical bonds, (i) linear amylose or (ii) branched amylopectin.

When eating, the starch polymer breakdown starts in the mouth, where it is acted on by a salivary enzyme, alpha-amylase. Further along the digestive system, in the small intestine, continued digestion occurs via alpha-amylase produced by the pancreas. This breakdown mostly produces the reducing sugars, maltose, maltotriose, and maltotetraose. The sugars are further digested by specific enzymes to produce individual glucose molecules, the end product of starch digestion.

Professor Goff and his team studied the effects of various soluble dietary fibres on the digestion of both native starch granules and pre-gelatinized plant starch. The concentrations of each dietary fibre were substantially different so that the starting viscosities were equivalent. Amylolysis, the enzymic breakdown of starch, was determined by both sample viscosity reduction and the concentration of reducing sugars (such as maltose) that were produced.

Professor Goff and his team found that, as a result of interrupted amylolysis, dietary fibre slows down the rate by which viscosity is reduced and concurrently slows the rate of maltose production. Importantly, they also showed that lowest rates of starch hydrolysis were correlated with highest viscosity retention results. *In vitro* testing showed that reduced amylolysis may be important in the dampening effect on the glycaemic response when dietary fibre is added to starch-rich meals.

They also looked at the ability of these fibres to reduce the diffusion rate of glucose after the breakdown of starch. In humans, diffusion of glucose within the small intestine and subsequent uptake of glucose into the bloodstream are linked to the release of insulin and, therefore, a factor in diabetes management.

From these *in vitro* analyses, the team identified several major outcomes: (i) all of the tested dietary fibres reduced the release and diffusion of glucose from starch, (ii) this correlated with

'intestinal' viscosity, (iii) the effects of the gums were more notable 60 minutes after enzymatic digestion, and (iv) xanthan gum resulted in the greatest modulation (inhibition) of glucose release and diffusion, and also the highest viscosity retention, when compared to control and all other test compounds.

The mechanism by which the dietary fibre components interfere with amylolysis is likely to be the result of reduced diffusion of either enzyme or its target (starch), or by inhibiting effective mixing. Professor Goff's team suggested that the absorption of dietary fibre directly to either enzyme or substrate is less likely to contribute to this process.

Gum-based Dietary Fibres and Control of Glucose Levels

Following on from their *in vitro* laboratory analyses, Professor Goff and his team conducted a controlled human clinical trial to test the efficacy of gum-based dietary fibres in controlling glucose levels following the consumption of pudding products. More specifically, they tested a range of mucilage gums, plant-derived components that can thicken, stabilise, and emulsify foods. These included yellow mustard mucilage, fenugreek gum, and flaxseed mucilage, which allow for excellent incorporation into foods.

Two different pudding products were prepared, using two different carbohydrate sources: tapioca starch or high maltose corn syrup. The fibre concentrations added to the puddings were designed to produce equal viscosity of gut contents after digestion, based on *in vitro* digestion analyses. The team ensured equivalent protein concentrations and removed all fat from the formulations.

Participants consumed a pudding with each dietary fibre, or the no dietary fibre control, on different test days. Participants provided blood samples at intervals between 15 minutes and 2 hours, by which point blood glucose levels were approximately equivalent to pre-eating baseline levels. Peak blood glucose occurred between 30 and 60 minutes.

Results showed that all soluble dietary fibre treatments produced significantly lower blood glucose levels compared to the control treatment at 30 minutes and at 2 hours. Both peak serum concentration of glucose and insulin were significantly lower for all treatments compared to control but there was no significant difference detected among any of the dietary fibre treatments. Starch had slightly less impact on blood glucose compared to maltose whereas gastric emptying rate, as measured by an added marker to the fibre-based puddings and control, had more of an impact.

The three dietary fibre test compounds were provided at concentrations to generate the same levels of viscosity within the small intestine by the addition of different quantities of fibre. However, consuming the varying amounts of dietary fibre resulted in equivalent alterations of blood glucose and insulin compared to control. This highlights the pivotal nature of viscosity in controlling the glycaemic response.

Mechanisms of Action and Rheology of Dietary Fibre

Rheology, the behaviour of non-Newtonian fluids like the viscous gels produced by the dietary fibres being tested, is likely to be significant to understanding the mechanism of action of these fibres in modifying the glycaemic response, and specifically, blood glucose levels and serum insulin levels after the consumption of food.

Professor Goff's team has found that all of the following mechanisms of action are involved in glycaemic control after eating with dietary fibre, and suggest these occur in the following order: (i) delayed emptying of the stomach, (ii) reduced digestive enzyme action in the small intestine, (iii) reduced or inhibited diffusion of the starch fragments or glucose molecules in the small intestine, and (iv) control of gut hormone release, responsible for digestion and absorption. By understanding the specific type of dietary fibre that best controls each action based on its molecular structure, fibre blends for glycaemia control can thus be optimised.

Future Health Benefits

Professor Goff and his colleagues propose that a greater focus on substantiating the health claims of dietary fibre supplemented foodstuffs is needed. They believe that profiling the specific actions and concentrations of individual dietary fibres is critical. To this end, they plan to continue investigating the relationship between the molecular structures and physical behaviour in the gut after consuming soluble dietary fibres. For meaningful health claims, the concentration of each soluble fibre needed to induce a glycaemic response would need to be specified, rather than relying on generalised intake values for all fibres as currently suggested.

Isolated and specific soluble dietary fibre can be consumed directly as a nutraceutical supplement or it can be added to foods during manufacture, to produce functional foods with potential for health claims. The challenge for food manufacturers is to incorporate sufficient soluble fibre per serving of product and not alter the taste and texture of the product, since viscous soluble fibres such as gums can have a significant impact on food quality. This is another area of active research for Professor Goff and his colleagues, with plenty of work left to do.

Meet the researcher

Professor Douglas Goff
Department of Food Science
University of Guelph
Guelph, Ontario
Canada

Professor Douglas Goff is a Professor of Food Science at the University of Guelph in Canada with over three decades of experience in his research field. After completing his PhD at Cornell University, USA, in 1987, Professor Goff returned to the University of Guelph where he had previously completed his undergraduate degree. His research program has since then focussed on ice cream and dairy systems, hydrocolloid functionality in food systems, and polysaccharide structure-function relations. For the last 10 years, he has also examined the relationship between physical and physiological functionality of polysaccharides as a source of dietary fibre. As a renowned scientist, Professor Goff shares his expertise internationally with Ice Cream Technology Industry members. To date, Professor Goff has supervised 60 graduate students and has published two books, 40 book chapters, and 180 refereed journal articles. Furthermore, in 2017, Professor Goff received the prestigious Food Hydrocolloids Trust Medal for lifetime achievement.

