Scientia

CELEBRATING DISCOVERY AND INNOVATION IN MEDICAL SCIENCE

EXCLUSIVE:

- Association of Medical Research Charities

HIGHLIGHTS:

- Preventing Rabies: A Deadly but Neglected Disease
- Bacteriophage Hunting: Searching for the Tiny Viruses That Kill Harmful Bacteria
- Understanding Fatigue: The Debilitating Side-effect of Cancer Treatment

Issue: #141

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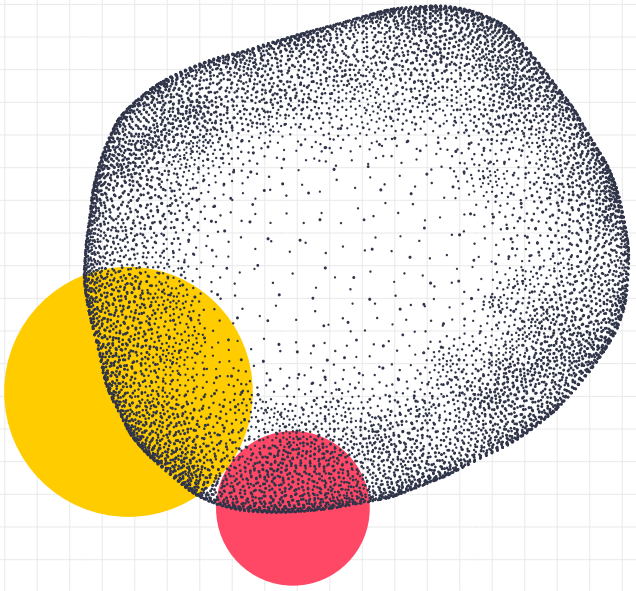

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WELCOME...

This riveting issue of Scientia provides an exciting insight into the future of health and healthcare with a vast array of new discoveries and innovative technologies in medical science.

The first section showcases the dedicated efforts of researchers working to improve the prevention and diagnosis of disease. Here, we can read of vital work conducted at all stages across the lifespan, from preventing sudden unexpected infant death to treating ophthalmic disease in later life. We can also read about the important research aiming to improve health in low- to middle-income countries by targeting rabies, neonatal hypothermia and exposure to environmental contaminants, among other key concerns. We close this section with an exclusive interview with Nicola Perrin, Chief Executive of the Association of Medical Research Charities, where we can read of their vital work saving and improving lives through research and innovation.

In the second section, we showcase the critical work of researchers pioneering the development of new drugs and interventions. From key methodological advances to support drug discovery efforts through to the identification of novel drug targets, we can read of the diverse ways in which the drugs of the future are being advanced. We can also read how gaining a better understanding of the mechanisms underpinning diseases such as diabetes is leading to the development of much-needed treatment interventions.

The third and final section is focused on the researchers boldly confronting the challenge of cancer. From understanding how tumours develop and progress, to how genetics and environmental factors can interact to influence the risk of cancer, to reducing the deleterious side effects of life-saving treatments, we can read of the inspiring, dedicated efforts to overcome this leading cause of death worldwide.

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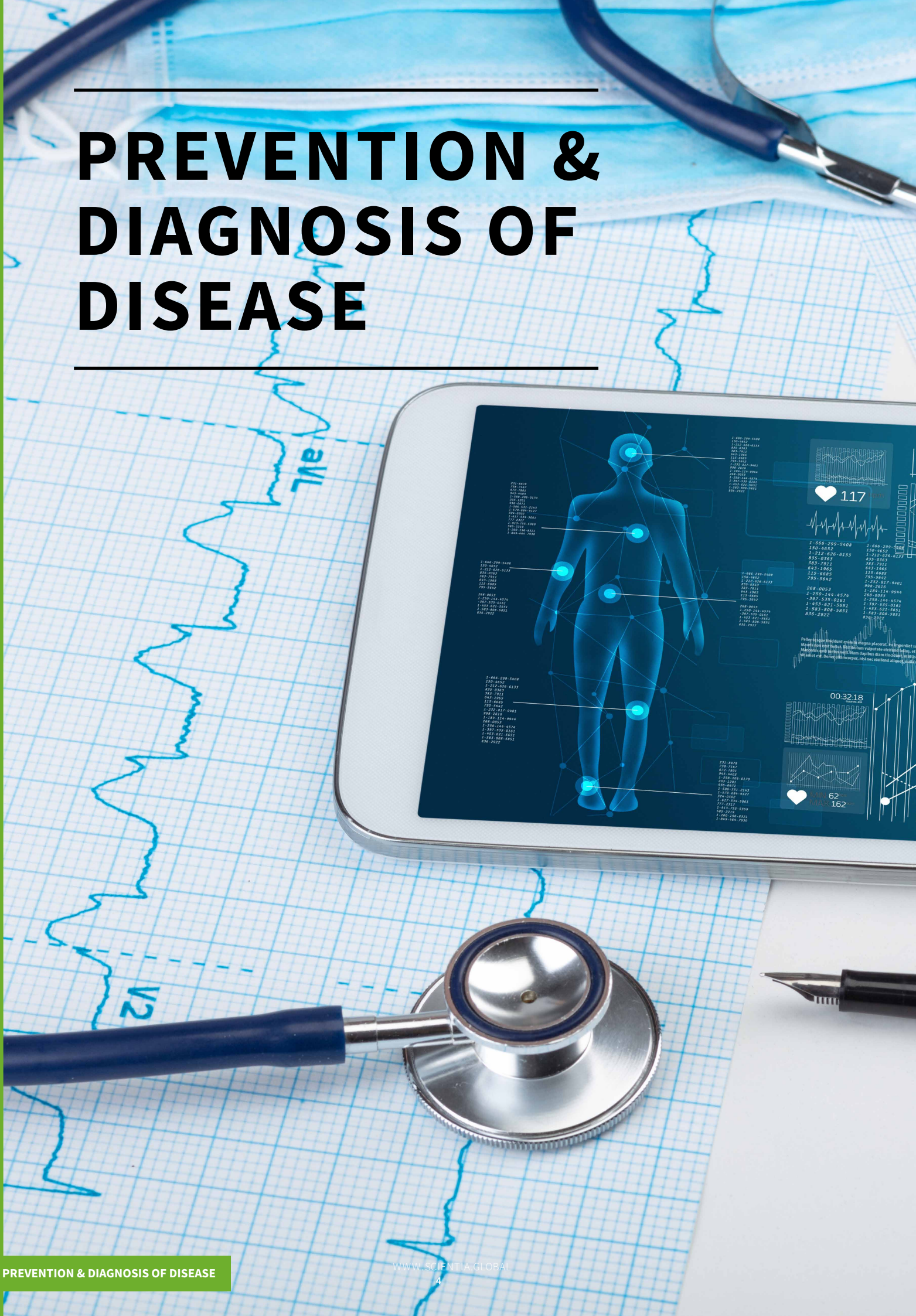

INNOVATION IN THE PREVENTION AND DIAGNOSIS OF DISEASE

Our first section of this issue is dedicated to the researchers committed to improving the prevention and diagnosis of disease. According to the World Health Organization, a significant proportion of disease-related deaths across the globe are preventable. While disease prevention focuses on keeping people healthy (and thus avoiding the need for treatment in the first place), refining the timing and accuracy of diagnosis facilitates more timely and often more effective interventions when needed. By driving change at both the individual and population levels, innovation in the prevention and diagnosis of disease across the lifespan is core to improving health and healthcare as well as reducing associated healthcare costs.

We open this section by meeting Dr Joanne Maki at Boehringer-Ingelheim Animal Health, who works in the important field of rabies prevention. Transmitted from animals to humans through the bite of an infected animal, rabies remains a potentially fatal disease. This is particularly problematic in the developing world, although the tools to eliminate human rabies already exist. Dr Maki has dedicated 30 years to better understanding how rabies can finally be defeated and we can read of her call to action embracing effective vaccination at the core of disease prevention efforts.

Exposure to environmental contaminants is common across the world although the consequences of such exposure are not always sufficiently understood. In Egypt, agricultural workers are frequently exposed to the commonly used pesticide chlorpyrifos when working in cotton fields. Dr W. Kent Anger, Dr Fayssal M. Farahat, Dr Pamela J. Lein and Dr Diane S. Rohlman have created a collaboration spanning several academic institutions to better understand the neurotoxic impact of this exposure. Their findings and recommendations have the potential to bring positive changes to the long-term health of employees working with pesticides and thus prevent future harm.

Another common environmental contaminant is fluoride. In low-income countries in particular, there is sometimes little control over the concentration of fluoride in drinking water. Unfortunately, excessive consumption can have dangerous consequences, including a bone disease known as skeletal fluorosis. We can read how Dr Tewodros Rango Godebo from Tulane University, USA, has used ultrasound technology to determine how fluoride exposure directly leads to reduced bone quality, and how his diagnostic method could be used for a widespread assessment of how fluoride (and other environmental contaminants) may harm bone health at the population level.

We then turn our attention to child health and the inspiring work of Dr Anne Hansen at Boston Children's Hospital, USA. In low- and middle-income countries, neonatal hypothermia is widespread, contributing to around one million baby deaths each year. To help overturn these shocking statistics, Dr Hansen has developed and tested a low cost, non-electrical warming mattress called the 'Dream Warmer'. We can read how her extensive work testing the Dream Warmer in Rwanda shows that it is safe, effective, and ready to help save the lives of young infants.

Our next group of featured researchers consists of Juan Lavista Ferres (from Microsoft), Dr Jan-Marino Ramirez and Dr Tatiana Anderson (both from Seattle Children's Research Institute), and Professor Edwin Mitchell (from the University of Auckland). The focus of their work is sudden unexpected infant death, which is defined as the death of a healthy baby aged under one year that is unexplainable without investigation. We can read how they have formed a unique collaboration to conduct vital and extensive research to better understand and ultimately prevent this tragic occurrence.

One of the most common birth defects across the globe is congenital heart disease (CHD). Although prospects and survival rates are improving, children with CHD are at risk of obesity and other chronic conditions later in life due to the widely held belief that physical activity is risky or even dangerous. We can read how Dr Marta Erlandson from the University of Saskatchewan in Canada has supported the development of CHAMPS, an innovative chronic disease management programme that aims to reduce preventable morbidity in children with CHD through physical activity.

Remaining on the topic of child health, we turn to the diagnosis of disease in babies and children. This can be challenging for clinicians, particularly when the disease is uncommon. Rare childhood diseases are the lifelong focus of Dr Michael Wangler at the Baylor College of Medicine and Jan and Dan Duncan Neurological Research Institute. Dr Wangler combines his expertise in paediatrics and genetics in his cutting-edge research identifying the genes responsible for rare and undiagnosed diseases, and we can read of the implications of his work not just for diagnosis but also for treatment.

Improving and progressing the diagnosis of ophthalmic disease across the lifespan is the specific focus of Dr Eric Buckland at Translational Imaging Innovations, Inc (TII). Dr Buckland has developed purpose-driven software systems to provide researchers with sophisticated tools to manage multifaceted imaging workflows and efficiently organise and analyse complex sets of images and data relating to the eye and vision. We can read how TII is facilitating the future of eye imaging research and dramatically improving health outcomes.

Our next featured researcher is Dr Peter Bretscher at the University of Saskatchewan, Canada. Having dedicated his career to the study of immunology, Dr Bretscher has proposed a novel framework for understanding the prevention and treatment of immune system-related diseases. His theorising is based on the linkage between basic science and medical innovation, and we can read here of the implications of his innovative work.

We conclude our first section with an exclusive interview with Nicola Perrin, newly appointed Chief Executive of the Association of Medical Research Charities (AMRC). The AMRC has united more than 150 medical research charities working across all areas of health and disease in the UK and overseas. We can read about their 2022–2025 strategic plan as well as gain an exciting insight into what the future holds for medical research charities.

PREVENTING RABIES: A DEADLY BUT NEGLECTED DISEASE

Rabies is transmitted from animals to humans through the bite of an infected animal, all too often with fatal consequences, particularly in the developing world. **Dr Joanne Maki**, Technical Director for the Veterinary Public Health Centre at Boehringer-Ingelheim Animal Health, has worked in rabies prevention for 30 years. With extensive background and experience gained in the rabies vaccine industry, Dr Maki shares her perspectives on the call to action to eliminate this deadly zoonotic disease.

The Origins of Rabies

The rabies virus has plagued mankind since the beginnings of recorded history. Its origins are postulated to be an insect virus that adapted to insectivorous bats and over time evolved to infect other mammalian species – including humans.

Dr Joanne Maki, Technical Director for the Veterinary Public Health Centre at Boehringer-Ingelheim Animal Health notes, 'The most striking feature of the rabies virus is how it is transmitted. Upon receiving a bite from a rabid animal, the virus replicates locally and invades local nerves. The infection continues with virus replication in neurons eventually invading the central nervous system and travelling to the brain.'

The infected host develops inflammation of the brain, known as encephalitis. This presents either as paralysis and recumbency or agitation and aggression. Dr Maki further explains, 'It is an evolutionary marvel that the virus modifies the host's behaviour to seek out and bite objects in the environment.' As such, the virus is transmitted in saliva from the infected host to another, and the cycle begins again.

As an RNA virus, the rabies virus replicates with low genetic fidelity meaning it can easily modify its genetic code and as a result, has developed different variants capable of infecting all mammalian species. The variants are identified through genetic sequencing. The fingerprints of the variants are unique by both geography and species. They survive by maintaining low levels of transmission in wildlife populations such as raccoons, foxes, skunks, bats, and mongooses. Although originating in wildlife, rabies has adapted readily to circulate in unvaccinated dogs. Dogs, often dubbed 'man's best friend' are the primary cause of human rabies deaths globally.

Can We Eliminate Rabies?

Dr Maki explains that the tools to eliminate human rabies already exist. Human rabies vaccines are routinely given to people bitten by animals in a treatment called post-exposure prophylaxis (PEP). She notes that injectable inactivated canine rabies

vaccines have proven time and time again to stop dog rabies outbreaks, and that oral rabies vaccines for wildlife have been used to eliminate fox rabies in Western Europe and the canine variant of rabies from the USA.

So, why are humans still dying from rabies? Dr Maki points to a lack of disease awareness, prioritisation of prevention, and a cultural willingness to vaccinate dogs to protect human lives as being the main obstacles. Despite this, almost all human rabies victims (>99%) have been bitten by a rabid dog. An estimated 59,000 human deaths occur each year due to rabies, and these are primarily in countries lacking adequate health care infrastructure. As many as 40% of these deaths are in children under 15 years of age living in Africa and Asia. Sadly, the Centers for Disease Control in the US estimates one person dies of rabies every 9 minutes worldwide.

‘As countries roll out mass COVID-19 vaccination programmes, one can only hope that lessons learned and investments made in health care can have a positive impact on preventing other infectious diseases, such as rabies.’

For these reasons, the World Health Organization (WHO) has classified human rabies as a neglected tropical disease. Shockingly, the global burden of rabies is estimated to be 8.6 billion USD/year, based primarily on human lives lost, the money spent on PEP, and the loss of economic productivity by those impacted by the disease.

The global rabies community is now challenging all countries to unite and invest in rabies prevention and ultimately, eliminate human rabies by 2030. Known as ‘Zero By 30’, this effort is spearheaded by a group called United Against Rabies (UAR). This effort will require a sustained One Health approach to repeatedly vaccinate dog populations against canine rabies in countries in which rabies is rife to dramatically reduce the risk of exposure to humans.

The Importance of Post-exposure Prophylaxis

Adequate post-bite medical care greatly increases the chance of a person surviving a rabid dog bite. In countries with good health care provision, human deaths from rabies are rare and the disease is routinely prevented through PEP. This consists of a series of injectable rabies vaccines and in high-risk bite cases, infiltration of the wound site with anti-rabies immunoglobulin.

Every year, more than 29 million people across the world receive post-bite medications. However, human deaths due to rabies still occur. Insufficient PEP supply in rural hospitals and clinics (such as in Africa) limits the potential number of dog bite victims treated, directly contributing to the death toll from human rabies. Sadly, in some situations, people cannot afford treatment or there are logistical roadblocks such as not being able to take time away from work or lack of transportation to the hospital for follow-up care.

Whether an individual can receive PEP after a dog bite is often geographically and economically driven. Dr Maki explains that WHO, in collaboration with Gavi, the Vaccine Alliance, has committed to providing PEP vaccine doses to low-income countries based on the country’s gross national income. A 2019 epidemiological and economic modelling study confirmed the value of providing anti-rabies vaccines through the potential to prevent an additional 489,000 deaths between 2020 and 2035.

WHO also investigated the impact of using a one-week course of intradermal vaccinations for PEP instead of the current intramuscular route protocol and approved this for human rabies PEP in 2018. However, as Dr Maki notes, the practice has not been uniformly adopted. This slow transition to intradermal PEP administration is thought to be partially due to a lack of proficiency in staffing and valid concerns about potential contamination of multi-use vaccine vials – both of which can be addressed through training. Benefits of intradermal PEP vaccination include the use of a lower dose per treatment and the need for fewer visits, in addition to an overall reduction in cost by 60–80%.

Integrated bite case management (IBCM) is a decision tool developed for physicians treating dog bite victims. In every case, the attending physician must decide if a patient is truly at risk of contracting rabies. If the dog that inflicts the bite runs away or cannot be identified, then the vaccination status of the biting dog is ‘unknown’. In such instances, PEP doses are routinely administered. IBCM gives physicians an algorithm to not choose to administer PEP if the biting dog is known and currently vaccinated against rabies, given that properly vaccinated dogs pose the lowest risk of rabies transmission. Any dog that has bitten a person should be quarantined and observed. If the dog remains healthy after 10 days of quarantine the PEP series of vaccines can be stopped. Dr Maki notes that incorporating IBCM into routine dog bite patient care can prevent unnecessary administration of rabies vaccines, thus improving cost and resource savings.

Critically, the Hampson/Trotter model hypothesises that if all PEP vaccines were given intradermally and integrated bite case management was implemented on a global scale the current supply of human anti-rabies vaccines would be enough to eliminate human rabies.

Development	Current situation	Change envisioned	Benefit
Intradermal PEP protocol	Intramuscular protocol uses much more antigen per doses and more doses	Acceptance and utilization of intradermal PEP protocol by all medical professionals	Increased efficiency of current PEP global supply to prevent human rabies
Funding of PEP vaccines in Gavi-approved countries	Lack of money prevents bite victims from receiving PEP	In Gavi eligible countries, funding will support PEP in areas of need	Improved access to necessary post-bite medical care
OIE Dog Vaccine Bank	Not all countries have access to quality dog rabies vaccines or have the ability to vaccinate during dog rabies outbreaks	Pilot programs using quality vaccine and training will lead to in-country support and increase mass dog vaccination programs	In-country cost/benefit justification of mass dog vaccination using quality vaccines will make dog programs sustainable
Vaccinated dogs wearing collars reduce unnecessary PEP	Uncollared dogs that bite people may or may not have rabies; so PEP is given as a precaution regardless of dog vaccination status	Properly vaccinated dogs wearing collars are less likely to transmit rabies, so if they bite a person PEP is avoided thereby saving resources and lowering medical costs	PEP doses are conserved and the bite victim does not undergo unnecessary treatment
The human/animal bond is improved by vaccinating and collaring community dogs	Dogs not wearing collars are a rabies risk and may be mistreated or killed	Dogs vaccinated against rabies and wearing a collar are viewed as protectors and companions	The welfare of community dogs is increased, and the number of dog bites is decreased

Table 1. Recent developments supporting the elimination of human rabies by 2030.

The Canine Rabies Vaccine Bank

Since vaccinating a sufficient number of dogs each year is critical to eliminating human rabies, the World Organization for Animal Health (OIE) created a canine rabies vaccine bank for countries, non-government organisations and other third parties interested in conducting mass dog vaccination programmes. To date, OIE has delivered 12.5 million vaccine doses to more than 22 countries (principally in Asia and Africa). Since the inception of the vaccine bank, Boehringer-Ingelheim Animal Health has provided doses of their injectable rabies vaccine RABISIN. Dr Maki is particularly proud of this contribution, not least because obtaining quality vaccines for mass dog vaccination programmes by individual countries can be financially very challenging.

Dr Maki hopes that as canine rabies comes under control, governments and policymakers will realise the societal benefits of healthier animals and fewer human deaths due to rabies. As rabies prevention is recognised as being an economically sound practice, mass dog rabies vaccination programmes become routine and canine rabies outbreaks prevented. As recently demonstrated in Mexico, a sustained and well-orchestrated canine rabies prevention programme can eventually eliminate human rabies deaths. As such, Mexico was recognised by WHO for eliminating human deaths due to canine rabies in 2019. RABIFFA, another Boehringer-Ingelheim Animal Health rabies injectable vaccine, was used for many years in this programme.

Recent Developments

Dr Maki recognises five recent advancements that have significantly bolstered the steps to eliminating human rabies. Three of these initiatives impact the delivery of PEP as discussed above: the funding of human rabies vaccines in impoverished countries, the implementation of IBCM, and the transition from intramuscular administration of PEP vaccines to intradermal delivery. The other two actions supporting effective mass dog vaccination are using collars to identify vaccinated dogs and the creation of a canine rabies vaccine bank (see Table 1).

Looking to the Future

Taking a reflective stance, Dr Maki suggests that only time will tell if the challenges caused by the COVID-19 global pandemic will bring a suitable investment in public health infrastructure and disease prevention to the world's stage. Can a positive outcome of our current suffering be the realisation that brings equality to improved access to vaccines and medical care? Dr Maki asserts, 'As countries roll out mass COVID-19 vaccination programmes, one can only hope that lessons learned and investments made in health care can have a positive impact on preventing other infectious diseases, such as rabies.'

Dr Maki further reflects that 'While we wait for better times, we can continue to raise awareness about rabies and do our best to vaccinate dogs. We can commit to building a global network that shares the benefits of intradermal PEP and IBCM to improve medical post-bite care.' Clearly, such changes are not complex and can greatly improve a person's chance of not dying of rabies. By working together in a One Health mindset, Dr Maki believes we can implement these changes and move closer to the global elimination of human rabies arising from the canine variant.

Meet the researcher

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Dr Joanne Maki is the Technical Director for the North America, Veterinary Public Health Center at Boehringer-Ingelheim Animal Health. She is a recognised global technical expert in rabies and rabies prevention. Her areas of expertise include supporting programs focused on the elimination of human rabies transmitted by dogs and wildlife rabies prevention. Dr Maki received her Doctor of Veterinary Medicine from Louisiana State University and her PhD from the University of Georgia. She was a National Institutes of Health principal investigator at the University of Georgia, Athens from 1996 to 2002 where she developed a model to study mucosal immunity. As a pharmaceutical technical director, she led efforts to license three commercial veterinary rabies vaccines including a non-adjuvanted injectable recombinant vaccine for cats, an oral vaccine for wildlife and a combination vaccine for horses.

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FURTHER READING

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ESTABLISHING THE NEUROTOXIC IMPACT OF CHLORPYRIFOS EXPOSURE IN WORKERS

Chlorpyrifos (CPF) is one of the most commonly used pesticides in the world. Agricultural workers in Egypt have relatively high levels of exposure to it when working in the cotton fields but until now, the neurotoxic impact of this has been uncertain due to a lack of evidence linking CPF dose and neurotoxicity. **Dr W. Kent Anger**, **Dr Fayssal M. Farahat**, **Dr Pamela J. Lein** and **Dr Diane S. Rohlman** have brought together their respective research expertise to collaborate on this issue. Their findings have the potential to greatly improve the long-term health of employees working with pesticides.

Chlorpyrifos: An Effective Pesticide

Organophosphorus pesticides (OPs) are the most common class of insecticides used in agriculture globally, and include a compound called chlorpyrifos (CPF). Sprayed on plants, animals and even buildings, CPF is used to kill pests by disrupting their nervous system. CPF inhibits the enzyme acetylcholinesterase (AChE), which would normally break down the neurotransmitter acetylcholine. This results in a build-up of acetylcholine in between neurons in the brain, which eventually stops the neurons from signalling. This build-up, known as acute cholinergic toxicity (or chlorpyrifos poisoning) causes the targeted insects, worms and other pests to die.

Although very effective at killing pests, CPF is also hazardous to the humans who apply it to agricultural crops. The same mechanism that causes acute toxicity in insects can also cause acute cholinergic toxicity in humans. However, a large amount of research suggests that repeated exposure to lower doses of OPs that do not cause acute cholinergic toxicity may cause a variety of neurological issues via mechanism(s) other than AChE inhibition. These range from psychiatric conditions including depression to neurodegenerative diseases like Alzheimer's disease and deficits in cognitive functioning.

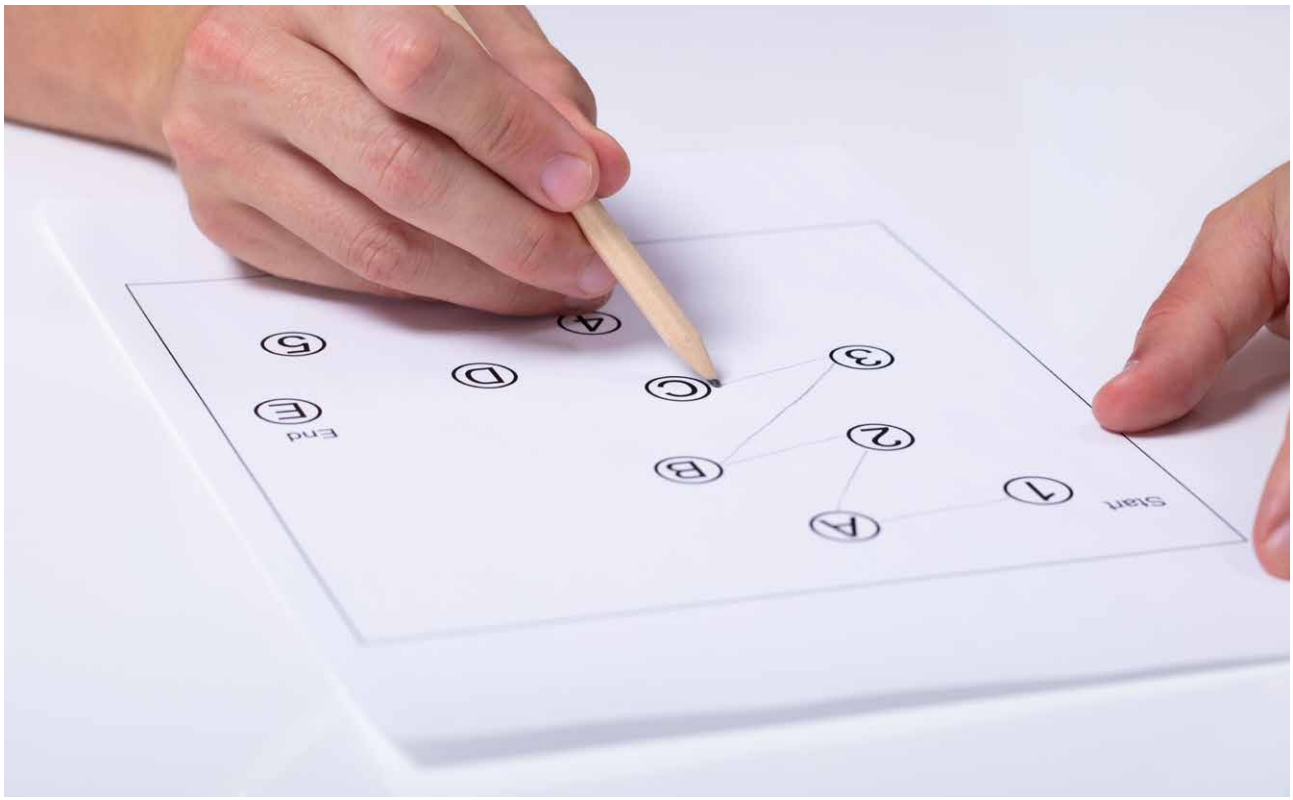
However, in the past, not all scientists have agreed that CPF is neurotoxic at doses that do not cause acute cholinergic toxicity due to a lack of evidence supporting a dose-response relationship, which is considered a central tenet of toxicology. In other words, there has been little evidence to confirm that the likelihood of a neurotoxic response to CPF increased as the levels of CPF exposure increased – a dose-response relationship. It was also unclear whether the classic biomarkers used to assess OP exposure in humans, specifically, cholinesterase activity in blood or levels of OP metabolites in urine, were relevant as biomarkers of occupationally-induced CPF neurotoxicity. Biomarkers are characteristics that can be measured to indicate biological processes occurring within the body in response to contaminants or interventions.

Cotton fields.

Establishing a definitive set of aims to investigate, Dr W. Kent Anger, Dr Fayssal M. Farahat, Dr Pamela J. Lein and Dr Diane S. Rohlman and their colleagues collaborated on an elegant set of studies to confirm the occupational health risks of CPF and to make suggestions for improvements to working conditions to mitigate health risks from CPF.

Patterns of Exposure

The first aim of the group was to establish patterns of exposure to CPF, which was achieved by Dr Farahat and his team studying different types of agricultural workers in Egypt. Based in the Nile delta region, the Ministry of Agriculture oversees cotton fields where they employ a number of different workers in different roles to ensure pest



Cognitive function assessment.

control. There are typically three groups of workers involved in the pesticide application process: applicators who spray the pesticide, technicians who walk in the fields to direct the applicators where to focus the spray, and engineers who usually stand to the side and oversee the operation.

The research team determined how much CPF each group of workers was exposed to by measuring levels of a molecule called 3,5,6-trichloro-2-pyridinol (simplified to TCPy) in their urine samples. TCPy is produced by the body during the metabolic breakdown of CPF, then eliminated in the urine. So, higher levels of TCPy in a sample indicates a higher intake of CPF. Applicators were found to have the highest levels of CPF exposure by far, followed by technicians and then engineers who had the lowest levels.

A previous study had shown that in these agricultural workers, CPF primarily enters the body through the skin (dermally), rather than from inhalation in the lungs. When the pesticide is being applied to cotton fields, the applicators walk through the plants they have just sprayed and as a result, their clothes and skin have sustained contact with the pesticides. Consequently, dermal exposure on their legs was found to be particularly high and, due to leakages on some of the applicators' backpack sprayers, some workers also experienced high exposure on their necks and backs.

The Effect of Dose on Behavioural Tests

Neurobehavioural tests provide a key tool for quantifying the biological effects of CPF on brain function in humans. In the next aim headed up by Drs Anger and Rohlman, behavioural tests were used to assess cognitive functioning and how this may be related to workers in different jobs exposed to differing levels of CPF. An established psychological assessment tool, the Trail Making Test, was used to assess participants' motor and cognitive speed, as well as their mental flexibility. The test consists of two parts, Trail Making A and B, with the latter being more mentally challenging.\ Participants included applicators, technicians and engineers as well as a control group that had experienced no occupational exposure to CPF. Participants were tested several times using Trail Making A and B throughout the summer growing season. The poorest performance was observed for the applicators who wore the backpack sprayers and who had contact with wet foliage. They were followed by the technicians who walked in the fields in front of the applicators, and then the engineers, who remained on the edges of the field during the application of CPF. The best performance throughout was observed for the control group, who had minimal exposure to CPF.

These results were clear – participants with the most contact with pesticides performed most poorly on the Trail Making Test. Testing of TCPy in the urine samples of participants further confirmed the dose-response between cognitive function and exposure to CPF based on job roles.

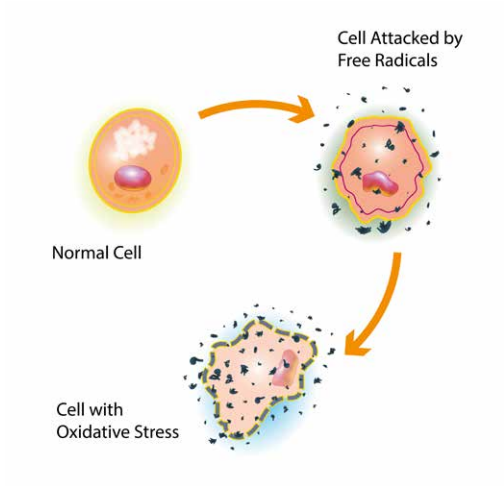


Illustration of oxidative stress.

Biomarkers

The team investigated potential biomarkers – TCPy in the urine, as well as AChE and butyl cholinesterase (BuChE) activity in the blood – and their relationship with human behavioural performance. However, they did not see a relationship between AChE and BuChE in blood samples and test performance, even though it is known that levels of these enzymes are reduced in the blood following repeated CPF exposure.

This knowledge allows us to use them as a biomarker for recent exposure, but the team concluded that they are not relevant biomarkers to determine the neurological effects of long-term, repeated exposure to CPF. That is, the repeated doses over time created damage that was measured by the behavioural test. The urinary TCPy measures reflect only the current year exposures, which accurately reflected the exposure differences between the job titles, but the behavioural test results were due to cumulative damage over many years of differing exposure levels, so any year's urinary measures would likely not, and did not, correlate directly with individual test performance in that year.

When the human CPF exposure conditions were mirrored in rat studies led by Dr Lein, similar biological effects (reduced AChE in the blood and reduced brain function as assessed by behavioural tests) were observed in the exposed rats. Using this model, Dr Lein's group identified one set of biomarkers that do appear to be linked to OP-induced neurotoxicity – those caused by oxidative stress. An imbalance between the production of reactive oxygen species in cells and tissues and the cellular mechanisms for detoxifying them causes oxidative stress. In humans, this can occur as a result of lifestyle choices like smoking, but as the team investigated here, it is also an effect of exposure to chemicals like pesticides.

Oxidative stress is frequently associated with cognitive impairment, neurodegenerative disease and now with neurotoxicity caused by OPs. Increased lipid peroxidation, protein nitration and decreased antioxidant capacity are all examples of these biomarkers. If they are proven to be indicators of OP-induced neurotoxicity in humans, this could be a useful way to diagnose neurologic damage in OP-exposed individuals and implement intervention strategies quickly.

Implementing Safer Work Practices

Gathering all of their findings over the years, Dr Anger and the team have endeavoured to use them to implement safer conditions in occupational settings. First of all, led by Dr Farahat, focus groups were held separately with applicators, technicians and engineers to provide education on their findings and discuss the use of personal protective equipment.

Employees were also given practical advice on how to better protect themselves from CPF. Rather than walking into crops that had just been sprayed with pesticide, they were encouraged to spray away from themselves and not walk into the plants. Plastic chaps made from cheap and easily accessible materials were demonstrated, which prevents CPF from coming into contact with the workers' skin on their legs. Chemical-resistant and protective clothing such as shoes and gloves have also been suggested as ways to reduce exposure, although these are expensive. During the intervention, employees were encouraged to reduce exposure, for example, by using a stick to mix pesticides.

Emphasising that these approaches are both inexpensive and simple, the researchers have helped employees working with pesticides to learn how to alter their routines to better protect themselves and their nervous systems from toxicity. As such, the dedicated work by Dr Anger, Dr Farahat, Dr Lein and Dr Rohlman on CPF exposure promises to lead to positive changes in many workers lives in the years to come.