CONTACT

E: dgoff@uoguelph.ca
W: https://www.uoguelph.ca/foodscience/people/douglas-goff

KEY COLLABORATORS

Dr Steve Cui, Agriculture and Agri-Food Canada
Professor Amanda Wright, University of Guelph
Professor Alison Duncan, University of Guelph

FUNDING

Natural Sciences and Engineering Research Council
Ontario Ministry of Agriculture, Food and Rural Affairs

FURTHER READING

N Repin, SW Cui, HD Goff, Impact of dietary fibre on in vitro digestibility of modified tapioca starch: viscosity Effect, Bioactive Carbohydrates and Dietary Fibre, 2018, 15, 2–11.

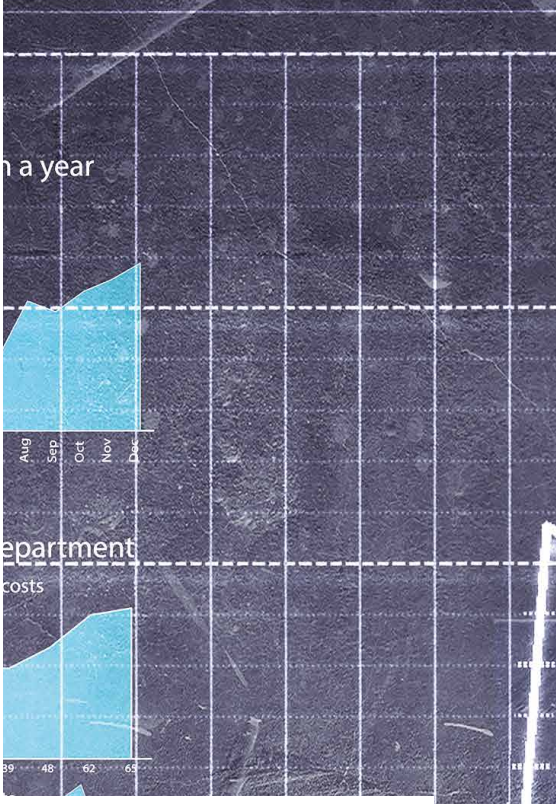
HD Goff, N Repin, H Fabek, D El Khoury, MJ Gidley, Dietary fibre for glycemia control: towards a mechanistic understanding, Bioactive Carbohydrates and Dietary Fibre, 2018, 14, 39–53.

BA Kay, K Trigatti, MB MacNeil, S L Klingel, N Repin, HD Goff, AJ Wright, AM Duncan, Pudding products enriched with yellow mustard mucilage, fenugreek gum or flaxseed mucilage and matched for simulated intestinal viscosity significantly reduce postprandial peak glucose and insulin in adults at risk for type 2 diabetes, Journal of Functional Foods, 2017, 37, 603–611.

HS Fabek, S Messerschmidt, V Brulport, HD Goff, The effect of in vitro digestive processes on the viscosity of dietary fibres and their influence on glucose diffusion, Food Hydrocolloids, 2014, 35, 718–726.

EDUCATION AND HEALTH DISPARITY ACROSS THE US

Adults living in certain US states suffer from more illnesses, more disability, and die sooner than residents of others. **Dr Mark D. Hayward** of the University of Texas at Austin and **Dr Jennifer Karas Montez** of Syracuse University are investigating these differences in health and exploring their relationship with educational attainment and state-specific policies, so they can work towards addressing the causes of this disparity.



Research into Health Disparities Across US States

People living in some US states have been found to experience poorer health and die sooner than those inhabiting others. In the year 2000 differences in life expectancy across US states were reported to be larger than those between high-income countries, such as Germany, Australia and Japan. These differences have been growing since the early 1980s, especially for women.

One of the most common explanations for these health disparities is that the different states are homes to people with different distinctive characteristics. For instance, people living in Mississippi, where there are high mortality rates, are more likely to be Black and less educated than those in Massachusetts, that has a relatively low mortality rate. In the US, being Black or less educated increases one's risk of death due to factors such as discrimination, poverty, and regressive tax policies.

A second possible explanation is that different states have different legislation and make varying degrees of investment in their population's well-being. Applying this theory to the same comparison, unlike Massachusetts, Mississippi does not offer a supplemental Earned Income Tax Credit (EITC), which boosts the income of families with low-wage workers, imposes only a negligible sales tax on cigarettes, and did not expand Medicaid coverage under the 2010 Affordable Care Act.

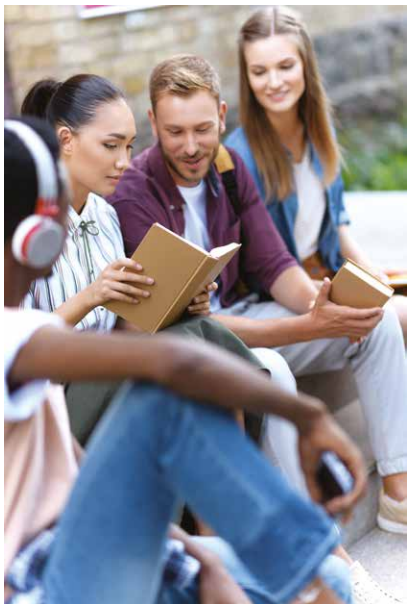
A team of researchers led by Dr Jennifer Karas Montez of Syracuse University and Dr Mark D. Hayward of the University of Texas at Austin have been carrying out research investigating the validity of these two theories.

The researchers analysed extensive data on adults living in the US from two different surveys: the National Longitudinal Mortality Study and the American Community Survey. The researchers also collected data on the policies and characteristics of all US states. For example, they collected information on the states' economic environment, income inequality, tobacco control policies, Medicaid coverage, and socio-political factors such as whether the state tends to vote for a Republican or Democratic presidential candidate, as well as characteristics of the states' populations such as age, sex, race, ethnicity, educational levels. The data collected was then analysed in an attempt to gain a better understanding of the factors behind cross-state health disparities.

The Relationship between Education and Health Disparities

Drs Montez and Hayward found education level to be one of the strongest predictors of health and mortality rates among US residents. This is aligned with past research findings highlighting the impact of education on an individuals' health. 'In the United States, one of the best predictors of how healthy and long someone will live is their education level,' says Dr Montez. 'More years of schooling generally translate into better health and longer life.'