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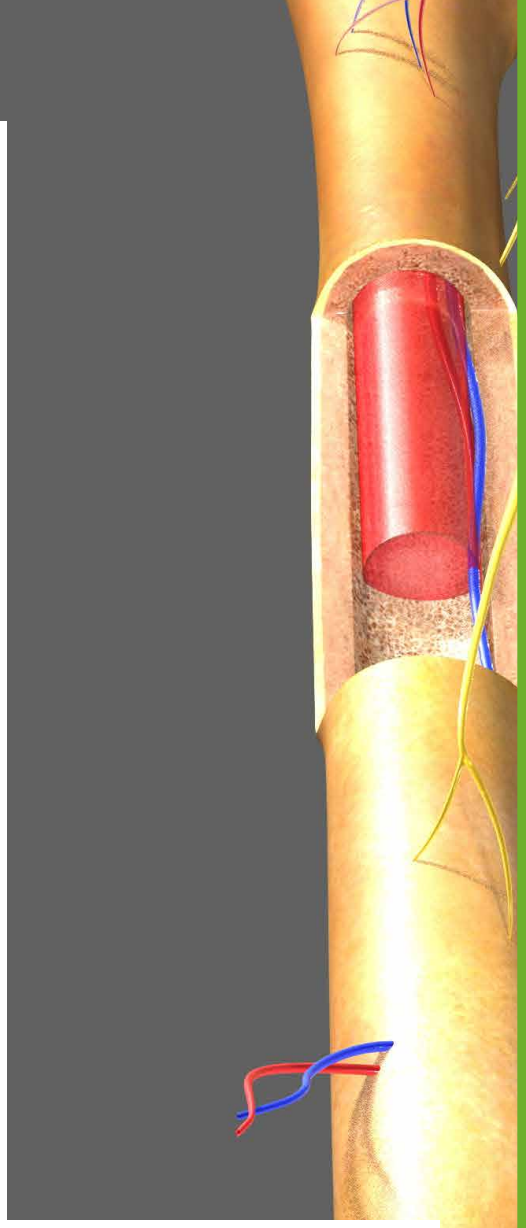
USING INNOVATIVE TECHNOLOGY TO INVESTIGATE BONE DISORDERS INDUCED BY ENVIRONMENTAL CONTAMINANTS



Throughout the world, especially in low-income countries, environmental contaminants such as fluoride have huge health consequences. While some countries add fluoride to their drinking water, others have little control over its concentration, resulting in dangerously high fluoride concentrations. Excess fluoride exposure is known to cause the bone disease skeletal fluorosis, but **Dr Tewodros Rango Godebo** from Tulane University in Louisiana, USA, believes that current diagnosis techniques are incomplete. Using ultrasound technology, he has determined for the first time how fluoride exposure leads to reduced bone quality, and how this diagnosis can be used in fluoride-exposed populations.

Fluoride in Our Water and Our Bodies

Our bodies are constantly exposed to mixtures of chemicals in the surrounding environment. The levels of each often depend on location and local regulations, meaning some people experience toxic levels and are consequently at high risk for health complications.

One such element that humans frequently consume is the negative ion of fluorine, called fluoride. Although it is found naturally in varying amounts, in many areas, fluoride is added to drinking water to help reduce tooth decay and improve dental health. However, an excess of fluoride consumption is linked to a variety of disorders. For example, if a child's teeth come into contact with too much fluoride while they are still developing, a condition called dental fluorosis may occur. Individuals with mild cases experience white marks on their teeth, whilst those with severe cases have discoloured and badly pitted enamel on the teeth surface. A severe form of enamel fluorosis is shown in Figure 1. This image is of a 14-year-old boy who grew up with 13mg/L of fluoride in drinking groundwater in the Ethiopian Rift Valley and who, as a result, experienced significant loss of enamel.

The skeletal system is most at risk for adverse effects resulting from fluoride over-consumption. Fluoride becomes incorporated into bone tissue which can cause a multitude of issues, depending on how much fluoride is ingested, how long an individual is exposed for and from what age, as well as genetic factors. For example, physiological processes can be disrupted, meaning the bone tissue is not maintained and repaired properly. This leads to a much higher risk of fractures and breakages. Even neurological development and functions of the reproductive system and cardiovascular system can be altered by the presence of excess fluoride.

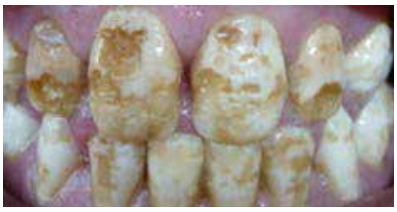
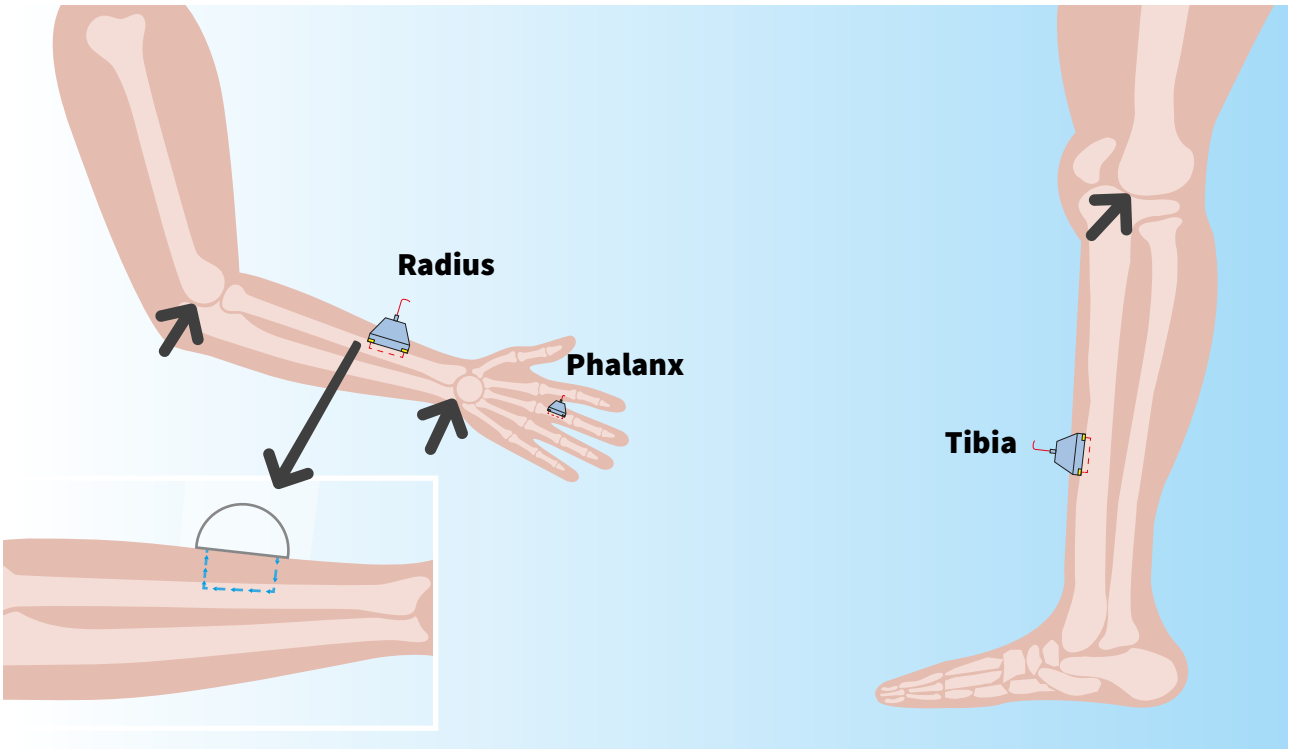
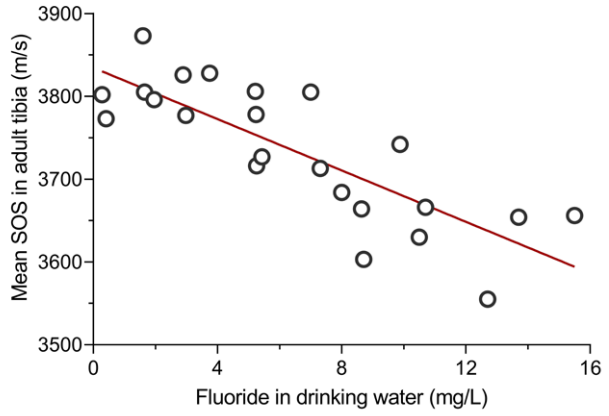


Figure 1. Enamel fluorosis in a 14-year-old boy. Credit Tewodros Godebo.

This surplus fluoride in the body changes both the physical and chemical properties of the bone minerals and the bone and enamel cells. If an individual is exposed for a sustained period at high fluoride levels while their bones are growing or repairing, they are highly likely to develop a disease called





Mean community-level SOS among adults and fluoride concentrations in drinking water from the sample communities in the Ethiopian Rift Valley.
Reproduced from TG Rango, et al., Bone quality in fluoride-exposed populations: A novel application of the ultrasonic method, Bone Reports, 2020, 12, 100235, under Creative Commons CC-BY-NC-ND license.
Credit: Tewodros Godebo

chronic skeletal fluorosis. This issue is one focus of Dr Tewodros Godebo's research at Tulane University in New Orleans, Louisiana. Serving as an Assistant Professor in the School of Public Health and Tropical Medicine, Dr Godebo studies long-lasting exposure to low- and high-fluoride and subsequent chronic skeletal fluorosis using novel diagnostic methods.

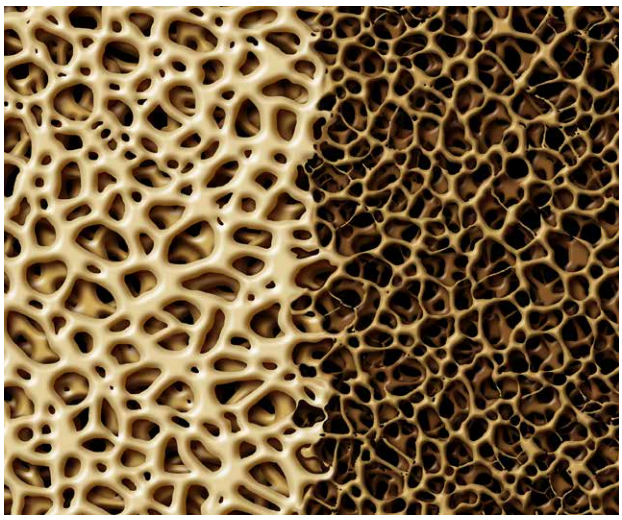
Chronic Skeletal Fluorosis

This chronic disease presents various bone complications and joint, tendon and ligament calcification that progressively worsen. This deposition of calcium crystals on the tissues that connect bone to muscle (tendons) and bone to bone around the joints (ligaments) leads to chronic pain and disabling movement in the long term. The hallmark of skeletal fluorosis is osteosclerosis – the thickening of tissue resulting in an abnormally thick bone. The disease also results in damage called bone lesions and osteoporosis which is the weakening of the bones.

Skeletal fluorosis also impacts the large, tightly bound molecules that help keep the bone tissue together, known as matrix proteins. Collagen is an example of one of these proteins, and when altered due to disease, the elasticity and biomechanical integrity of the bone is damaged. This means that people suffering from skeletal fluorosis have a very high tendency to suffer from fractures and breaks, which can be exacerbated by the effect of fluoride on the function of the parathyroid glands. This results in hyperparathyroidism, which means that the hormones that normally regulate the calcium concentration in the body are imbalanced. The consequent depletion of calcium in the bones further diminishes their flexibility and thus increases susceptibility to fractures.

The physical structure of bone tissue is complex as it includes an intricate network of mineralised fibrils, cells, proteins and water. The different bone parameters, from its density and collagen composition to the microstructures inside, all influence how resistant a bone is to fracture. These components come together to determine the mechanical properties of bone.

Therefore, even though it is well-understood how fluoride impacts bone forming and resorbing cells, it is unclear how it



Bone with osteoperosis.

affects bone quality in humans. A skeletal fluorosis diagnosis still depends on using X-ray imaging to determine bone density. However, this method of testing does not account for alterations to the microstructures, collagen composition and elasticity due to fluoride exposure. This is where Dr Godebo is making strides in his field by investigating how innovative technology can be used to improve the diagnosis of chronic skeletal fluorosis.

An Ethiopian Population with High Exposure to Fluoride

In an important study, Dr Godebo and his colleagues analysed data from a population of 341 participants, aged 10–70 years old, living in the rural location of the Rift Valley in Ethiopia. Around 14 million of those living in Ethiopia are thought to be at high risk of dangerous levels of fluoride exposure. However, the Rift Valley region experiences an unusually high exposure to natural fluoride from the groundwater wells for which they depend on their drinking and cooking water.

A wide range of fluoride concentrations in the drinking water was found, spanning from 0.3 mg/L to 15.5 mg/L. To put this into context, the typical low level of fluoride in most high-income countries (including where it is added to prevent tooth decay) is 0.7 mg/L and the World Health Organization recommends that drinking water should not contain a fluoride concentration any higher than 1.5 mg/L. This means that shockingly, some people were ingesting ten times the recommended amount of fluoride. This was represented in urine samples from the participants which showed an unusually high concentration (up to 39.5 mg/L) of fluoride, suggesting a significant amount of fluoride is also retained in the bone.

The team used a non-invasive, portable ultrasound method to measure the bone density of the population in the first study of its kind. All the previously mentioned parameters of the bone quality (density, collagen and microstructure) affect the speed of sound (SOS) measurement. Because there was previously no existing research on using ultrasound and SOS to measure the bone quality in groups with varying levels of exposure to fluoride, Dr Godebo aimed to investigate how useful and applicable it could be in real-world situations.

Before beginning the experiment, Dr Godebo hypothesised that SOS would increase as bone density increased due to fluoride exposure. This is because the X-ray diagnosis technique that is used in countries where skeletal fluorosis is common tends to detect increased bone density (osteosclerosis) in these patients.

An Unexpected Finding

Dr Godebo measured the SOS in a type of bone tissue called cortical bone, which is a protective, dense outer surface layer of tissue that surrounds the internal cavity. It comprises nearly 80% of the skeleton and is dense and solid, making it a good target for measuring bone quality and strength. They tested the tibia in the lower leg, the radius in the forearm and phalanx in the fingers (see illustration above titled *'Bone with osteoperosis.'*

Intriguingly, Dr Godebo discovered that his initial hypothesis was incorrect and that higher fluoride exposure in fact decreased SOS measurements in all three of the cortical bone sites. This suggests that because ultrasound measurements are able to measure and characterise the overall quality and inner properties of the bone, these decreased measurements actually indicate the deterioration of the bone. According to Dr Godebo, this reveals that the physical effects of excessive fluoride exposure are complex and diverse, as is bone quality.

Most importantly, it was found that individuals sourcing their drinking water from community wells with elevated levels of fluoride had significantly lower bone quality compared to those from communities with low fluoride levels. Dr Godebo's work has shown that this fluoride-induced bone deterioration can be confirmed and quantified in rural settings, outside of medical establishments. X-ray techniques are relatively expensive, they expose patients to potentially dangerous radiation, and are difficult or even impossible to use in remote settings. In comparison, Dr Godebo's ultrasound method is portable, low-cost and effective. As such, the method would be useful for a widespread assessment of how fluoride and other environmental contaminants may harm bone health at the population level. However, it will be important to test the feasibility of this as a diagnostic method through additional research in clinical settings and other locations.

Dr Godebo's research is a vital component of understanding how fluoride impacts the health and lives of people who have little control over their water source and how this might be investigated in the future. Furthermore, this work and methods have the potential to be applied to other environmental contaminants as well as other induced bone disorders.

Meet the researcher

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Dr Tewodros Rango Godebo is an Assistant Professor at the Department of Environmental Health Sciences, School of Public Health and Tropical Medicine. He received his PhD in Environmental Geochemistry from Ferrara University, Italy. Prior to joining Tulane, he was a postdoctoral fellow in the Nicholas School of the Environment, Duke University. His research interests lie in identifying the origins and mechanisms of water-soil-food contamination, as well as utilising and developing elemental biomarkers to understand the links between exposure to contaminants and human health.

EXPERTISE

Environmental Geochemistry, Water and Food Quality, Biomarkers and Health

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DREAM WARMER: AN INNOVATIVE, SAFE AND EFFECTIVE COMPLEMENT TO SKIN-TO-SKIN CARE FOR NEONATAL HYPOTHERMIA

Dr Anne Hansen is the Medical Director of the Neonatal Intensive Care Unit and Associate Chief of the Division of Newborn Medicine at Boston Children's Hospital. With her colleagues, Dr Hansen has developed and tested a low cost, non-electrical warming mattress called the 'Dream Warmer' to help prevent and treat neonatal hypothermia in countries with limited medical resources. Her team has conducted extensive testing in Rwanda with results demonstrating that this medical device is safe, effective and ready for use on a wider scale.

Neonatal Hypothermia

Neonatal hypothermia occurs when a newborn baby's body temperature drops below 36.5°C, and brings with it the risk of serious adverse effects on the baby's health and even death. Babies who do survive may suffer from stunted growth, which can also impair brain development. While the ability to keep babies warm is taken for granted in rich countries, this can be surprisingly challenging in low and middle-income countries (LMIC), resulting in widespread neonatal hypothermia. Something of a silent killer, neonatal hypothermia is estimated to contribute to around 40% of, or one million baby deaths each year in LMIC.

Sick and premature infants are more likely to be affected. These babies rely on external heat sources to maintain a normal core body temperature. For preterm babies, it may take weeks to months for them to be able to maintain a normal temperature on their own. In countries with good medical resources, at-risk babies are placed in incubators or on warming tables. However, in LMIC these resources are often not available; neonatal hypothermia is estimated to occur in up to 85% of babies receiving hospital care in LMIC.

Dr Anne Hansen is the Medical Director of the Neonatal Intensive Care Unit and Associate Chief of the Division of Newborn Medicine at Boston Children's Hospital. She has spent over a decade working with medical professions in Rwanda and through this work, recognised the overwhelming need for better neonatal care and treatment of hypothermia in LMIC.

Current Interventions

Currently, the World Health Organization recommends that Kangaroo Mother Care (KMC) should be used to provide heat for low birth weight infants. This method involves placing the baby directly on a family member's chest (usually the mother's) using direct skin-to-skin contact to provide warmth, as a

Credit: Anne Hansen

mother kangaroo would do for the 'joey' in her pouch. This method is low cost and effective, also helping with bonding and milk production.

Dr Hansen fully supports this method as the gold standard for thermoregulation. She also acknowledges that mothers need to be supported to succeed at KMC because it heavily relies on the mother to provide a continuous source of heat over a potentially long period of time. There are scenarios in which KMC does not provide sufficient heat, and when the mother cannot be available for KMC due to illness or the need to conduct incompatible activities. In addition, if a baby is unwell, he or she may require

Credit: Anne Hansen

medical examinations or interventions during which KMC would be difficult or infeasible. In response to these challenges to KMC, Dr Hansen and her colleagues looked to develop a low cost, non-electric warming device.

The team initially undertook a review to see if any adequate products were already in existence. They identified two neonatal warming devices, and these had similar specifications. However, several issues were identified involving preparation, cleanliness, compatibility with KMC, ability to access an infant for assessment or treatment, and finally, cost. Dr Hansen and her colleagues realised that to overcome these deficiencies they needed to develop and test a new product. This led to their collaboration with the Rwanda Ministry of Health to design and test an infant warming device.

Dream Warmer

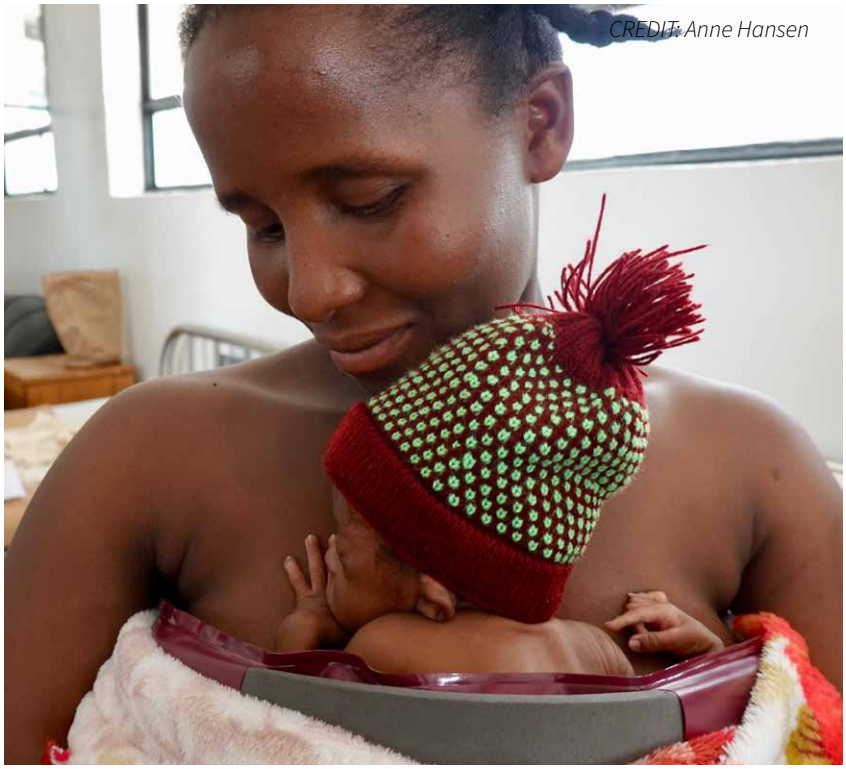
Dr Hansen and her colleagues developed the Dream Warmer, a reusable mattress that is low cost and does not require electricity, operating similarly to a heating pad. The warmer is made of a wax phase change material that changes from liquid to solid at skin temperature. This plastic-encased, wax-based mattress is placed in a thermos of hot water for around 30 minutes until the wax is melted, and then into an insulating sleeve. Once the temperature indicator shows that it is at a safe temperature to be used, the baby can be placed directly on it like a mattress, or it can be wrapped around the baby's back during KMC to provide additional heat. It stays at skin temperature for around 6 hours.

Unlike other warmers, the Dream Warmer allows medical staff and parents easy access to the baby, and because it has no attached fabric, it can be cleaned and reused even in settings without diapers or washing machines. The mattress is easy to prepare, clean and maintain; simple instructions are provided on the insulating pad and thermos to ensure comprehension even by those with low literacy skills. It can also be used in the delivery room for neonatal resuscitation, on transport to a higher level of care, or in the home setting.

Promising Results for Dream Warmer

Initially, the team undertook two pilot studies to test the safety, efficacy and feasibility of using the Dream Warmer. This testing took place in rural Rwandan hospitals and health centres where resources are often limited. In the first pilot study, published in 2018, the Dream Warmer was used on 102 different occasions with a total of 33 hospitalised infants who were either hypothermic or at risk of developing hypothermia based on their low birth weight. These early results were very promising: hypothermia was corrected in 95% of cases and prevented in 100% of at-risk infants.

In 2019, Dr Hansen and her team published the results of a second pilot study, this time in the health centre setting, and



CREDIT: Anne Hansen

included interviews with mothers and nurses to understand their experiences with the warmer. Mothers reported that the warmers worked well and they liked being able to remain close to their babies and continue to breastfeed. Nurses reported that the warmer allowed good access to the baby and was beneficial for resuscitation. Both caregivers and nurses found the warmer intuitive to use and effective in keeping babies warm.

The biggest concern that was reported by some nurses was that they found the preparation time challenging. Dr Hansen has built this feedback into educational materials, emphasising the value of preparing the warmer in advance. In addition, after multiple uses, the research prototype warmers showed some signs of wear and tear leading the research team to find stronger materials and manufacturing techniques as they move to commercially available devices.

Building on Success

Following these two successful trials, Dr Hansen and her team brought the Dream Warmers to ten hospitals in a phased approach called a 'cluster randomised stepped wedge' study design. This approach was chosen because it is more robust and efficient for introducing a novel intervention in multiple sites in a low resource setting compared to the more common method in which all sites implement the intervention simultaneously. The researchers compared data obtained before and after introduction of the warmer, which from an ethical perspective avoided the need to deny infants use of the warmer once it was available. All infants were eligible to use the intervention if they either had, or were at risk of hypothermia based on low birth weight.

As with the previous studies, mothers were encouraged to use KMC alongside Dream Warmer. The primary outcome was the infants' temperature, assessed by a nurse every 30 minutes until it had returned to normal, and then hourly. The secondary outcomes were survival to discharge, safety and feasibility.

Throughout the study period, 464 babies used the warmers on a total of 892 different occasions. In around half of these encounters, the warmer was being used by low birth weight infants. The results were once again very positive with the primary outcome showing that when the Dream Warmer was used, 79% of babies achieved euthermia (normal temperature), compared to 59% without. Interestingly, infants who did not use Dream Warmer also experienced an increase in rates of euthermia. A potential explanation is that this study increased the awareness of hypothermia, leading to the improved use of other interventions such as KMC and hats to improve the temperature of at-risk babies.

In terms of the secondary outcomes, mortality rates were much lower in those who had used Dream Warmer at least once; specifically, those who used the warmer had a mortality rate one third that of those who never used the warmer. Regarding safety, there were no adverse side effects such as burns, rashes or other skin irritation. Feasibility was also very good, with nurses having no instances of incorrect preparation, usage or cleaning of the warmer. The only problem noted was some minor leaking of wax on the inside layer of the plastic mattress (but with no wax leaking outside of the mattress).

Future Implementation of Dream Warmer

This was the first time a large-scale study had assessed a low-cost infant warming intervention. Overall, the Dream Warmer demonstrated good effectiveness, safety and feasibility, having undergone over 1,000 uses across all three studies. No adverse safety effects were identified and the warmer left only 8% of babies hypothermic. 'Based on our extensive testing, we now have very strong evidence to support using the Dream Warmer on a wider scale. We are confident that this frugal technology will be an invaluable addition to the treatment of hypothermia in LMICs. With the appropriate equipment, neonatal hypothermia is a preventable condition and should be a key focus to reduce neonatal mortality and ensure that these vulnerable patients not only survive but thrive' says Dr Hansen.

Meet the researcher

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Dr Anne Hansen is the Medical Director of the Neonatal Intensive Care Unit and Associate Chief of the Division of Newborn Medicine at Boston Children's Hospital. She is a graduate of Harvard Medical School and completed a residency in paediatrics at Boston Children's Hospital, as well as a Fellowship in Neonatology at the Joint Program in Neonatology of Boston Children's Hospital and Harvard Medical School. Dr Hansen's research focuses on improving the care of seriously ill infants with an emphasis on global health, medical device development, and innovative approaches to treat neonatal conditions. Since 2010, she has worked in collaboration with the Rwanda Ministry of Health and Partners In Health to develop and implement their National Neonatal Protocol. Dr Hansen has developed a medical device called the Dream Warmer, to reduce neonatal hypothermia in countries with limited medical resources.

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FUNDING

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HARVARD UNIVERSITY

UNDERSTANDING SUDDEN UNEXPECTED INFANT DEATH: A UNIQUE COLLABORATION

When a supposedly healthy infant passes away, it can be hard to understand why. **Juan Lavista Ferres** (Microsoft), **Dr Jan-Marino Ramirez** and **Dr Tatiana Anderson** (both from Seattle Children's Research Institute), and **Professor Edwin Mitchell** (University of Auckland), form the core of a novel collaboration to conduct vital and extensive research into the risk factors and mechanisms behind sudden unexpected infant death. This unique collaboration spanning across disciplines, industries and continents, is providing the deeper understanding that is needed to prevent unnecessary infant deaths.

The Tragedy of Sudden Unexpected Infant Death

Sudden unexpected infant death (SUID) is the tragic occurrence of the death of a healthy baby aged under one year old that is unexplainable without investigation. Sudden infant death syndrome falls under the umbrella of SUID in addition to accidental suffocation, often during sleep, and other ill-defined causes.

The exact reasons for SUID are not known but the risk factors include maternal smoking during pregnancy and around the baby once born, premature birth, environmental stresses, socioeconomic disadvantage and ethnic differences. In order to prevent SUID, measures such as placing a baby on their back to sleep and in their own bed, avoiding thermal stress from excess bedding or clothing, breastfeeding and use of a pacifier are recommended.

Over 3,700 infants die from SUID each year in the USA alone, a tragic statistic that emphasises the necessity of dedicated research. The team of collaborators leading this vital work consists of data science expert Juan Lavista Ferres (Microsoft), esteemed researchers Dr Jan-Marino Ramirez and Dr Tatiana Anderson (both at the Seattle Children's Research Institute in Washington), and world-renowned SUID expert Professor Edwin Mitchell (University of Auckland).

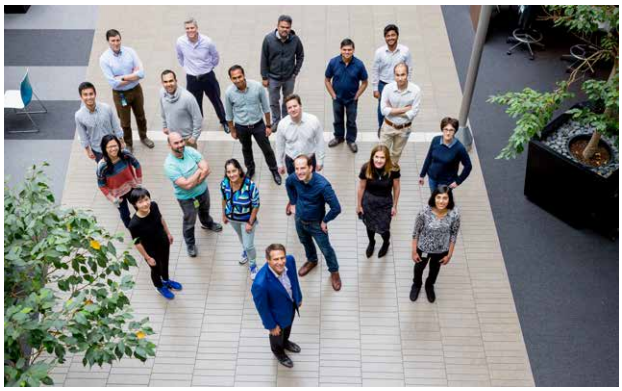
Real-world Data: The Risks of Maternal Smoking

Although both pre- and post-natal smoking have been long associated with SUID, evidence from real-world data was distinctly lacking. In a ground-breaking study published in 2019, the team revealed a clear statistical link between a mother smoking while pregnant and SUID.

In this study, data were obtained from the Centers for Disease Control and Prevention (CDC) for every birth in the USA over a period of five years. In total, this dataset represented over 20 million

live births, of which 19,000 resulted in SUID. Data on prenatal smoking was gathered on 12.4 million of these pregnancies and 11,000 of the SUID cases. Due to the large sample sizes, the researchers were able to analyse the data at a more granular level than previous studies had been able to. Shockingly, they discovered that a mother smoking just one cigarette a day during her pregnancy doubled the risk of SUID. In addition, they found that smoking before pregnancy could also have a big impact. The risk of SUID was 50% higher in women who smoked but quit by their first trimester, compared to women who did not smoke at all.

The team found that every single cigarette a pregnant woman smoked



increased the risk of SUID, up to 20 a day, at which point the risk plateaued. Around 11.6% of expecting mothers smoked during their pregnancy and only a quarter stopped.

Previous studies investigating the physiological basis of the link between maternal smoking and SUID suggest that smoking damages key neurotransmitters and their receptors in the foetal brain. Nicotine is likely to alter normal respiration, sensitivity to chemicals and sleep. Combining this information with their own research, the authors concluded that if no mothers smoked during their pregnancy in the USA, there would be 22% fewer SUID cases each year – equating to over 800 infant lives saved. Consequently, minimising maternal smoking is essential for reducing the occurrence of SUID, which the authors believe should be facilitated through education on smoking cessation before and during pregnancy.

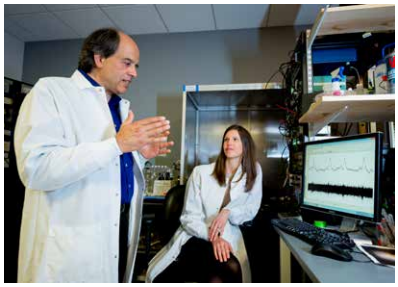
Sudden Unexpected Infant Death: The Impact of Age

In another study, the team aimed to determine whether there are significant subcategories of SUID depending on the age of the death of an infant. Of all the deaths documented in the neonatal period (up to 28 days old), 73% occur within the first week of life. This time period is, therefore, a critical one for a newborn.

Sudden unexpected postnatal collapse (SUPC) and sudden unexpected early neonatal death (SUEND) are both recognised as causes of neonatal death occurring in infants up to 7 days old. Sadly, around half of all infants that experience SUPC pass away, and survivors often suffer from long-term neurological issues. Even though these occurrences are well-documented, they currently do not have a standardised definition in the medical world and are consequently grouped under SUID as the cause of death.

The team wanted to find out whether there were statistically different subcategories of SUID based on the age of death of an infant, and if so, characterise the different risk factors for each group on this basis. For this study, the team again used CDC data of every birth over an 11-year period, representing over 41 million births with 37,600 cases of SUID. Using Microsoft's sophisticated data analysis software, the authors found that cases of SUEND (death within the first week of life) were statistically distinct from all the other SUID deaths after this time period (post-perinatal). They found that the greatest risk for SUEND is on the first day of life and this risk decreases exponentially over the course of the first week.

Importantly, deaths after the first week of life up until the end of infancy were statistically indistinguishable from each other. This led the researchers to conclude that SUEND and post-perinatal SUID reflect two distinct entities and should be categorised as such. Furthermore, the team believes that differentiating between SUEND and SUID will allow a better understanding of their different underlying mechanisms and potential genetic causes.



Moreover, this discovery will also require the development of an educational campaign to inform parents of best practices and behaviours that specifically avoid risk factors associated with SUEND. Indeed, the team's analysis uncovered first insights into the differing risk factors for SUEND versus SUID. One of these related back to their previous research into maternal smoking; this behaviour was identified as the most significant modifiable risk factor for SUID, but less so for SUEND.

Further Understanding Risk Factors

The team has now completed multiple other studies related to SUID. In one study, they used the CDC data to take a deeper look into the risk factors of SUID at different times of the day. Critically, different risks for SUID were identified during the day compared to at night. The majority of SUIDs occur when babies are sleeping at night and this is the time when younger infants were at higher risk, in comparison to older infants who were at higher risk during the day. This may be linked to the fact that younger infants spend more of their time asleep in deep rapid eye movement sleep, a state of autonomic instability. Sharing a bed with a parent and the sleeping position of an infant are also risk factors for night-time SUID.

Another study from the team investigated the effect of altitude at birth on SUID rate. They found a small, yet statistically significant, increase in SUID at very high altitudes greater than 8,000 feet, possibly due to the lower partial pressure of oxygen or lower environmental temperature at altitude. Through additional collaborative research, the team has outlined that younger age of SUID is associated with maternal smoking and factors related to lower socioeconomic status. In contrast, older age of SUID is associated with prematurity, low birth weight and admission to a neonatal intensive care unit.

Looking to a Brighter Future

All of this work has culminated in exciting developments for the collaboration. Dr Ramirez explains that they are now building 'the world's first genomic database dedicated to sudden unexpected infant death'. DNA extracted from tissue donations from recent SUID cases and buccal swabs from their parents are being collected, as well as medical records and autopsy reports. The DNA is extracted and submitted for whole-genome sequencing, which means each nucleotide in the entire genome is decoded.

This work has created a more optimistic, brighter future for SUID research. Dr Anderson further explains, 'What really sets us apart is our unique collaboration between academia with a deep knowledge of the literature and scientific/medical writing experience, and industry with professional data scientists using state-of-the-art data analytics.' The team's goal is for scientists all over the world to be able to access the vital data being gathered to progress our understanding of the devastating occurrence of SUID. Of particular interest to the team is the identification of gene variants that put a foetus at risk of SUID, which they hope could lead to consequent prenatal testing.

The larger team have also made a huge difference in their political landscape after successfully lobbying for better funding, data collection and parent support within the field of SUID. Named in honour of a baby lost just before her first birthday, the members of this important collaboration hope that Scarlett's Sunshine Act will help them make a significant difference in the lives of many families to come.

Meet the researchers

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Dr Tatiana Anderson received dual Bachelor of Science degrees in Neuroscience and Physiology from the University of Minnesota in Minneapolis, USA. She went on to complete her PhD in Neuroscience at the University of Washington in Seattle. Dr Anderson has fulfilled multiple scientific roles, including her current post managing the joint collaborative program between Seattle Children's Research Institute and Microsoft.

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Professor Edwin Mitchell obtained his medical degree at St George's Hospital Medical School in London and has worked in the UK, Zambia and New Zealand over the course of his career. He has received several awards for many landmark studies of sudden infant death syndrome and was made a fellow of the Royal Society of New Zealand in 2009.