A recent study in 2016 found that a 25-year old white man living in the US could expect to live up to 70 without a high school diploma, but his life expectancy would rise to 82 with a college degree.



The reasons for the association between people's educational level and their health is not, as some would assume, limited to differences in resulting income.

'Education provides people with a large bucket of resources that they can use to create a healthy life,' explains Dr Montez. 'For example, people with more schooling tend to be employed in jobs they enjoy and that stimulate their minds, to marry and stay married, to have large and beneficial social networks, to feel in control of their life, and to engage in healthy behaviours like exercising and avoiding tobacco.'

‘While college-educated adults have similar health no matter which US state they live in, the same is not true for people with low levels of education. It turns out, having low levels of education is much worse for health and longevity in certain states.’

The positive effects of education on health, therefore, go beyond those derived from generally higher salaries, such as access to more expensive medical services. While imparting field-specific knowledge or skills, education also teaches people how to navigate modern society and look after themselves as well as their families and friends. This tends to improve their health and wellbeing, while also opening a broader range of social and economic opportunities for these individuals.

State-dependent Effects of Low Education on Health

Since the 1970s, social and economic policies have varied considerably across US states. Around that time, the federal government began to give states greater discretion over which policies, programs, and services they fund (a movement called the ‘Devolution Revolution’), and states began to take away the authority of local governments to make their own laws (a process called Preemption). In their research, Drs Montez and Hayward have explored whether the resulting cross-state differences influenced the extent to which residents’ health was affected by their education level.

‘The relationship between education and health (and longevity) has been replicated in so many studies for so many years that it is has been referred to as a social fact,’ says Dr Montez. ‘A few years ago, our team started questioning whether this social fact was true in all US states. Is having a college degree equally beneficial for health and longevity in all states? Is not graduating from high school equally bad for health and longevity in all states?’

The researchers found that people with college degrees had similar health, regardless of what state they lived in. ‘This makes a lot of sense,’ says Dr Montez, ‘the skills and resources that individuals acquire through schooling inhere within the individuals. A college degree is like a personal firewall. One can take it just about anywhere and it will have similarly large benefits for health.’

However, the same pattern was not observed for residents with low educational attainments. Dr Montez says that, ‘while college-educated adults have similar health no matter which US state they live in, the same is not true for people with low levels of education. It turns out that having low levels of education is much worse for health and longevity in certain states. This too makes a lot of sense. Because people with low levels of education have a much smaller bucket of social and economic resources, they are more affected by the resources – or lack of resources – around them.’

As an example of this, residents of New York and Mississippi with college degrees had similar health, yet those who never graduated from high school had worse health if they lived in Mississippi. The researchers hypothesise that this is probably due to differences in legislations and policies adopted by these two states.

For instance, the state of New York imposed a hefty excise tax on cigarettes, implemented its own Earned Income Tax Credit (EITC), participated in the Affordable Care Act’s Medicaid expansion, and chose not to preempt local governments from introducing health-promoting legislation.

On the other hand, Mississippi retained a negligible cigarette tax, does not offer its own EITC, did not take part in the Medicaid expansion, and chose to preempt the same health-related policies that were accepted in New York. ‘The social and economic policy contexts of these two states are now dramatically different,’ Dr Montez explains, ‘and these differences are more salient to low-educated adults than they are to college-educated adults.’

Cross-state Inequalities in Women’s Mortality and Disability

Drs Montez and Hayward also examined in detail the mortality rates of US residents between 45–89 years of age. Their analyses

revealed that the probability that a woman or man died in a given year differed according to the state in which they resided. The researchers quantified differences in the mortality rates across all 50 US states and then separated them into those due to individuals' characteristics (such as race) and those due to the states' characteristics (such as tax policies).

In men, 34% of observed mortality differences were due to their individual attributes, while 23% was due to different state characteristics. For women, however, this was quite different, with 30% of the differences due to their attributes and 53% due to the states'. In other words, different states' characteristics appear twice as important in influencing the mortality of women than they were for men.

The main state-level factor influencing male mortality appeared to be their state of residence's tobacco environment, but for women state-level social and economic characteristics mattered more. The researchers describe how, 'women are more likely than men to be living in poverty, raising children, caring for aging parents, and interacting with the medical care system. State health, education, childcare, and workplace policies can ease any or all of these challenges – and these challenges can be more difficult for women to navigate if states do nothing or make matters worse.'

Another study carried out by Dr Montez and her colleagues revealed that the probability of having a disability also differs across states, both because of the varying attributes of residents and the states they live in. Overall, the probability of having a disability was found to be lower in states with stronger economic output, more income equality, state supplemental EITC, and for middle-aged women, higher cigarette taxes.

The Path Towards Improving Health for all Americans

Research has consistently highlighted the presence of significant health and longevity disparities across different US states. These differences have been found to be associated with a host of factors, including the educational attainment of the states' residents. Drs Montez and Hayward's work confirms the role of education in fostering health, while also suggesting that, due to varying policies and regulations across US states, poor education has more serious effects in certain states.

Government initiatives or legislations that impede young people's access to education could therefore have serious consequences on their adult life. In an Opinion Editorial published in the Huffington Post, Dr Montez highlighted the potential health risks of bills proposed by the House and Senate, which would tax graduate students on the cost of their tuition and move investments in education, which are largely derived from public tax dollars, away from public schools to private ones.

In their work, Drs Hayward and Montez have also suggested steps that could pave the way towards better health for all American citizens, such as policies that ensure equal access to quality education, measures aimed at closing income gaps, and initiatives fighting tobacco or drug use.

'The next step in our project is to explain why certain US states are so hazardous for individuals with low levels of education,' says Dr Montez. 'What policies, or combination of policies, explain why low-education is more hazardous to your health in some states than others? Ultimately, we want to develop a set of recommendations that states can implement to improve the health and longevity of their residents.'

Meet the researchers

Dr Jennifer Karas Montez
Department of Sociology and
Aging Studies Institute
Syracuse University
Syracuse, NY
USA

Dr Jennifer Karas Montez is a Professor of Sociology and the Gerald B. Cramer Faculty Scholar of Aging Studies at the Maxwell School of Citizenship and Public Affairs at Syracuse University. She holds an MA in Sociology from the University of Houston and a PhD in Sociology from the University of Texas at Austin, as well as a BS in Mathematics and an MS in Statistics from Purdue University. She completed her postdoctoral training in the School of Public Health at Harvard University. Dr Montez investigates trends and inequalities in adult mortality in the United States. Her work aims to explain why trends since the 1980s have been particularly troubling for women and for adults with low levels of education.

CONTACT

E: jmontez@maxwell.syr.edu
W: https://www.maxwell.syr.edu/soc/Karas_Montez,_Jennifer/
@jennkarasmontez

Maxwell
Syracuse University

Dr Mark D. Hayward
Department of Sociology and
Population Research Center
University of Texas at Austin
Austin, TX
USA

Dr Mark Hayward is a Professor of Sociology, a Centennial Commission Professor in the Liberal Arts, and a faculty research associate of the Population Research Center at the University of Texas at Austin. He attained a BA in Sociology from Washington State University, as well as an MA and a PhD in Sociology from Indiana University. Dr Hayward has served as the president of the Southern Demographic Association, chair of the Sociology of Population and Aging and Life Course sections of the American Sociological Association and sits on several other scientific advisory boards around the US. His research primarily addresses how life course exposures and events influence health and mortality in the adult population.

CONTACT

E: mhayward@prc.utexas.edu
W: https://liberalarts.utexas.edu/prc/directory/faculty/mdh745
@mdhayward

TEXAS
The University of Texas at Austin

KEY COLLABORATORS

Dr Anna Zajacova, Associate Professor of Sociology, Western University, Ontario Canada
Dr Robert A. Hummer, Professor of Sociology, University of North Carolina, Chapel Hill
Dr Steven H. Woolf, Professor of Family Medicine and Population Health, Virginia Commonwealth University
Dr Jason Beckfield, Professor of Sociology, Harvard University

FUNDING

US National Institute on Aging
US Eunice Kennedy Shriver National Institute of Child Health and Human Development
Robert Wood Johnson Foundation
Carnegie Corporation of New York
American Sociological Association

FURTHER READING

MD Hayward, RA Hummer and I Sasson, Trends and group differences in the association between educational attainment and US adult mortality: Implications for understanding education's causal influence, Social Science and Medicine, 2015, 127, 8–18.

JK Montez, A Zajacova and MD Hayward, Explaining inequalities in women's mortality between US states, SSM – Population Health, 2016, 2, 561–571.

JK Montez, A Zajacova and MD Hayward, Disparities in disability by educational attainment across US states, American Journal of Public Health, 2017, 107, 1101–1108.

JK Montez, MD Hayward and DA Wolf, Do U.S. states' socioeconomic and policy contexts shape adult disability? Social Science and Medicine, 2017, 178, 115–126.

JK Montez, Deregulation, devolution, and state preemption laws' impact on US mortality trends, American Journal of Public Health, 2017, 107, 1749–1750.

CANNABIS USE: THE NEW NORMAL?

As more countries begin to decriminalise and legalise cannabis, understanding attitudes towards its use will be essential in anticipating the risks and benefits of these legislative changes. **Professor Patricia Erickson** of the University of Toronto and **Professor Andrew Hathaway** of the University of Guelph provide new insights into the attitudes and practices of both cannabis users and non-users in order to better understand the ongoing normalisation of cannabis use.

Cannabis: Friend or Foe?

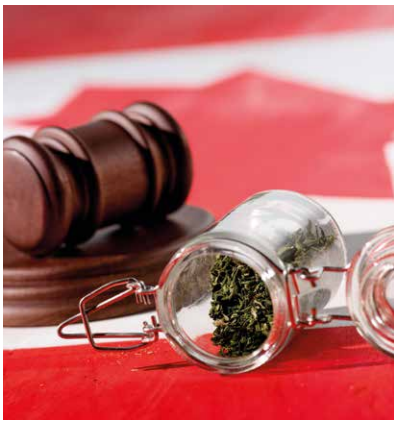
Cannabis is the most widely used illicit drug worldwide by a wide margin. The United Nations Office on Drugs and Crime estimate that approximately 4% of the world's population use cannabis on an annual basis. Recent years have seen a growing body of literature concerning the health effects of cannabis, both positive and negative. Studies have linked smoking cannabis to lung conditions and the worsening of pre-existing mental health conditions; conversely, cannabis has been investigated and used as a treatment for chronic pain, loss of appetite caused by chemotherapy, and a host of other health issues. Considerable research is underway to obtain a more precise picture of effects.

The prevalence of cannabis use in society and the volume of contemporary studies into its potential medical benefits have led to the widespread reform of cannabis laws in recent years. Some countries, such as the UK, have approved the use of cannabis as a treatment for specific medical conditions. A number of countries around the world have gone on to decriminalise the possession of cannabis, while very few have legalised its production and sale; in Canada, where statistics for use have consistently been among the highest in the world, cannabis has been legally available for medicinal purposes since 2001, and for recreational access to adults since 2018. However, individual attitudes vary greatly on the acceptability of its use according to age, location and social context.

Professor Patricia Erickson from the Department of Sociology and the Centre for Crime and Socio-legal Studies at the University of Toronto, and Professor Andrew Hathaway at the University of Guelph, have published prolifically regarding harm reduction policies and the normalisation of attitudes towards cannabis in Canada. They aim to understand the perspective of young people regarding cannabis use, particularly the stigma associated with the use of cannabis and the shift in attitudes towards regarding cannabis use as a 'normal' activity.

What's the Harm?

Harm reduction measures aim to reduce the negative impact of drug use, whether controlled, recreational forms



or more problematic dependencies. Examples of harm reduction measures include needle exchange programs, used to reduce the risks of blood-borne infections for intravenous heroin users, and safer consumption sites to prevent overdoses. A number of studies have argued that an increase in the incidence of high-strength strains of cannabis means that appropriate harm reduction approaches are now needed to mitigate their possible negative health effects, particularly to protect youthful consumers.

In a 2003 review, Professors Erickson and Hathaway called this approach into question and argued that there is little evidence that cannabis smokers experience greater toxicity from more

potent strains. They argued that most evidence shows that the harms associated with cannabis use tend to be minor compared to those associated with the criminalisation process. Of course, current forms of consumption such as edibles and concentrates pose additional concerns to consider in relation to health and safety effects.

Professors Erickson and Hathaway argued that in addition to being harmful, prohibition was also not an effective deterrent. The origins of global prohibition were based on misconceptions or misinformation, exaggerating the harms and addictive potential of illicit drugs overall.