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IMPROVING THE OUTLOOK FOR CHILDREN WITH CONGENITAL HEART DISEASE

Congenital heart disease (CHD) is one of the most common birth defects across the globe. Although prospects and survival rates are improving, there is scant understanding or help available to get children with CHD active. Many believe physical activity is risky or outright dangerous, and as a result, children with CHD are at risk of obesity and other chronic conditions later in life. **Dr Marta Erlandson** from the University of Saskatchewan has aided the creation of CHAMPS, an innovative program for children with CHD, where researchers and children are learning how to manage the disorder from each other.

Congenital Heart Disease

Congenital heart disease (CHD) is a term used to describe a range of birth defects that impact the normal function of the heart. It is one of the most common types of birth defect and is often diagnosed through an ultrasound scan before a baby is born. Although there is no clear-cut reason for the development of CHD, Down's syndrome and other alterations to the chromosomes (genetic make-up) are often risk factors. The behaviours of an expectant mother can also increase the risk of CHD. These may include smoking and drinking, having poorly uncontrolled type 1 or type 2 diabetes and taking certain medications such as statins during pregnancy.

The various types of CHD can be found both alone and in combination with others. Examples include having a hole in the heart between the two chambers, which is known as a septal defect, and having an abnormally narrow pulmonary valve which usually controls the flow of blood from the heart to the lungs, which is known as pulmonary valve stenosis. These structural defects can cause various symptoms in babies and children such as a rapid heartbeat and breathing problems especially when feeding, severe fatigue, swelling in the abdomen, legs or eyes, and a blue tinge to the skin called cyanosis. These issues are usually obvious when a baby is still very young, but if the heart defects are mild, the child might not show related problems until they are older.

Mild defects do not always create major problems and can even improve without intervention. However, many children with CHD require surgery (or even multiple surgeries) to restore more normal structure and function to the heart. Thanks to advances in medicine and surgery, this is often successfully achieved, but those living with CHD tend to require monitoring and treatment throughout their life due to the potential for developing future issues. CHD increases the risk for associated health complications such as blood clots, heart valve and rhythm issues and endocarditis, which is an infection of the heart and valve lining.

Credit: Marta Erlandson.

Improving the Outlook for Congenital Heart Disease

The detection and treatment of CHD have greatly improved over the years, so much so that within the next decade, 1 out of every 150 young adults is expected to be living with the disorder. Although these promising survival rates provide the hope of a long life for children living with CHD, facing the possible complications can prove challenging.

Children undertaking swimming as part of CHAMPS. Credit: Marta Erlandson.

Critically, many children with CHD are significantly less active than their peers, resulting in lower aerobic fitness and higher risk of early-onset cardiovascular diseases. Understanding and living with such a serious disorder can also cause mental health issues in children as such, both short- and long-term anxiety.

Investigating how these issues can be improved is Dr Marta Erlandson at the University of Saskatchewan in Canada. In the College of Kinesiology, Dr Erlandson and her colleagues are working to better understand how the needs of children with CHD can be met. According to Dr Erlandson, 'comprehensive chronic disease management (CDM) specific to the physical activity and mental health needs for children with CHD is currently lacking and requires resolution to prevent future health problems associated with CHD and physical inactivity.' There is a growing body of evidence that CHD disproportionately increases morbidity, mortality, and health care costs in adulthood.

Therefore, researching and recognising how children with CHD can be aided in the development of healthy lifestyle behaviours earlier rather than later is an essential step in improving their lives. 'We aim to understand the physical and psychological health of children with CHD and create a sustainable comprehensive chronic disease management program for these children,' explains Dr Erlandson.

The Children's Healthy-Heart Activity Monitoring Program of Saskatchewan

To carry out this CHD research, Dr Erlandson and her team created the Children's Healthy-Heart Activity Monitoring Program of Saskatchewan, also known as CHAMPS. This program involves an annual week-long summer camp style CDM for children with CHD all across Saskatchewan – the only one of its kind in Canada. Initiated in 2014 and funded by the Jim Pattison Children's Hospital Foundation and other supporters, the camp now welcomes around thirty 7–15 year-olds each year.

CHAMPS gives these young people the opportunity to experience a supportive and safe summer camp that aims to build their confidence and encourage them to be physically active. Most of them have a large surgical scar on their chest about which they may felt self-conscious and perhaps did not realise others shared. But through the chance to have conversations about their experiences and activities like swimming, the children are able to connect with others like them and come to see they are not alone. In the past, children with CHD were told they should avoid too much exercise and many are nervous or unable to participate in strenuous sports. However, at CHAMPS, the health of the children is monitored closely and they are encouraged to try new activities and sports to push their existing boundaries.

Every day, the CHAMPS team plans activities that are based on a specific chronic disease management and prevention program for children with CHD. In this way, the children learn what exercises they can do, how to do them, and what their nutrition should

Children undertaking team exercise as part of CHAMPS. Credit: Marta Erlandson.

look like. From the older children at the camp, the younger ones see what they can be capable of with the right tools and also that they can successfully manage their chronic condition. Additionally, they are able to talk about any health anxiety and emotions that result from their CHD and Dr Erlandson says that this environment helps to alleviate some of this stress.

Dr Erlandson further emphasises how important an improved understanding of their ability to exercise is to the later-life health of these children. Many young people with CHD go on to develop obesity and other disorders because of their lack of knowledge. As such, Dr Erlandson believes dedicated, comprehensive, and affordable chronic disease management programs are essential for reducing preventable morbidity in this population.

The Multiple Aspects of CHAMPS

The team more recently received additional funding from the Saskatchewan Health Research Foundation to expand their program. This involves extending the program to six months in duration and extending the availability of sessions to parents as well as children. As a result, parents can learn from clinical child psychologists how to communicate with their children about their condition and consequent emotions. As there is little to no infrastructure to support chronic disease management other than standard cardiology visits, this should be a great help for families dealing with CHD.

During CHAMPS camps, children and their families are given the opportunity to be involved in the team's research. So far, Dr Erlandson has helped to elucidate different aspects of CHD and is continuing to study the disorders. An early study from CHAMPS investigated CHD in relation to arterial stiffness, which is the loss of elasticity and consequent stiffening of artery walls. This creates a high risk of hypertension (high blood pressure) and other serious problems like strokes and children with CHD appear to have a higher risk of arterial stiffness than their healthy counterparts. They studied groups of children with CHD who had low physical activity with those that had high physical activity. Interestingly, she found that those with low activity had clearly increased arterial stiffness compared to those with high activity. Consequently, she concluded that regular physical activity is a beneficial method of minimising arterial stiffness and enhancing the future health of children with CHD.

Another study by the CHAMPS research group examined the health anxiety that children with CHD experience in comparison with children and adolescents that were developing more typically. As predicted, they demonstrated that young people with CHD have significantly higher anxiety surrounding their health. Dr Erlandson says this shows that they have a clinical level of risk for anxiety and therefore, require thorough interventions to help them deal with their condition properly.

Striving for better CHD management

The American Heart Association recommends that children living with CHD should have the same level of physical activity as their healthy peers, as it is both safe and beneficial. However, these children clearly have specific needs that are not currently met by existing programs. Dr Erlandson's team discovered this through their parents, who also cite financial constraints as an issue.

As a result, Dr Erlandson and the CHAMPS research team are striving to, 'create a sustainable comprehensive chronic disease management program for children with CHD' that will improve the current and future lives of the many children living with CHD both in Canada and throughout the world.

Meet the researcher

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Dr Marta Erlandson achieved her BSc, MSc, and PhD from the College of Kinesiology at the University of Saskatchewan. She also completed a Postdoctoral Research Fellowship at the University Health Network/University of Toronto as part of the College of Medicine's Osteoporosis and Women's Health Program. Dr Erlandson now serves as an Assistant Professor in the College of Kinesiology at the University of Saskatchewan. This is also where she also carries out her research into congenital heart disease (CHD). Through her work, Dr Erlandson aims to better understand the physical and psychological needs of children with CHD and develop interventions to help them.

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UNIVERSITY OF SASKATCHEWAN

USING GENETICS TO DIAGNOSE RARE METABOLIC DISEASES

Identifying the cause of an illness in a sick baby or child is not always easy, particularly if the disease is rare. Throughout his career, **Dr Michael Wangler**, at the Baylor College of Medicine and Jan and Dan Duncan Neurological Research Institute, has investigated rare childhood diseases. Combining his expertise in paediatrics and genetics, Dr Wangler utilises genomics, metabolomics and the humble fruit fly to identify the genes responsible for rare and undiagnosed diseases to improve both diagnosis and treatment.

Genetics and Genomics and Disease

Many genetic disorders are due to a mutation in one or more genes coding for a protein. Dr Michael Wangler at the Baylor College of Medicine and Jan and Dan Duncan Neurological Research Institute seeks to identify genetic mutations in individuals with previously undiagnosed medical conditions. For a sick baby or child without a diagnosis, not knowing the root cause of the illness understandably creates considerable stress for worried parents. Clinicians work to provide a diagnosis as doing so can help them identify appropriate medical care. Unfortunately, diseases with a genetic cause can be extremely difficult to diagnose. One of the key tools in this process is DNA sequencing.

The human body is a collection of trillions of cells, in which collections of specialised cells are organised into tissues and organs. Within each cell is the 'blueprint' or code held by deoxyribonucleic acid (DNA). Genes are sections of DNA and each gene encodes specific proteins that are required to perform all the functions of the human body.

Genetically coded proteins are extremely important to the structure and function of cells, organs and tissues and perform the bulk of the work within individual cells. Many proteins are involved in body chemistry or metabolism. Metabolism refers to the various biochemical processes which take place in the body. This includes the breakdown of carbohydrates, fats and proteins in food and the release, use or storage of energy. Metabolic disease or disorder occurs when one or more of the biochemical processes are disrupted such as those due to mutations in metabolic genes.

A large proportion of the human genome is comprised of DNA that exists outside of genes and does not encode protein, so the term 'exome' refers to the ~3% of the genome containing the protein-coding genes. Whole exome sequencing (WES) is a key diagnostic tool to sequence the DNA for the protein-coding genes for an individual. WES is more focused than sequencing the entire genome, and it not only facilitates diagnosis for individual patients but also can contribute to the identification of new causes of

genetic disease particularly when there are additional causes for a particular disorder.

One group of diseases that Dr Wangler has focused on for years is peroxisomal disorders. Peroxisomes form a key part or organelle of our cells, and they are generated and function due to the action of particular genes. Genetic changes causing defects in the production of peroxisomes can affect the liver and brain amongst other organs, whilst mitochondrial defects can affect the brain, muscles, vision and hearing. Together with his team, Dr Wangler contributes to the expanding knowledge of genetic disorders by identifying genetic changes and the associated defects they cause in cell organelles.

An alteration or 'variant' of a gene alters the code it holds. Every individual has hundreds of variants in their genome that affects proteins but not every variant is pathogenic – the term used to describe a mutation's ability to cause a disease or disorder. If a variant affects the code for a protein and leads to a significant change, it can alter the structure of the resultant protein. The result can be a defective protein, or the protein may not even be produced.

The mutation of even a single gene can have severe clinical outcomes. Cystic fibrosis and haemophilia are both well-known examples of diseases caused by a pathogenic variant of a gene. WES can be a powerful tool for diagnosis in patients. However, it is not simple to interpret. The careful review of the data provided by WES in the detection of disease can uncover other changes in the DNA sequence known as 'variants of unknown significance' which are not obviously benign or pathogenic. If WES identifies two or more genes that could be the cause of the disease, it is a challenge to ascertain if one or even both are responsible for the disease.

Metabolomics and the Humble Fruit Fly

To understand which genetic variants are pathogenic and to further study diseases like peroxisomal disorders, Dr Wangler and his team have developed *Drosophila* models which are used to test the function of rare variant genes which they have identified as potential causes of disease. This process assists in the verification of findings and is a key focus in the work of Dr Wangler and his team. *Drosophila*, or to give it the full Latin name, *Drosophila* melanogaster, is better known as the fruit fly. At first glance, the fruit fly is not an obvious substitute for the human body – but looks can be deceiving. Genetically, the genomes of flies and humans share many essential genes, particularly for the function of the nervous system. *Drosophila* shares approximately 60% of its DNA with humans, and internally, many of the organ systems and cells function in the same way.

In simple terms, the suspect gene is inactivated in the DNA of fruit fly embryos and the resulting flies are examined for the effects compared to normal flies. If the resulting disease in the fly can be rectified by inserting a normal, healthy version of the human gene into the fly but not by inserting the human variant version, it corroborates the variant as being the genetic cause of the illness.

In research published in 2016, and again in 2019, Dr Wangler and his team identified variants of *DNM1L*, a gene which has been indicated to be crucial in mammalian development, in peroxisomal and mitochondrial function and used *Drosophila* mutant studies to determine which variants were pathogenic.

With his team, Dr Wangler also conducts metabolomic research using animal models, studying small molecules like metabolites and their substrates, and the effects that genetic changes can have on their production and interactions.

Using mice in addition to fruit flies, Dr Wangler has investigated the metabolism and genetics of peroxisome production. Conducting such investigations is complex, and

incorporates gene manipulation, biochemical analysis and monitoring of the animal reactions in various tests and comparing them to normal animals. Dr Wangler's research into peroxisomal biogenesis disorders (PBD) uncovered an unforeseen link between peroxisomes and carbohydrate metabolism. Critically, peroxisomes are known to be vital to fat metabolism. The discovery of the link between peroxisome function and carbohydrate metabolism suggests it could be a new target for the treatment of PBD.

Metabolomic Profiling in Patients

In further research into metabolic diseases, Dr Wangler and his team studied 19 patients with mild to intermediate peroxisomal biogenesis disorders-Zellweger spectrum disorders (PBD-ZSD). The team combined biochemical investigation for the diagnosis of peroxisomal dysfunction with untargeted metabolomic small molecule profiling. More than 650 compounds were detected, and the results identified a reduction in plasma sphingomyelin as a consistent feature indicating it has the potential to be a novel biomarker for PBD-ZSD. Findings also indicated that characteristic metabolite changes decrease with age, suggesting that untargeted metabolomic profiling is useful in detecting abnormal peroxisome function in young patients but the usefulness decreases with the age of the patient.

Further to this, in a study published in 2019, Dr Wangler and colleagues used fruit flies to verify a diagnosis of peroxisomal disorder when the patient's metabolic profile did not indicate PBD, possibly due to their age. The team successfully identified a new *DNM1L* variant gene and verified the effect using the *Drosophila* functional studies. The researchers identified the patient, age 27, as the first adult reported with a pathogenic variant of DNML1 to exhibit neurological symptoms. The team concluded that *DNM1L*-related disorders are associated with different variations of the gene and a broad range of symptoms and severity.

Future Research Plans

Currently, Dr Wangler and his team are involved in several projects. They are part of an international collaboration with the Model Organisms Screening Center for the Undiagnosed Disease Network (UDN). In this project, DNA sequencing of undiagnosed patients is performed in the search for additional cases of rare disorders and further develops *Drosophila* models for testing the functionality of possible disease-causing variants.

Work to date completed by Dr Wangler and his team using WES and genomic and metabolomic studies to investigate the genetic causes of rare diseases holds much-needed promise for the diagnosis and treatment of genetic disorders in humans. Further work will undoubtedly drive forward this complex yet critical area of research.

Dr Michael Wanger with his team.

Meet the researcher

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Dr Michael Wangler is a licensed, practising physician, board-certified in both paediatrics and medical genetics. With a career focussed on rare childhood disease, he uses genetics to understand human health. Currently, Dr Wangler is an Assistant Professor in the Department of Molecular and Human Genetics at Baylor College of Medicine, and the Jan and Dan Duncan Neurological Research Institute. Dr Wangler specialises in research into the underlying mechanisms of Mendelian disease, and the clinical and genetic aspects of rare human disease. Dr Wangler has contributed to numerous peer-reviewed scientific articles regarding gene function in peroxisomal disorders and undiagnosed disease. His various achievements include the Molecular and Human Genetics Most Outstanding Fellow Award in 2011 and, since 2014, a seat on the Scientific Advisory Board of the Global Foundation for Peroxisomal Disorders.

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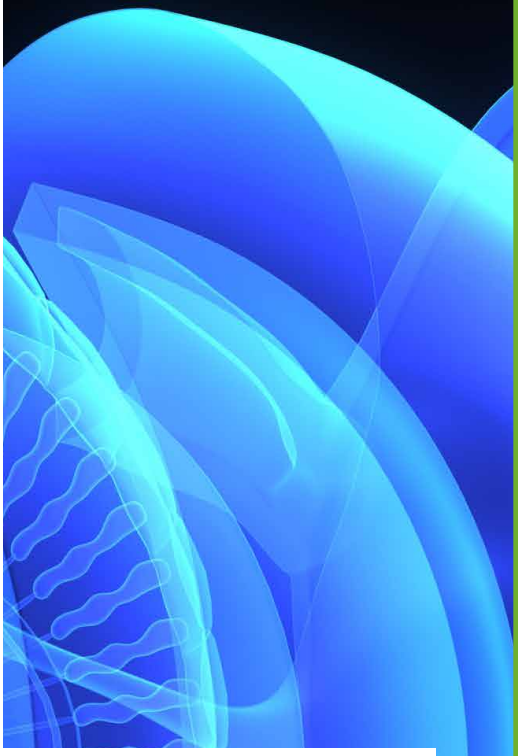
Baylor College of Medicine

TRANSLATIONAL IMAGING INNOVATIONS: ACCELERATING OPHTHALMIC RESEARCH THROUGH AN INTEGRATED ONLINE PLATFORM

Led by **Dr. Eric Buckland**, Translational Imaging Innovations, Inc. (TII) provides purpose-driven software systems that drive such ophthalmic research forward. The TII image management platform provides researchers with the tools to manage multifaceted imaging workflows and efficiently organize and analyse complex sets of images and data to accelerate the development of new diagnoses and treatments for eye diseases. By unleashing the power of the eye, TII aims to transform medicine.



The Window to Our Soul and Our Health

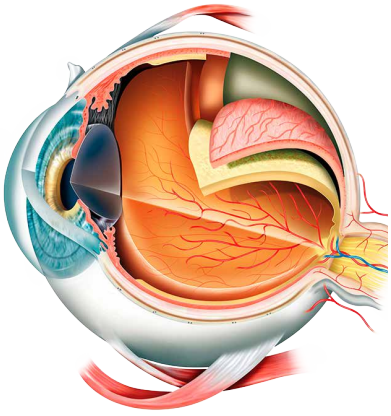
Like a digital camera, the eye captures images of our world by focusing light through the lens at the front of our eye to the sensor – our retina – at the back of the eye. Our retina is part of our central nervous system, circulates blood within our cardiovascular system, and enjoys a privileged role in our immune system. We count on our eyes to interact with the world around us, and in return, our eyes constantly tell us about our health.

Clinicians and scientists rely on advanced imaging technologies for patient care as well as for the development of new treatments to slow down progressive vision loss and prevent blindness. Increasingly, these same ocular imaging tools are making their way into the study of neurological and systemic disease.

Degenerative Eye Disease: Diagnosis and Prognosis

Degenerative eye diseases worsen progressively over time. One of the most common forms is macular degeneration in which an area of the retina (the macula) wears down, leading to vision loss. The exact cause is not well understood, but we believe the same factors that lead to heart disease contribute to it. And much like heart disease, macular degeneration advances with age. Currently, age-related macular degeneration (AMD) is the leading cause of blindness in the Western world and the incidence is expected to rise considerably in the coming years.

Optical coherence tomography (OCT) is a three-dimensional imaging technique that has dramatically improved the management of macular degeneration. OCT provides clear images for the assessment of dry macular



degeneration, characterized by deposits of a fatty substance called drusen that builds up in the retina gradually over time. There are currently no treatments for dry AMD but the dry condition may develop into the wet form. Wet macular degeneration is caused by abnormal blood vessels developing in the retina. Although this condition worsens more rapidly, it is treatable. For most people, eye injections of monoclonal antibodies

called anti-VEGF prevent the condition from worsening. OCT has played a critical role in the management of wet AMD, providing immediate insight that the clinician uses to determine the timing of eye injections to preserve sight, maximize patient comfort, and control costs.

Glaucoma is a degenerative eye disease that is caused by obstructed flow and drainage of fluids that circulate through the eye. This obstructed flow increases the pressure inside the eye, causing damage to the optic nerve which connects the eye to the brain. Loss of this nerve function slowly results in vision loss in the peripheral visual field and blindness if left untreated. Unfortunately, the early stages of glaucoma do not present with symptoms. While current estimates suggest more than 3 million Americans have glaucoma, only half are thought to have received a diagnosis. Without a diagnosis and in the absence of treatment, the condition continues to irreversibly progress.

Glaucoma is typically identified before symptoms develop at routine optician visits and is confirmed through various tests. These can include an eye pressure test, a gonioscopy, a visual field test, or an optic nerve assessment that uses optical coherence tomography. Glaucoma remains a significant medical challenge, and advanced imaging technologies are in constant development to improve decision-making in glaucoma management.

Inherited retinal diseases (IRDs) are caused by the mutation of at least 1 of more than 270 associated genes. As a group, IRDs are diverse with some leading to a gradual loss of vision over time and others resulting in vision loss much earlier, such as in infancy or young adulthood. Although relatively rare, IRDs are the leading cause of blindness in people aged 15 to 45, affecting 1 in every 2,000 individuals. The effective diagnosis and tracking of the progression of IRDs in individuals requires the identification of sensitive and specific biological markers, or in simpler terms, measures of a biological or pathogenic state.

This can be achieved using an advanced imaging technique called adaptive optics (AO), enhanced retinal imaging that allows for a direct view of photoreceptors in the retina. AO retinal imaging is increasingly being used to support gene research into genetic vision loss and as a tool to support the development of gene therapy. AO imaging shows how photoreceptors (rods and cones) are distributed in the eye. While two patients may present the same clinical conditions, the pattern of rods and cones may differ significantly. The analysis of these differences may prove essential in determining the type of genetic defect and in assessing the performance of new gene therapies.

Even More Than Meets the Eye

Eye examinations can reveal much more than just diseases of the eye. Systemic diseases like diabetes and even Alzheimer's disease can also be detected through the examination of eye damage. For example, diabetic retinopathy occurs as a result of high blood sugar levels seen in diabetes. The retina requires a constant supply of blood, but if the blood is full of sugar, the vessels that transport it become damaged and the retina becomes impaired, potentially leading to retinal detachment, vision loss, and blindness without intervention. With regard to Alzheimer's, direct diagnosis is not yet fully functional, but research suggests that observing a diminished density of blood vessels in the retina can be a signal of neurodegenerative disease. Researchers and innovative young companies are using the eye-brain connection to study neuromuscular diseases such as multiple sclerosis. Small involuntary motions of the eye, called micro-saccades, are impacted by neurological disease progression. These micro-saccades can now be extracted through precision tracking of retinal motion using imaging technologies. Artificial Intelligence (AI) methods to analyse the motion used to monitor slight changes in saccades associated with neuromuscular disease progression are opening a new field in diagnosis of central nervous system disease through non-invasive imaging of the eye.

Translational Imaging Innovations, Inc.

As demonstrated above, eye imaging is essential in the identification and management of pathologies directly and indirectly related to visual impairment. AI is growing in importance for the analysis of images. However, systems developed to use images for clinical patient care lack the coherence and organization required for more advanced research.

Dr. Eric Buckland is overcoming these limitations and transforming the field of ophthalmic research through his company, TII. Dr. Buckland explains, 'Our mission is the provision of better diagnostics and better therapies that provide more predictable benefits to patients faster, at a lower cost, and with less frustration.'

Before TII, Dr. Buckland was the CEO and Co-founder of Bioptigen. This company enhanced the use of optical coherence tomography by dramatically improving image quality and increasing system flexibility. Bioptigen innovations have made a lasting impact in early-stage ocular research and pediatric ophthalmology. Bioptigen was the first to introduce real-time OCT imaging of the retina and the cornea into ocular

surgery. Bringing a new class of imaging to researchers and clinicians, Bioptigen manufactured and distributed their advanced OCT machines around the world, improving the use of imagery for both research and patient care.

Through this early work, Dr. Buckland identified the 'tremendous untapped potential to extract latent medical information from the exquisite images we can acquire through the eye.' Building on this, Dr. Buckland is now creating innovative and marketable software solutions to fulfill unmet ophthalmic needs through TII.

An Online Platform for Accelerating Ophthalmic Research

Quantitative biomarkers are needed to fully utilize the retinal images from techniques like optical coherence tomography for clinical diagnosis. To facilitate research into finding these biomarkers, the team at TII created an online, integrated platform to collect, store, share, and manage ocular images and data. This center, called Lattice, allows researchers to access and analyse considerably more information than before.

AI is becoming fundamental to medical advancements. Complex algorithms process huge amounts of images and data to automatically identify patterns related to targeted disease or condition. In ophthalmology, AI can be used to identify disorders of the eye by essentially comparing a new patient's scan to its database of known pathologies. AI-enabled techniques for automated diagnoses of diabetic retinopathy using ocular imaging, for example, were the first medical AI solutions to be cleared by the FDA. The field of AI in medical imaging is still in its infancy. AI requires the systematic management and analysis of large numbers of images validated by experts, to uncover 'explainable' patterns in images to guide effective clinical interpretation.

Dr. Buckland's work means that annotated images from all over the world can be stored in a distributed but uniform manner, providing remote accessibility to large volumes of images and data that for AI programs to work with. TII systems are designed to make data access easier, more transparent, and less frustrating. TII enables the free flow of actionable ophthalmic information and empowers researchers to develop devices and therapeutics faster and at a lower cost. The goal is faster, more reproducible results and improved communication and collaboration across the field of ophthalmic research. Ultimately, this may save the eyesight and improve the overall health of millions of people.

Ensuring the Software is User-friendly

The Translational Imaging Innovations platform is supported by four pillars. Lattice is an easy-to-use Web application that incorporates electronic record keeping, protocol adherence, scheduling, subject and exam tracking, and team communications. Lattice is an information system built around our proprietary data model: our Data Genome. ocuvault™ is a unique data transport and storage system for the secure transfer of images and data. ocuVault maintains data provenance and security, ensuring accessible, actionable, and auditable data throughout a project lifecycle. oculink™ is a visual software solution that aggregates data and images for rapid curation, visualization, and annotation, designed by researchers for researchers. Mosaic is a work environment for studying the impact of disease and therapeutics on photoreceptors. Mosaic was originally developed by Dr. Robert Cooper and Dr. Joseph Carroll at the Dennis P. Han, MD Advanced Ocular Imaging Program (AOIP) at the Medical College of Wisconsin (MCW).

In addition, TII is licensed to the AOIP Image Bank, which houses AO and OCT images and data from more than 1,800 real patients with one or more degenerative retinal. TII continues collaboration with the Advanced Ocular Imaging Program (AOIP) at MCW and the Ocular and Computer Vision Laboratory at Marquette University to research imaging biomarkers of inherited retinal disease.

Real-world Applications

The National Eye Institute (part of the National Institutes of Health in Washington, DC) has recognized the capabilities and vision of TII and has awarded grants to aid in the continued development of its initiatives. TII software is already used for managing 80 clinical research projects. Mosaic is integrated into an international research project sponsored by the Foundation Fighting Blindness®. ocuVault and ocuLink are in early deployments through relationships with innovative imaging system startups, ophthalmic biophysics research collaborators, and drug development organizations. As these programs continue to be utilized, their impact will become more recognized and widespread.

Through his dedicated work, Dr. Buckland is truly transforming ophthalmic research. Lattice, Mosaic, and ocuVault and ocuLink promise to become invaluable tools for finding and improving new quantitative biomarkers for degenerative eye diseases. By creating a dedicated space that pulls together images and data and combines them with AI to push forward diagnostics, Dr. Buckland is facilitating the future of eye imaging research. Now—and in the coming years—these platforms will be used to improve diagnoses and therapies so that people suffering from degenerative eye disease will benefit from dramatically improved health outcomes.

Meet the researcher

Translational Imaging Innovations, Inc.
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Dr Eric Buckland completed his Bachelor and Master of Science degrees in Physics at North Carolina State University in Raleigh. He went on to receive his PhD in Optics from the University of Rochester's Institute of Optics in New York state. Throughout his impressive career, Dr Buckland has taken on a variety of leadership positions in global science innovation companies. As an entrepreneur, Dr. Buckland co-founded Bioptigen, which he managed from inception to exit. Currently, Dr Buckland is the Chief Executive Officer and Founder of Translational Imaging Innovations, situated in Hickory, North Carolina. He has assembled a great team to work with great collaborators on developing the next generation of software systems for discovering, developing, and deploying new markers of disease through the window of the eye. His passion is enabling his community to deliver the benefits of their research to patients, faster, at lower cost, and with better outcomes. Dr. Buckland is a Fellow of the American Institute of Medical and Biological Engineering (AIMBE) and a Senior Member of Optica.

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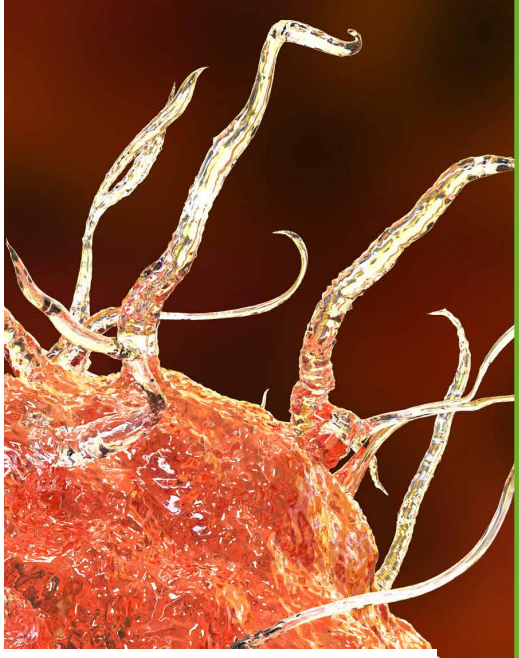
FURTHER READING

https://sbir.nih.gov/statistics/success-stories/bioptigen

TRANSLATIONAL
IMAGING
INNOVATIONS

A SUBSTANTIATED FRAMEWORK FOR THE PREVENTION AND TREATMENT OF IMMUNE SYSTEM-RELATED DISEASES



Dr Bretscher has articulated his views, as expressed here, in a fuller manner that is accessible to the layperson and the interested immunologist, in a document called ***Spiral Immunology***. This consists of a 1-, a 10- and a 20-pager, designed to be read successively. The full text is available on his website.

Immunity is generated normally against invaders, such as viruses and cancer cells, but not against parts of the body to which the immune system belongs. In 1970, Dr Peter Bretscher and Dr Melvin Cohn proposed a theory to account for how this is achieved. Importantly, immune responses against invaders can take one of two main forms, and Dr Bretscher (currently at the University of Saskatchewan) also proposed an explanation for how the choice of immunity is made. These two proposals are supported by diverse findings. Here, we outline and justify these proposals and explain how they lead to strategies to prevent and treat diverse diseases.

The Clonal Selection Theory

Animals can make an astronomical number of chemically distinct antibodies, produced by the body's immune system when foreign substances (known as foreign or non-self-antigens) are detected. But how can the body produce so many diverse antibodies? The Clonal Selection Theory, formulated in the 1950s and 1960s, addressed this and a second question: How does the body normally manage to respond against foreign but not against self-antigens? Unfortunately, this attribute of self-nonself discrimination can occasionally fail, leading to immunity against self-antigens. This occurs, for example, in autoimmune diabetes.

According to the **Clonal Selection Theory**, one cell can produce only one chemically unique antibody. The cell bears this antibody on its surface as a receptor for antigen. As the body can produce so many different antibodies, the cell with the potential to produce an antibody specific for a particular antigen (an antibody precursor cell) is scarce indeed. According to Clonal Selection Theory, an antigen can ***select*** this ***cell*** by binding its antibody receptor, causing it to multiply over days to form a clone of cells, some of which secrete copious amounts of antibody. This theory explains why it takes days to produce significant antibody and how scarce cells, through cellular multiplication, can produce substantial amounts of antibody.

These antibody precursor cells belong to a class of white blood cells called lymphocytes, on the basis of their microscopic characteristics. Antibody precursor cells are generated in a particular organ. If they interact with an antigen, as generated, they die; if not, they give rise to mature antibody precursor cells. Most self-antigens are present in this organ. The lymphocytes specific for the self-antigens present in the organ are thus obliterated. This process is called *central* tolerance, resulting in a population of lymphocytes primarily specific for foreign antigens.

A Model for the Activation/ Inactivation of Mature Lymphocytes

Lymphocytes are the cells of the body that can generate immune responses. While most anti-self lymphocytes are eliminated by central tolerance, some self-antigens, such as insulin, are not sufficiently present in the organ where lymphocytes are generated, to obliterate their lymphocytes.

Mature lymphocytes, specific for *peripheral* antigens such as insulin, a target self-antigen in autoimmune diabetes, also exist. Immunologists realised in the 1960s that antigens can interact with mature lymphocytes in two ways, one leading to their activation and immunity, the other to their inactivation, i.e., foreign and peripheral self-antigens would, respectively, activate and inactivate their corresponding lymphocytes in the best of circumstances. What determines 'To Be or Not To Be?'

Dr Peter Bretscher (currently at the University of Saskatchewan) and Dr Cohn proposed in 1970 how these two ways of interaction differ: an antigen inactivates a single lymphocyte, whereas its activation requires its antigen-mediated interaction with other, 'helper' lymphocytes. These proposals explained many observations. In addition, they explain how peripheral antigens, such as insulin, normally inactivate their mature lymphocytes. Insulin, being a peripheral self-antigen, is present when the first insulin-specific lymphocyte is generated. This loner lymphocyte is therefore inactivated. Further insulin-specific lymphocytes (see Figure above) are obliterated as generated, either one or a few at a time. Lymphocytes specific for a foreign antigen accumulate in its absence. When this antigen impinges upon the body, it mediates the lymphocyte interactions required to generate an immune response against the foreign antigen.

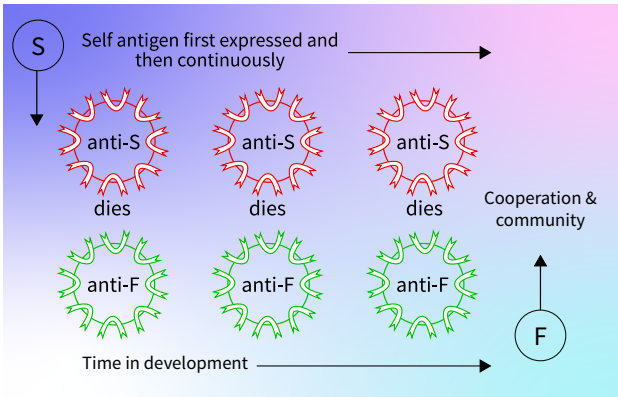
These ideas led to the Two Signal Model of Lymphocyte Activation. The interaction of an antigen with a lymphocyte's receptors generates signal 1, leading to its inactivation, unless helper lymphocytes recognise the antigen and deliver signal 2, leading to its activation.

The Two Signal Model also explains how autoimmunity arises. For example, infection by bacteria known as group A streptococci can result in autoimmunity against heart tissue. This is because the bacteria are foreign but also contain some structures similar to a peripheral heart antigen. Thus, scarce lymphocytes specific for these similar structures can be activated by the bacteria but would normally be inactivated by the heart tissue!

If To Be, What To Be?

There are three types of lymphocytes. B cells, generated in the bone marrow, multiply when activated by an antigen and some of their progeny secrete antibody. The CD4 T and CD8 T cells are generated in the thymus gland. The CD8 T cells, when activated, multiply and produce cytotoxic T lymphocytes that bind to the surface of cancer and virally infected cells, destroying them, thereby protecting against cancer and viral infections. The cover illustration shows cytotoxic T cells killing a cancer cell.