Social Stigma

In a 2011 study, Professors Hathaway and Erickson surveyed a large cohort of frequent, adult cannabis smokers in four cities in different provinces in order to obtain meaningful data regarding attitudes towards cannabis use and gauge the effectiveness of the prohibition laws in Canada at that time. Their data showed that although some frequent users were somewhat worried by a threat of criminal punishment, others were concerned with the social stigma associated with their cannabis use being 'outed' to employers or family; however, most felt little disapproval among their friends and colleagues.

The perception of many of those interviewed was that use of the drug, in the larger society, was still seen to be associated with 'deviant' behaviour, including the use of other drugs and criminal activity. Most were sensitive to the importance of restricting use to social situations where others would not be bothered by use, such as avoiding use around children, for example. These findings supported their previous conclusion that criminal threats were not necessarily the most effective deterrent towards the use of cannabis, and indicated that social attitudes were more likely to limit or prevent use and channel where and how the drug was consumed.

In a subsequent study in 2015, Professors Hathaway and Erickson and their team conducted interviews with undergraduate students, both users and non-users, from the Universities of Toronto, Alberta, and Guelph. The first analysis was mostly focused on those students who abstained from cannabis use. Questions asked by the team considered each student's reasons for abstaining and their perceptions of responsible cannabis use, and the perceived differences between users and non-users.

The general perception of non-users was that cannabis is a relatively safe, unproblematic drug. Most students indicated that use was inappropriate when it clashed with commitments or priorities such as doing schoolwork. Non-users also indicated that a major reason for abstaining from cannabis use was due to worries regarding the possibility of arrest, and worries about it affecting future job prospects. Family and cultural issues also factored into decisions to abstain from cannabis use. Some abstainers saw cannabis smokers as less mature or foolish, or irresponsible, echoing the results of the previous studies in which cannabis smokers expressed concern about social stigma emanating from certain audiences, and hence their caution in disclosure.

Cannabis Use and Gender

Interestingly, the researchers' findings showed a difference in attitudes towards the use of cannabis by men and women. More men use cannabis – and use it more frequently – than women according to surveys. Gender inequality and biases seemed to contribute to the attitudes of non-users towards users. The social consequences of using cannabis were perceived to be greater for women than men, men, with both men and women suggesting that women are more concerned with the potential risks of cannabis use and that they are more vulnerable to gendered social criticism (e.g., being stigmatised as sexually promiscuous, immature, or attention-seeking).

The Social Network: Cannabis Distribution in Peer Groups

In a later analysis of the campus study data in 2018, Professors Erickson and Hathaway investigated the role of social supply networks in normalising the use of cannabis by undergraduate students. The team interviewed students who were regular or occasional cannabis smokers, and asked them questions regarding how they usually acquired the cannabis that they smoked.

The team found that 44% of their respondents reported that they bought cannabis from a friend or that a friend bought it on their behalf. Like the previous study, they found a gendered difference in attitudes, noting that women were more likely to have someone purchase the cannabis for them. Use of such a 'broker' was seen to be beneficial through providing a safe distance between the buyer and dealer. Users saw social supply networks and the availability of free cannabis from peers as advantageous as a way of reducing consumption and keeping its use occasional and limited to social functions.

The prevalence of these social networks led Professors Erickson and Hathaway to conclude that legalisation would be unlikely to reduce the high levels of cannabis use among young people in Canada, as individuals would still be able to obtain cannabis through these social networks, and that in many cases, these illicit contacts would be desirable over legally available sources of cannabis.

Professors Erickson and Hathaway also pointed out issues with the recent legislative approaches enacted in Canada. They noted that one of the main objectives of legalisation measures is to prevent access of drugs to younger people; following legalisation, cannabis products are now age-restricted in Canada, available for purchase from the age of 18 in Alberta and 19 in other provinces. The authors noted that this approach is unlikely to prevent the use of cannabis by youths, as legal supplies of cannabis can be diverted to the black market for distribution to younger people, much as alcohol is. Moreover, the age restrictions or high retail prices of the regulated product could drive younger users back to their already established peer networks.

These findings have very important consequences for legalisation measures. For example, overly restrictive guidelines following legalisation could lead to users finding alternative routes to save money or get better access to cannabis. The authors concluded that formal drug policies, no matter whether they may favour legalisation or prohibition, tend to be less effective at curtailing or moderating use than the informal control of cannabis use practised by the users themselves.

Legalisation and the Future

The work of Professors Erickson and Hathaway has provided a nuanced insight into the use of cannabis. Their rigorous studies have helped them recommend more effective harm reduction and legislative measures. It is not yet clear how legalisation will impact the views and social preconceptions of users and non-users alike. Normalisation has been accompanied by the reduction of stigma, but it has not been eliminated. Understanding attitudes towards cannabis both before and after legalisation will be especially important as other countries around the world begin to reconsider their policies on cannabis use and legal availability.

Meet the researchers

Professor Patricia Erickson
Department of Sociology
University of Toronto
Toronto, Ontario
Canada

Professor Patricia Erickson completed her PhD at the University of Glasgow, Scotland, after working as a research scientist at the Addiction Research Foundation (ARF), and on her return she became the Head of the Drug Policy Research Program at ARF. In 1992, she became a Professor of Sociology and Criminology at the University of Toronto. In addition to her professorship, Professor Erickson is also Scientist Emerita at the Centre for Addiction and Mental Health (CAMH). Professor Erickson's research interests include illicit drug use and drug policy, with a recent focus on examining ways of improving screening for youths with substance use and mental health problems in custodial facilities. She has published over 150 articles, chapters, books, and monographs on drug policy, illicit drug use, and drug and mental health issues in marginalised groups.

CONTACT

E: pat.erickson@utoronto.ca
W: http://sociology.utoronto.ca/tag/patricia-erickson/

Professor Andrew Hathaway
Department of Sociology and Anthropology
University of Guelph
Guelph, Ontario
Canada

Professor Andrew Hathaway is an Associate Professor in the Criminal Justice and Public Policy program of the Department of Sociology and Anthropology at the University of Guelph. His PhD is from McMaster University. Professor Hathaway conducts research in the areas of illicit drug use, harm reduction, human rights and Canadian drug policy. His main focus is the study of cannabis for medical use and understanding the normalisation of cannabis consumption. Professor Hathaway is the author of the textbook Drugs and Society published in 2015 and has published a host of articles over the past 20 years concerning trends in and attitudes towards drug use in Canada.