The CD4 T cells are the helper lymphocytes. When activated, they help the activation of B cells and CD8 T cells; in their absence, the antigen inactivates these cells, in accord with The



Two Signal Model. Thus, answering the question of 'What To Be?' requires us to understand what different circumstances result in the activation of B cells to produce antibody, or CD8 T cells to express cell-mediated immunity.

The role of the absence/presence of CD4 T cells in determining whether an antigen inactivates or activates B cells and CD8 T cells has been broadly accepted by the immunological community for the last three decades. Dr Bretscher argues strongly that single CD4 T cells are inactivated by antigens, and that their activation requires CD4 T cell cooperation, on both conceptual and observational grounds. An alternative, currently more popular idea is that the signal 2 for the activation of a CD4 T cell is not a result of CD4 T cell collaboration, but rather the engagement of pattern recognition receptors with pathogen-associated molecular patterns (PAMPs), representing an evolutionarily much older form of defence than the immune system. As explained below, these different ideas on the activation/inactivation of CD4 T cells are central to considering the question of 'If To Be, What To Be?'

The conditions of immunisation leading to strong cytotoxic T lymphocytes, an expression of cell-mediated immunity, and to antibody responses, are different. This raises two important questions: What is the biological advantage of such differential regulation and what is its mechanistic basis?

The Threshold Hypothesis

Immunologists realised in the 1980s that the activation of CD4 T cells can give rise to two main types of T helper cells, Th1 and Th2 cells. Th1 cells facilitate the generation of cytotoxic T lymphocytes, i.e., of cell-mediated immunity, whereas Th2 cells facilitate B cells to produce antibodies. Thus, the question, 'If To Be, What to Be?', can be translated into 'What determines whether Th1 or Th2 cells are generated?'

Dr Bretscher proposed in 1974 that Th1 and Th2 cells are respectively generated when antigen mediates tentative and robust CD4 T cell interactions. This 'Threshold Hypothesis' accounts for how various variables of immunisation affect the Th1/Th2 nature of the ensuing response.

Minimally foreign antigens are only able to induce naïve CD4 T cells to generate Th1 cells, as there are only a few CD4 T cells specific for such antigens; even with an optimal amount of antigen to support CD4 T cell collaboration only tentative CD4 T cell collaboration can occur. An optimal level of more foreign antigen, for which there are more CD4 T cells, supports robust CD4 T cell collaboration and so the generation of Th2 cells, whereas a lower level of the same antigen only supports tentative CD4 T cell collaboration and so Th1 cells.

Moreover, responses often evolve with time from a Th1 towards a Th2 mode. This too can be explained by Dr Bretscher's account. The CD4 T cells multiply when an antigen impacts the immune system and so, as long as the antigen level is sustained, the strength of CD4 T cell collaboration increases, accounting for this evolution.

One example shows the biological importance of the generalisation that minimally foreign antigens should only induce cell-mediated immunity. Cancers are minimally foreign, being derived from and only slightly different from their parental self-cells, and are only susceptible to cell-mediated attack. This naturally raises the question of how they sometimes escape the immune response by deviating it into an antibody mode?

A widely held hypothesis, an alternative to the Threshold Hypothesis, is that PAMPs are required not only to activate CD4 T cells, but the nature of the particular PAMPs associated with an antigen determines the Th1/Th2 phenotype of the response. Dr Bretscher discusses in *Spiral Immunology* many reasons why this is implausible. One reason is that this theory does not explain the evolution of the response from a Th1 towards a Th2 mode by either PAMP-containing and PAMP-free foreign antigens, as in the former case the PAMPs do not change during the course of the response and, in the latter case, there are no PAMPs.

The Threshold Hypothesis and Medical Strategies for Prevention and Treatment

Vaccination procedures currently in use increase the rapidity of the antibody response upon infection. They are effective against pathogens contained by antibodies but ineffective against invaders only contained by cell-mediated immunity, such as cancers and the pathogens responsible for AIDS and tuberculosis. Critically, work in Dr Bretscher's laboratory has developed ways of protecting against and treating infections caused by pathogens best and uniquely contained by cell-mediated immunity.

One such parasite causes human cutaneous leishmaniasis. Resistance and disease, respectively, correlate with Th1 and Th2 or mixed Th1/Th2 responses. Infection of mice results in a similar pattern as in humans. Infection of 'susceptible' mice with one million parasites quickly results in a Th2 response and progressive disease, and a sustained Th1 response and containment of the parasite in 'resistant' mice. Dr Bretscher's laboratory has shown that infection of susceptible mice with 300 parasites results in a stable Th1 response and, in time, a Th1 imprint. Reinfection with a million parasites, sometime after this first infection, results in a Th1 response and resistance. The same strategy works in mice with tumours and mycobacteria, responsible for tuberculosis and leprosy. These studies underpin the low dose vaccination strategy.

Visceral leishmaniasis, which is fatal if untreated, is caused by a parasite related to the one that causes cutaneous leishmaniasis. Visceral leishmaniasis is also only contained by a Th1 response. The mixed Th1/Th2 response of patients is modulated to have a Th1 phenotype on a short treatment with drugs that kill the parasite. Dr Bretscher suggests this change occurs because the reduced antigen load following parasite killing modulates the immunity to have a Th1 phenotype.

Less than 1% of individuals infected with HIV contain the virus without treatment. These 'elite controllers' generate a stable cytotoxic T lymphocyte, Th1 response and produce little antibody. Most HIV-infected and untreated individuals will, sometime after infection, produce antibody as the response acquires a Th2 component, and then experience the successive stages of AIDS.

Dr Bretscher proposes that the drop in viral load, caused by anti-retroviral therapy, modulates the immune response from a non-protective, mixed Th1/Th2 response at the initiation of therapy, to a protective, Th1 mode, similarly as the administration of anti-parasite drugs results in the cure of visceral leishmaniasis. A simple assay, involving an examination of the nature of the anti-HIV antibodies, can be employed to assess when the response is of a predominant Th1 phenotype, and when anti-retroviral therapy should be stopped. Given the presence of protective immunity, viral rebound will not occur, as most often happens upon cessation of treatment at random times.

The work of Dr Bretscher is an inspiring example of the linkage between basic science and medical innovation. It is clear that Dr Bretscher has presented foundational ideas that are on course to facilitate the development of improved strategies to prevent and treat diverse diseases.

Meet the researcher

Professor Peter Bretscher
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Dr Peter Bretscher, Professor at the University of Saskatchewan, Canada, studied physics at Cambridge, England, as an undergraduate, and obtained his PhD in protein X-ray crystallography in 1968, at the Cambridge Laboratory of Molecular Biology. Here, he became fascinated by immunology and discussed his early ideas with Francis Crick. In 1970, Bretscher and Cohn proposed the Two-Signal Model of lymphocyte activation, a theory made to explain how the immune system fights foreign invaders but not constituents of the body to which it belongs. In 1974, he proposed how the immune system chooses one of the two major classes of immunity with which to attack an invader. This theory is pertinent to understanding how immune responses against diverse antigens, including cancers and infectious agents, are regulated, and so to achieving vaccination against AIDS, tuberculosis and cancers. In 2017, Professor Bretscher published the short book, 'The Foundations of Immunology and Their Pertinence to Medicine'. It provides an overview of contemporary immunology and its pertinence to strategies of prevention and treatment of diverse immune-system related diseases. It is written to provoke immunologists but be accessible to non-specialists.

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ASSOCIATION OF MEDICAL RESEARCH CHARITIES

The Association of Medical Research Charities (AMRC) is dedicated to supporting medical research charities in saving and improving lives through research and innovation. Founded over 30 years ago, the AMRC has united more than 150 medical research charities in all areas of health and disease throughout the UK and overseas. In this exclusive interview, we follow up our 2019 interview with the new Chief Executive, Nicola Perrin, to hear their latest news.

When we last spoke to AMRC in 2019 and since then, the arrival of COVID-19 changed the world as we knew it. What has been the impact of the pandemic on medical research charities and how have they fared?

Since the pandemic started, medical research charities have lost at least £292 million in income, forcing them to cut £270 million of their research spend. This is the first substantial decrease in fundraising charity research spend in the last decade, a drop that is over seven times greater than the drop after the 2008 recession.

To honour existing research commitments many charities had to dig into precious reserves, adapt their fundraising activities, make redundancies, and cut funding for other activities. Our charities plan to cut their research spend by over £148 million over the next year and expect it to take three years for their research spend to return to normal levels.

With these funding uncertainties, the UK risks losing a generation of talented young scientists, potential future research leaders. In an AMRC survey, 40% of charity-funded early-career scientists indicated that they were considering leaving research. This would have a severe impact on decades of research crucial to finding new ways to diagnose, manage and treat diseases.

At the height of the pandemic, 70% of trials conducted in the NHS funded by AMRC members were on hold. That figure is now coming down but, despite best efforts, non-COVID research trials are struggling to recruit patients. In April 2019, about 200,000 people were recruited to trials. This April, the figure for non-COVID research was half that. The pandemic also forced charities to invest additional funding into ongoing trials to keep them going, funding that may have otherwise been used to start new clinical trials.

Despite these hardships, the sector stepped up to support the national research effort; providing a skilled workforce, infrastructure and support for testing and vaccine development, and they pivoted to fund new COVID-19 research. Cancer Research UK donated equipment and reagents to national COVID-19 testing and have set up their own testing hubs and the Asthma UK and British Lung Foundation Partnership co-funded a £1.3 million national research programme to evaluate coronavirus tests in hospitals, general practices and care homes and launched a post-COVID Hub and helpline for people left with breathing difficulties after COVID-19.

Between June 2020 and May 2021, AMRC and over 150 of our charities campaigned for Government to provide financial support to protect charity-funded research at risk. Over 105 MPs and Peers supported our call, 7,000 letters were sent to the Prime Minister from the public, and the campaign featured in over 150 news pieces. We raised awareness of the breadth of research that charities support and highlighted the vital role they play in the ecosystem. Finally, in May this year, Government announced a £20 million allocation for early-career researchers supported by charities. We're keen to keep working with the government to make sure they understand the value of medical research charities and ideally to encourage them to continue to support and collaborate with them.

As new CEO, what are your next steps for the sector?

AMRC does such important work to support medical research charities and so I feel incredibly honoured to take on this role. At a difficult time for the sector, I am lucky to be able to build on the fantastic legacy of Aisling Burnand's leadership over the past seven years.

Key products and innovations supported by charity funding

Insulin pen	Cochlear implant	Precision medicines for cancer	Statins to lower cholesterol
			
Diabetes UK	Action against Hearing Loss	Breast Cancer Now; CRUK; YCR	British Heart Foundation
Gene therapy to treat eye conditions	Sleep on side campaign to tackle still birth	The FAST test to spot a stroke	Polio vaccine
			
Fight for Sight	Tommy's; Action Medical Research	The Stroke Association	Action Medical Research

Throughout the next few months, I will be meeting with all 151 member charity CEOs to hear what matters most to our charities and how we can continue to improve our services.

We have three priorities as an organisation, moving forward. The first is to provide our charities with the evidence and narrative to champion their vital role in the UK life sciences ecosystem. While we've done a lot over the last few years to advocate for medical research charities, our recent engagement with Government demonstrates that we still have a job to do to explain why medical research charities are so important.

I am also keen to make sure we are supporting our charities to collaborate effectively. The pandemic has demonstrated the power of partnerships; we need to do more to help AMRC charities identify potential partners and highlight opportunities to connect and collaborate.

The research landscape is changing and there is, rightly, increasing emphasis on the culture of research. We want to help you consider what those changes mean for charities, and to think about how we can support our charities to be responsible funders in a rapidly changing environment. We have already launched our Equity, Diversity and Inclusion Resource Hub, but we are looking at what else we can do to help share best practice.

I'm really excited to start working on these priorities with the amazing AMRC team.

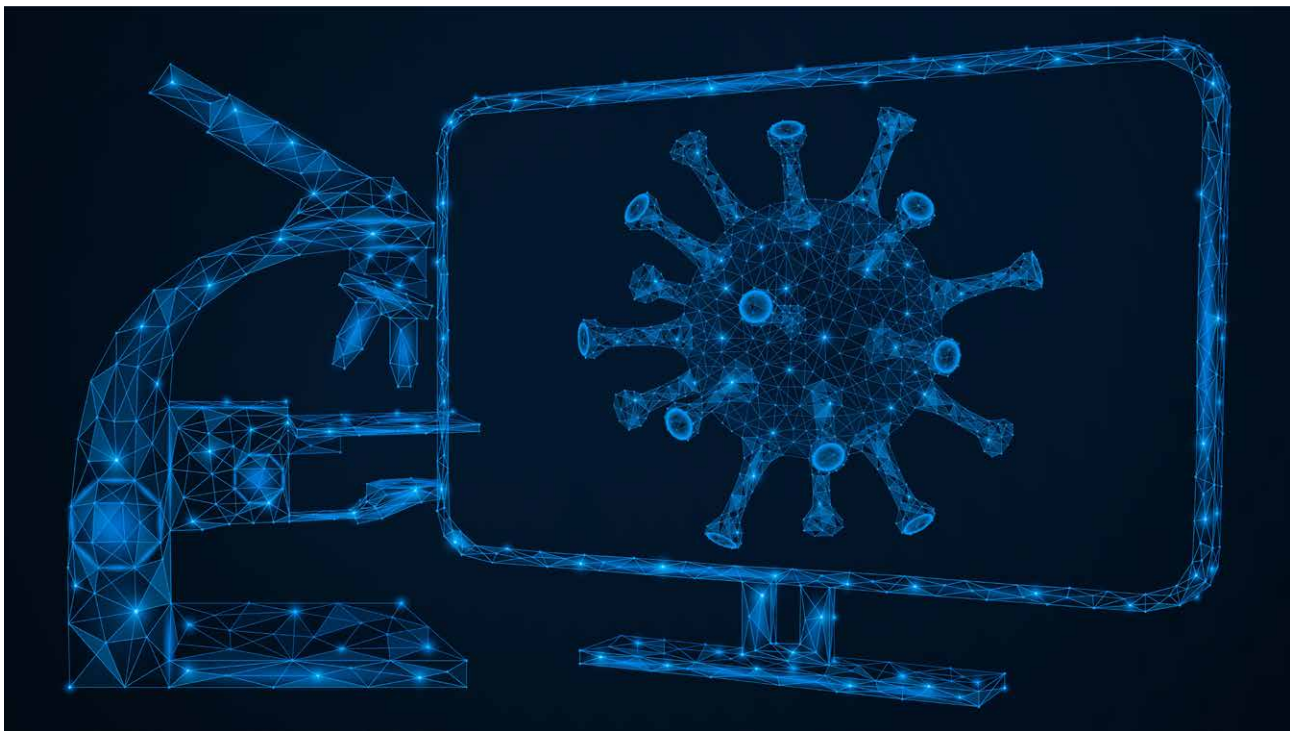
Can you tell us about your 2020–2025 Strategic Plan?

In the run up to 2020, it was clear there were challenging times ahead: unprecedented political instability, economic uncertainty and an increasingly polarised society created a difficult environment for charities and the communities they represent. However, there were also new opportunities and hope to improve the life and experience of patients.

To assist with the development of our 2020–25 Strategic plan, we undertook a survey of our members. The survey highlighted that securing income had overtaken demonstrating impact as the most significant challenge for member charities. It gave a clear indication that making medical research funding more accessible to charities and research and innovation in the NHS should be higher priority focus areas.

With these insights in mind, we brought together a framework for our work and activity, setting out five overarching priorities:

1. To champion the unique voice of the medical research charity sector by informing and educating internal and external stakeholders across the changing life sciences and digital health landscape.
2. To influence the research and health funding landscape to ensure that medical research charities investment is leveraged efficiently for patient impact.
3. To foster and enable better collaboration to address the needs of patients by working with industry, academia, NHS, regulators, our members, and other stakeholders.



4. To maximise the potential for research and innovation in the NHS to ensure the investment from medical research charities has the greatest impact for patients.
5. To drive forward the quality and future-focused membership offering which addresses changing needs and enhances AMRC's delivery capability.

Why are medical research charities so important to the UK?

There's this view of medical research charities as fluffy things doing good, whereas they're actually an integral contributor to the UK's life sciences: responding to the public's priorities, tackling areas of unmet need, and accelerating health impact.

Responding to the public's priorities

Charity research is shaped by the public's priorities. Over 83% of AMRC charities are using patient voice in their research strategy or influencing work. This ensures funding is directed where it will make the most difference and lead to more efficient products and interventions that help prevent, diagnose, treat, cure, and improve quality of life for people. AMRC charities contribute to a quarter of medical products, interventions and clinical trials reported by public funders on the research evaluation platform Researchfish.

Areas of charity research are chosen by the fundraising public, identifying diseases that matter to them. AMRC charities account for 66% of public investment into cancer and cardiovascular research.

Tackling areas of unmet need

Charities invest in underfunded conditions, including rare diseases. 67 AMRC charities fund research on rare diseases, and 27 exclusively fund research into a rare disease.

Medical research charities also support research in common conditions where there is low investment, for example over a quarter of public funding into mental health research is provided by AMRC charities.

Charities are also helping to address geographical health inequalities, by funding research and creating infrastructure and networks that benefit people and institutions throughout the UK.

Accelerating health impact

Medical research charities' sole purpose is to improve human health, not to make profits or cut costs. They help to convene and attract the necessary funders to move promising research forward and deliver benefits to patients sooner. AMRC charities account for a quarter of new collaborations and partnerships in the UK that have been reported by public funders on the research evaluation platform Researchfish. They have also leveraged more than £7 billion in further funding for UK research during the last 20 years.

Charities use their research to advise and shape policy and practice, helping to advance healthcare. They provide expert advice to Government committees, citations in clinical guidelines, and influence the training of health professionals, ensuring that decisions that impact patients are based on evidence. Over the past two decades, 10,000+ grants from 49

charities have led to 4,000+ unique influences on policy and practice that help advance healthcare.

What challenges and opportunities do you see on the horizon for medical research charities?