CONTACT

E: hathawaa@uoguelph.ca
W: https://www.uoguelph.ca/socioanthro/people/andy-hathaway
@GuelphSOAN

KEY COLLABORATORS

Mark Asbridge, Dalhousie University
Serge Brochu, University of Montreal
Marie-Marthe Cousineau, University of Montreal
David Marsh, Northern Ontario School of Medicine
Elaine Hyshka, University of Alberta
Geraint Osborne, University of Alberta
Amir Mostaghim, University of Ontario Institute of Technology
Kat Kolar, University of British Columbia
Mark van der Maas, Rutgers University
Cameron Duff, Melbourne University

FUNDING

Social Sciences and Humanities Research Council (SSHRC), 2007–2011, $116,895
'Drug normalisation and stigma: Canada's experience with cannabis and tobacco'

SSHRC, 2011–2014, $103,500
'Cannabis, stigma and policy change: A 3 campus study of normalisation among university students'

FURTHER READING

AD Hathaway, A Mostaghim, PG Erickson, et al, "It's Really No Big Deal": The role of social supply networks in normalizing use of cannabis by students at Canadian universities, Deviant Behaviour, 2018, 39, 1672–1680.

K Kolar, P Erickson, A Hathaway, G Osborne, Differentiating the drug normalization framework: a quantitative assessment of cannabis use patterns, accessibility and acceptability attitudes among university undergraduates, Substance Use & Misuse, 2018, 201, 8, 53(14), 2339–49.

P Erickson, M van der Maas, A Hathaway, Revisiting deterrence: Legal knowledge, use context and arrest perception for cannabis, Czech Sociological Review, 2013, 49(3), 1–22.

P Erickson, A Hathaway, Normalization and harm reduction: Research avenues and policy agendas, International Journal of Drug Policy, 2010, 21, 137-139.

PLAYING VOICE MESSAGES TO IMPROVE HYGIENE AND HEALTH

Hand hygiene is the most cost-effective approach to preventing the transmission of infectious diseases in hospitals. While there has been much effort towards improving hand hygiene by healthcare workers, patients are seldom targeted. To this end, **Dr Stephen Lane**, working with Stargates Inc. a small business in Arlington, VA, US, and Johns Hopkins Hospital, of Baltimore, MD, US, has spearheaded efforts to improve patient hand hygiene using a novel approach involving voice reminders, called Hand Hygiene for Patients.

Hand Hygiene for Hospital Patients – A Neglected Opportunity

Hospital surfaces are commonly contaminated with infectious organisms that can be cross-transmitted between healthcare workers, the environment and patients. Given that many patients also have compromised immunity due to their disease or treatment, they often acquire infections during their hospital stay.

These infections, which can be fatal, are known as hospital-acquired infections. According to recent data from the US Center for Disease Control and Prevention, approximately 722,000 infections are acquired in US hospitals annually, resulting in 75,000 deaths and over $9.8 billion in healthcare costs. The problem is further exacerbated by the rise in drug-resistant bacteria, which pose a serious, growing and global concern to public health. More work is urgently needed to prevent these infections, protect sick patients and ultimately, save lives.

One of the best approaches to prevent hospital-acquired infections and break the chain of bacterial transmission is hand hygiene; i.e., removing bacteria from the hands using soap and water or disinfectant. Currently, most research has been directed towards increasing hand hygiene in healthcare providers, such as doctors or nurses.

However, efforts aimed at promoting hand hygiene by patients are rare. Nevertheless, patients' hands – more than any other part of the body – are likely to contact environmental surfaces, dressings, healthcare workers' hands and other patients in hospital facilities, all of which can be contaminated by bacteria. A 2016 study published by *JAMA Internal Medicine* showed that nearly one-quarter of patients had at least one multidrug-resistant strain of bacteria on their hands upon discharge from hospital and admission to a post-acute care facility.

Alarmingly, 10% of those patients acquired one or more new multidrug-resistant strains during their stay at the post-acute care facility, suggesting that current measures are inadequate to address patient hand hygiene.

These and other studies indicate that patient hand hygiene is a greatly underappreciated prevention method to decrease hospital-acquired infections. Interestingly, only a minority of studies have directly measured the hand hygiene rate of patients as an outcome. While measuring different health outcomes, such as infection rate, is clearly important, methods to directly measure hand hygiene are also crucial.

To this end, Dr Lane and his team at Stargates Inc. have begun to develop

‘Hand Hygiene for Patients (HHfP) involves simple sensors in a hospital patient’s room, reporting to a central computer. When HHfP detects toilet use by a hospital patient, it waits to see if the patient washes or disinfects his or her hands voluntarily. If they do not, HHfP plays a non-shaming voice message in the patient room, which only the patient can hear, encouraging good hand hygiene. Results to date are that those voice messages increase hand hygiene by men.’

methods to both measure and improve hand hygiene rates in patients. Dr Lane explains that: ‘Hospital surfaces are reservoirs of infectious organisms, left there by patients. Those same patients have compromised immune systems, owing to the disease that brought them to the hospital. The result is that, far too often, patients acquire a disease during their hospital visit. These hospital-acquired infections can be fatal. They cost billions of dollars and tens of thousands of lives each year in the USA.’

Their unique approach, called Hand Hygiene for Patients (HHfP), specifically targets hospital patients using a novel computer-based system to both measure and increase hand hygiene, thereby ultimately reducing hospital-acquired infections.

Hand Hygiene for Patients

In general, there are four occasions when patient hand hygiene may reduce the risk of hospital-acquired infections – after toilet use, before eating and when entering and leaving their room. HHfP targets hand hygiene after toilet use, due to the possibility that faecal matter can contaminate the hands at that time.

Data from the largest observational study of patient hand hygiene by Srigley and colleagues in 2014 found that following toilet use, men washed their hands about 23% of the time, women about 36% and that the combined average was 30%. Given these low hand hygiene rates, there is substantial room for improvement and an opportunity to decrease hospital-acquired infections by encouraging hand hygiene following toilet use.

Dr Lane describes how: ‘Hand Hygiene for Patients (HHfP) involves simple sensors in a hospital patient’s room, reporting to a central computer. When HHfP detects toilet use by a hospital patient, it waits to see if the patient washes or disinfects his or her hands voluntarily. If they do not, HHfP plays a non-shaming voice message in the patient room, which only the patient can hear, encouraging good hand hygiene. Results to date are that those voice messages increase hand hygiene by men.’

To determine hand hygiene compliance, HHfP uses simple electronic sensors without requiring patients to wear an identifying or tracking device. These sensors report lavatory door position (open or closed), toilet use and hand hygiene. The sensors pose no risk to patients. Their data are electronically transmitted to and recorded by a central computer, where they are integrated to determine hand hygiene after toilet use, using software that Dr Lane has developed over 20 years in the field.

Once toilet use has been detected, HHfP waits to determine whether the patient voluntarily washes his or her hands. If they do, HHfP records that fact and takes no further action. However, if they do not wash, HHfP plays a voice message through a small loudspeaker in the patient’s room to remind them to wash their hands. These messages are motivational, inviting the patient to action, rather than admonishing. HHfP then waits to see if the patient washes his or her hands in response to the message and records their wash or its absence.

To avoid monotony and hence patients ignoring the message, HHfP plays multiple different voice messages, thus avoiding patients hearing the same voice message twice during their stay. A sample message is: ‘Soap and sanitiser

are here to make it easy for you to clean your hands. Will you?' HHfP's message texts can be easily modified, and an almost unlimited number of different messages can be played. To ensure their clarity, the messages are spoken by trained actors.

Testing HHfP in a Patient Ward

To show that HHfP increased patient hand hygiene, Dr Lane and his colleagues completed a National Institutes of Health-sponsored Phase I trial at the Cardiovascular Progressive Care unit at Johns Hopkins Hospital, in Baltimore, MD, USA. The unit had 32 single patient rooms – each with a soap and a sanitiser dispenser in the room, and a soap dispenser in the lavatory. Since previous studies have shown that there are different rates of hand hygiene between males and females, they were studied separately.

HHfP was installed in 12 patient rooms to collect data from patients enrolled in the study. Patients were either enrolled in the 'Baseline group' (with no voice reminders) or the 'Intervention group' (with HHfP voice reminders), but not in both. The number of toilet uses with and without hand hygiene was recorded for both patient groups to determine the effect of HHfP on patient hand hygiene. Only toilet use with the lavatory door closed was considered, in order to exclude healthcare workers emptying waste into the toilet, for example. Hand hygiene after message delivery was counted the same as voluntary hand hygiene.

One of Dr Lane's informal observations from his previous work is that voice messages are effective toward changing behaviour if the listener both a) knows that the message subject is important and relevant, and b) believes that he or she is not meeting the standard set in the message. HHfP's Phase I results are consistent with those findings.

He found that there was no change in the hand hygiene compliance of females following toilet use. Regardless of whether voice messages were played, their hand hygiene averaged about 55%, suggesting that they knew that hand hygiene is important – they washed a lot – but believed they already washed their hands enough and so they didn't wash any more. Therefore, HHfP had a limited impact on women's hand hygiene in Phase I.

However, men notably increased their hand hygiene after toilet use with the door closed from 47% to 60% when voice messages played. This suggests that men also knew that hand hygiene following toilet use is important but were also aware that they did not wash their hands enough. As a result, men increased their hand hygiene compliance to the common value of about 55%.

Summary and Future Directions

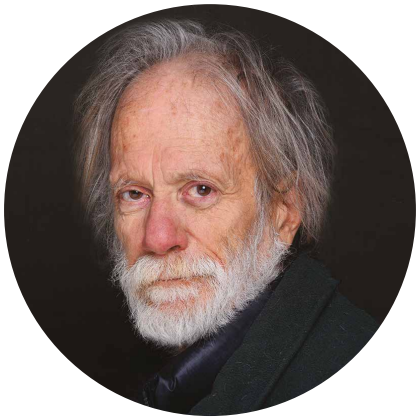
While Dr Lane has experienced some success in increasing hand hygiene by men, the data also show that voice messages alone will achieve only about 55% compliance – thus, there is still considerable scope for improvement in hand hygiene by hospital patients. Combining HHfP with education emphasising the need to wash after every toilet use may further improve hand hygiene in both men and women. Dr Lane, therefore, plans to include patient education to raise their hand hygiene standards in a future Phase II trial.

The crucial question that remains after the Phase I trial is whether hospital-acquired infections are reduced through the implementation of HHfP. Dr Lane states that: 'Our next step is to investigate the effect of HHfP's increased hand hygiene on the hospital-acquired infection rate.'

Therefore, in Phase II, infection rates will be compared between the Baseline and Intervention groups to show that HHfP can significantly lower the number of infections acquired in hospitals. Phase II will include a number of hospital units from several different hospitals and a long-term facility. He explains that, 'we expect to find that the increase in hand hygiene by patients due to HHfP's voice messages leads to a significantly lower hospital-acquired infection rate, with consequent savings in money, and most importantly, in human lives.' He is interested in collaborating with researchers in US hospitals to achieve that end. He also seeks a manufacturer and distributor of hospital products to help him commercialise Hand Hygiene for Patients after Phase II trials have shown its value.

In summary, Dr Lane and his team have successfully used HHfP to target the patient population for hand hygiene – a group that has been previously overlooked as research has predominantly focused on hand hygiene by healthcare workers.

Through harnessing the missed opportunity to focus on this population, Dr Lane and his team hope that HHfP can be a cost-effective and novel technology-based approach to further improve hand hygiene amongst hospital patients and consequently reduce hospital-acquired infections, decrease healthcare costs and ultimately save thousands of patients' lives each year.



Meet the researcher

Dr Stephen Lane
Member of Technical Staff, Stargates Inc.
Arlington, VA
USA

Dr Stephen Lane completed his PhD in Physics at the University of Maryland (US) in 1970. From 1970 to 1985, he worked with a number of companies on monitoring nuclear power plants for leaky cooling tubes, and processing seismic data to distinguish signals between nuclear explosions and earthquakes. In 1985, he became Vice President, Research and Development, of Amron Corporation (US) and began working in healthcare. He has been the Principal Investigator on a variety of health-related projects, including developing devices to infer potentially dangerous circumstances in the home of an elderly person, without the need for him or her to wear an identifying device or to push a button, and to summon help if needed; to deter an elderly or confused person's dangerous attempt to leave their bed until help can arrive; to deliver voice messages to remind healthcare workers in a hospital to wash their hands after patient contact if they did not do so voluntarily; to encourage their use of alcohol based gel rather than soap and water; to remind them to wash with soap and water for at least 15 seconds if they failed to do so voluntarily; and to praise them when they performed hand hygiene. Currently, he is working with Stargates Inc., taking the lead to promote hand hygiene in hospital patients using hardware and software developed from his previous research. His work on promoting hand hygiene compliance has been published in peer-reviewed journals such as *Critical Care Medicine* and the *American Journal of Infection Control*.

CONTACT

E: slane@stargates.com
W: http://www.stargates.com

FURTHER READING

J Cao, L Min, B Lansing, B Foxman and L Mody, Multidrug-resistant organisms on patients' hands: A missed opportunity, JAMA Internal Medicine, 2016, 176, 705–6.

M Pokrywka, M Buraczewski, D Frank, H Dixon, J Ferrelli, K Shutt and M Yassin, Can improving patient hand hygiene impact Clostridium difficile infection events at an academic medical center? American Journal of Infection Control, 2017, 45, 959–63.

JA Srigley, CD Furness and M Gardam, Measurement of patient hand hygiene in multiorgan transplant units using a novel technology: an observational study, Infection Control and Hospital Epidemiology, 2014, 35, 1336–41.

E Zimlichman, D Henderson, O Tamir, C Franz, P Song, CK Yamin, C Keohane, CR Denham and DW Bates, Health care-associated infections: A meta-analysis of costs and financial impact on the US health care system, JAMA Internal Medicine, 2013, 173, 2039–46.

STARGATES INCORPORATED

LISTEN TO THE STORY BEHIND THE **SCIENCE**

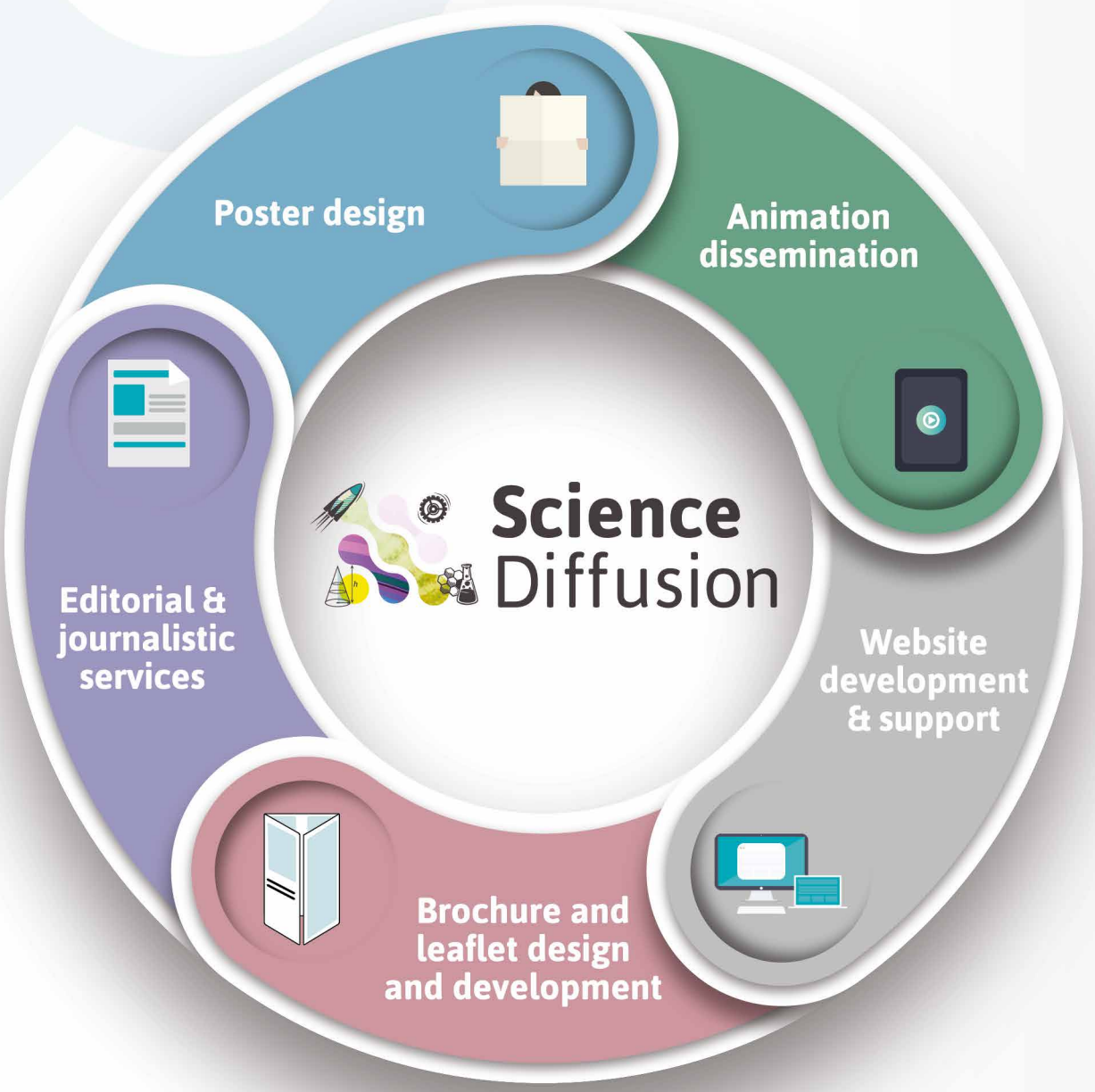
SciPod is moving science communication into the 21st century, providing you with an unlimited, informative and comprehensive series of scientific research audiobooks to keep society and science connected.
So what are you waiting for?
It's free, it's fun, it's only one click away:
www.scipod.global

SCIPOD

For more information, visit www.scipod.global

Do you want to increase the **visibility** and **accessibility** of your **research?**

Contact us today to discuss your dissemination needs and find out how we can help.



W: www.sciencediffusion.com **E:** info@sciencediffusion.com

worldwide cancer research

EVERY DAY, MILLIONS OF LIVES ARE TURNED UPSIDE DOWN BY CANCER

#LivesTurnedUpsideDown

Every day millions of lives are turned upside down by cancer.

Whether they are one of the estimated 1 in 2 people worldwide diagnosed with some form of the disease or one of those forced to watch a much-loved friend or family member fight it…

However, there is hope. Worldwide Cancer Research funds pioneering research projects around the world, working tirelessly to find better ways to prevent, diagnose and treat the disease.

To find out more, please visit www.worldwidecancerresearch.org