As a result of the pandemic, medical research has never been more visible, across government, the NHS, and the public. We need to build on that, to ensure continued investment in medical research, and to learn the lessons from the pandemic, including removing unnecessary bureaucracy, fostering collaborations, and engaging patients and NHS staff in the importance of research. Recent government reviews – the Spending Review settlement, the Life Sciences Vision, the Clinical Research Vision – are all moving in the right direction, and we hope there will be opportunities for partnerships with charities, but we need to see the detail of implementation plans.

At the moment, there is increasing focus on the sustainability of universities, and revisiting discussions about full economic costing. The partnership between government, charities and universities is very important for us, but there have been tensions about the current model of government support for charity-funded research in universities – the Charity Research Support Fund (CRSF). There are concerns that the size of the CRSF has not kept up with charity investment. This will be a key priority for us – we have just published a report exploring alternative models. We recognise there will be no easy answers but look forward to the start of a conversation between universities, government, and charities to find a sustainable approach.

While the pandemic has demonstrated the importance of research, there are still real challenges with capacity in the NHS workforce and dealing with the ongoing backlog from COVID-19. The Health and Social Care Bill currently passing through parliament provides an opportunity to mandate the need for research for Integrated Care Systems, but unfortunately, the amendment was not adopted at Committee stage. We hope there will be further discussions as the Bill passes through the Lords.

The UK-EU agreement reached Christmas 2020 left several issues unresolved. It is essential that charity-funded researchers in the UK can continue to collaborate with European colleagues. The uncertainty over association with Horizon Europe is a concern but the government announcement to provide a short-term scheme to cover the first wave of grant calls is a step in the right direction. The UK must also continue to attract talented science and research professionals. Unfortunately, the fast-track visa scheme to attract prize-winning scientists to the UK has received no applications over the last six months.

It's also important to remember that amongst the challenges there are also opportunities, with proposed revisions both to clinical trials regulations and data protection. However, this needs to be done with care to make sure we don't lose the potential for collaboration and data adequacy.

A positive new relationship between the UK and EU that benefits patients depends on resolving the outstanding problems. As a member of the Brexit Health Alliance and on behalf of our charities, we will continue to call on the government and EU to take steps to safeguard the interests of patients, and the healthcare and research they rely on.

W: https://www.amrc.org.uk/
@AMRC

DRUG DEVELOPMENT

PIONEERING NEW DRUGS AND INTERVENTIONS

The second section of this issue showcases the vital work of researchers dedicated to improving medicine and healthcare through the development of novel drugs and innovative interventions. In the UK, for example, it typically takes longer than a decade and in excess of £1 billion to bring a new drug to the market. The journey from laboratory to patient is often a long and complex process, and here we read of researchers working at various stages in the scientific development of a wide range of drugs and interventions. Despite the inherent challenges and diversity of this critical field, the potential for revolutionising patient care and saving patient lives is a shared goal for our featured researchers.

We open this section by meeting Professor Leon WMM Terstappen from the University of Twente in the Netherlands. From a drug development perspective, cultures of cells ('cell lines') are an important component of the scientific tool kit. We can read how Professor Terstappen has refined key methodological techniques in his laboratory to produce high-quality cell lines with the potential to benefit the discovery and development of important new treatment targets.

In the field of drug development, covalent drugs are molecules that irreversibly bind to specific, targeted sites in the body. Dr Mikail Abbasov from Cornell University, USA, is developing and utilising new technologies to allow the mapping of novel molecular targets for potential covalent drugs. We can read how covalent drugs can inhibit the disease-causing functions of certain proteins and in doing so, hold tremendous promise for application in cancer, autoimmune disorders and other diseases.

Dr Fritz Markwardt at Martin-Luther-University Halle-Wittenberg in Germany takes a different approach in driving forward the development of novel therapeutics. His specific focus is on the P2X7 receptor which is located within our cells, particularly in the immune and inflammatory systems. We can read how by elucidating the structure, function and mechanisms of the P2X7 receptor, Dr Markwardt is informing the development of much-needed drugs for inflammation and pain.

Kidney disease is prevalent throughout the world and can sometimes present in a severe form of nephrotic syndrome in which too much protein is passed in the urine. Professor Ferruh Artunc from the Tübingen University Hospital in Germany studies one classic symptom of nephrotic syndrome in particular – oedema, which is the extreme swelling of the legs and eyelids. His work is

advancing our understanding of how oedemas are formed as well as potential treatment interventions.

Diabetes mellitus is another prevalent disease that can often bring additional complications for patients. Heart disease is a particular risk due to the high levels of sugars, lipid and cholesterol causing thickening of the blood vessels. We can read of the pioneering team from the Centre of Molecular Immunology, Cuba, working in collaboration with Dr Spencer Proctor from the University of Alberta in Canada, to test a novel antibody to prevent the binding and accumulation of cholesterol in the blood vessels.

We then turn to the pressing issue of drug resistance. Due in large part to the overuse and misuse of antibiotics, antibiotic resistance (in which bacteria change in response to these critical medicines rendering them ineffective), is an urgent and global health concern. Dr Kristin Parent from Michigan State University, USA, is exploring an intriguing alternative – bacteriophages. We can read how these tiny viruses infect and kill bacteria but are harmless to humans, and how Dr Parent's exciting collaborative projects are leading to a wealth of new discoveries in this field.

The successful treatment of human immunodeficiency virus (HIV) hinges upon antiretroviral drugs, allowing patients to live longer and healthier lives. However, like other viruses, HIV will mutate, leading to resistance to therapies that had previously been effective. Dr Eric Freed at the USA's National Cancer Institute is working to better understand how exactly HIV becomes resistant to antiretroviral drugs, and in doing so, is paving the way for the development of new drugs to overcome this critical obstacle to effective treatment.

The design of new and effective antiviral drugs was (and remains) a key priority in the efforts to manage the SARS-CoV-2 pandemic. The FDA-approved drug Remdesivir (RDV) acts by interfering with the SARS-CoV-2 viral replication mechanism, but how precisely this is achieved has been uncertain. We can read how Dr Jin Yu from the University of California, Irvine, USA, adopted a computational approach to determine how RDV effectively halts viral replication and how her work is also paving the way for the development of new antiviral drugs.

In addition to developing new drugs, sometimes an existing drug can be repurposed to treat a condition or disease other than for which it was developed or typically used. *In silico* drug discovery allows the screening and identification of large numbers of drug candidates for novel purposes, and is being used by Dr Y-H Taguchi at Chuo University, Japan. By developing a computational technique known as 'tensor decomposition-based unsupervised feature extraction', Dr Taguchi has successfully identified various known antiviral drugs to be viable candidates for the successful treatment of SARS-CoV-2.

Unfortunately, some very effective drugs, including powerful pain relievers such as opioid drugs, also come with significant and highly problematic side effects. Our last featured researcher in this section is Dr Ying-Xian Pan from the Rutgers New Jersey Medical School, USA. We can read of his dedicated efforts towards developing novel strategies and opioid analgesics that can successfully treat pain without the side effects and critically, without the abuse potential of traditional opiates.

A NOVEL APPROACH TO SINGLE CELL IDENTIFICATION, ISOLATION AND CHARACTERISATION

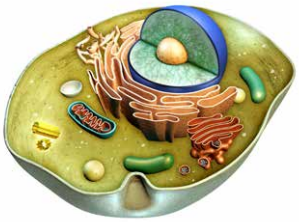
The establishment of cell lines capable of producing high-quality monoclonal antibodies is imperative for the development of therapeutic agents and the advancement of biomedical research. **Prof Leon WMM Terstappen** and his team from the University of Twente in the Netherlands have developed a highly efficient method of identifying and isolating cells that produce high concentrations of specific antibodies. With his team, Prof Terstappen has further honed this method to aid in the refinement of molecular cloning techniques to increase the yield of superior quality monoclonal cell lines.

Optimising Cell Line Production

Monoclonal antibodies that recognise particular antigens are routinely used for therapeutic and research purposes and are predominantly manufactured using mammalian cells. There is an increasing need for rapid, cost-effective and accurate methods to isolate and characterise single cells to create and maintain stable cell lines. Cell populations can be notoriously diverse, and the necessity to ensure that cell lines are derived from a single cellular source is crucial when producing high-quality monoclonal antibodies.

Traditional methods for screening and isolating candidate cells include limited dilution sedimentation, micropipette cell-picking, fluorescence-activated cell sorting and so-called 'lab-on-a-chip' technologies. All have proven to be valuable contributors in the field of single-cell analysis, but are time-consuming, labour intensive, and limited by the requirement for large cell numbers, many of which are lost during processing. Additionally, since these approaches tend to utilise entire cell colonies, the probability of achieving monoclonality is greatly reduced. This discounts their use clinically and hampers the swift detection of rare cells which may ultimately impact patient diagnosis and treatment. Indeed, the understanding of resistance to certain drugs and the identification of alternative therapies is vital to improving disease outcomes.

Prof Leon WMM Terstappen of the University of Twente leads the renowned Medical Cell BioPhysics Group at the Techmed centre. The group strives to overcome the limitations of other cell isolation and characterisation methods by investigating and implementing innovative techniques, with the specific aim of isolating and clonally expanding rare cells. Consequently, the research undertaken by Prof Terstappen and his colleagues has led to the development of an extremely effective procedure for

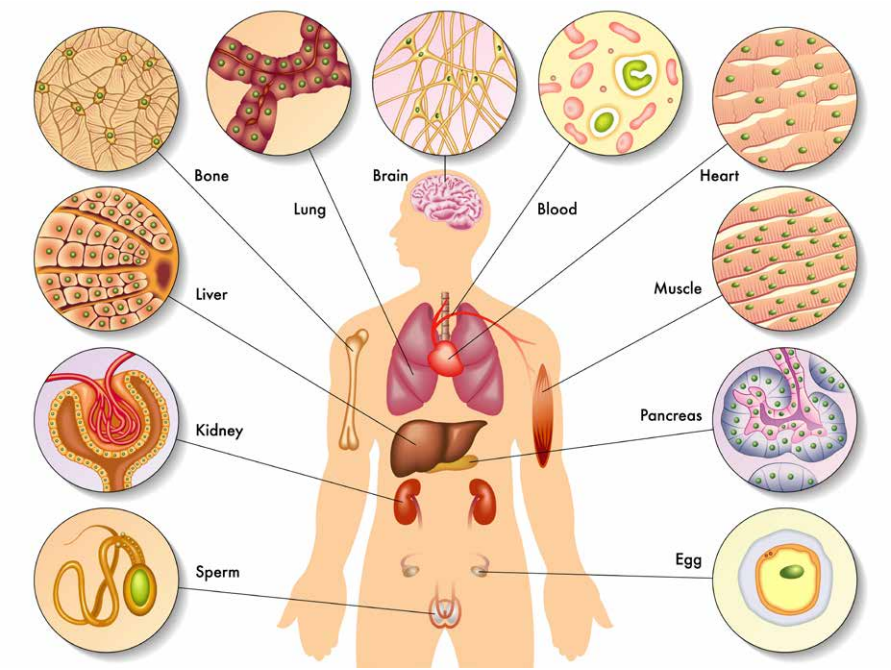


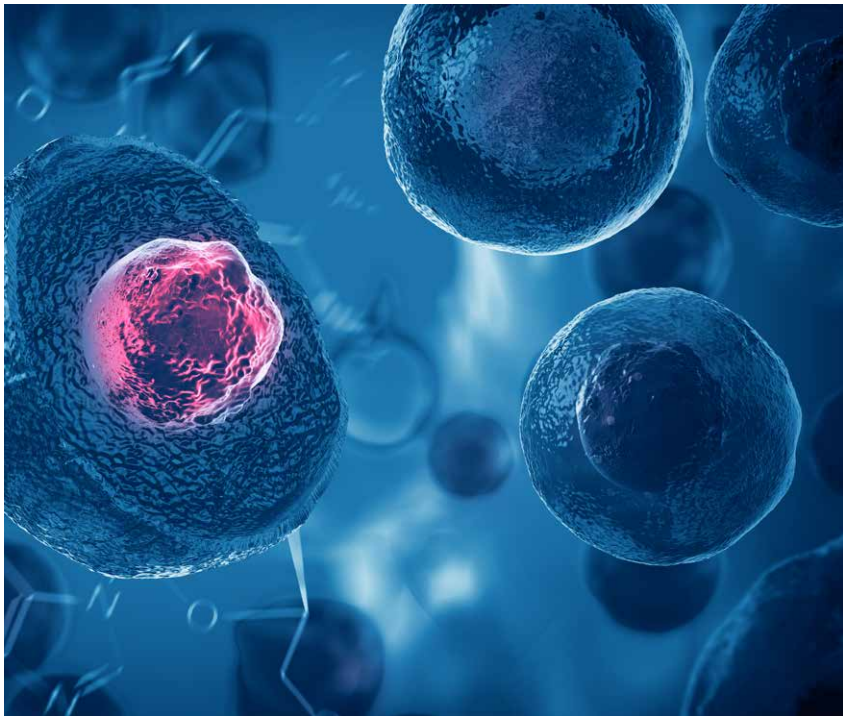
Basic cell structure

the isolation and interrogation of single cells using a self-seeding microwell chip and Fluorescent or Surface Plasmon Resonance imaging (SPRi) readout. Furthermore, Prof Terstappen's group has proposed a novel technology combining several processes which optimises throughput by enabling vast numbers of cells to be precisely screened simultaneously over a significantly reduced time period.

Single Cell Isolation

The preliminary research conducted by Prof Terstappen and his team provides an account of the introduction of a self-





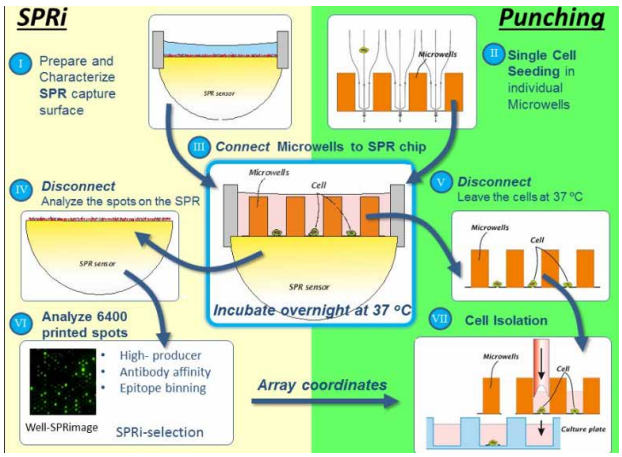
seeding microwell chip to isolate single cells in a semi-automated fashion with minimal manipulation.

Microwell chips comprised 6,400 individual wells, each with a small pore embedded into a thin membrane on the bottom. A solution containing fluorescently labelled target cells was applied to the microwell chip, and a small negative pressure was asserted to gently force the fluid through the well, dragging a single cell to settle on the pore. The fluid then exited via the pore, leaving the cell behind, which effectively sealed the well and prevented further cells from entering. The remaining solution was diverted to the adjoining well, and the next individual cell was deposited. This process was repeated until all microwells contained a single cell, and was completed within approximately 60 seconds. Each cell was then imaged through the membrane on the bottom of the well, using fluorescence microscopy.

The next step of the procedure was to place underneath the microwell chip a thin, high-binding capacity polyvinylidene fluoride membrane which had been pre-treated with a ligand to promote binding with the cell secretions of interest. The microwell chip with the membrane attached had been incubated overnight at 37°C and during the incubation period, the cellular secretions diffused through the pore in the bottom of the well and were captured onto the membrane.

Following incubation, the membrane was detached from the microwell and the membrane stained with fluorescence-conjugated antibodies recognising the secreted products. The cellular secretions could now be seen as tiny spots printed on the surface and quantified by fluorescence microscopy.

The amount of antibody secreted per cell per day was determined and based on this analysis, the cells could be categorised as high-, medium-, low- or non-producing cells. The data from these experiments confirmed that the majority of cells produced no or only a small number of antibodies, and only a few cells produced high quantities. This further emphasises the need for accurate cell isolation techniques with minimal cell destruction. Since the coordinates of the spots matched those of the microwells containing each cell, secretions could be accurately tracked to a specific cell, the wells containing high antibody producing cells could be



CREDIT: Leon Terstappen

identified, and a thin needle was used to punch the bottom of the microwell containing the desired cell into a small cup positioned below the microwell. Each individual cell could then be expanded to develop monoclonal cell lines with increased antibody production.

Introducing a Novel Technology

Having refined this pioneering technique, the team turned their attention to SPRi. Conventionally, SPRi is used to measure label-free real-time biomolecular interactions to provide insights into the affinities of interactions between antibodies and their targets (ligands). The SPRi process occurs in real time and uses a primed sensor to capture secretions of interest for analysis. However, only recently has SPRi been utilised for cell analysis and protein secretions from intact single cells quantified.

Prof Terstappen and his collaborators were able to combine the self-seeding microwell chip with SPRi technology to precisely isolate single cells and not only accurately characterise the quantity of antibodies produced by the individual cells, but also their affinity – a previously unreported feat. More specifically, following self-seeding of the wells with individual cells, the microwell chip was attached to the SPRi sensor and incubated overnight. The cellular secretions diffused out through the pore and were captured by the ligands on the sensor, creating a series of small spots printed on the surface directly below each well. Using imaging software, the kinetics of antibody production including the pg antibody produced per cell per day and their affinity to the ligand was determined and revealed those cells that likely could be expanded into monoclonal cell lines with high quantity and quality of antibodies.

Characterising Cell Secretions and Monitoring Dynamics

A further extension of Prof Terstappen's work includes the development of a microwell printing method that circumvents production issues encountered with other cell line manufacturing methods. Having tirelessly researched effective single-cell selection and isolation methods, and the successful capture and analysis of specific cellular secretions, the group proceeded to categorise cells into high-, medium-, and low-producing cell populations. They also monitored cell cultures over a period of 48 hours to evaluate whether their secretion status remained constant over time.

Following the determination of the cell secretion concentrations, Prof Terstappen and the team then investigated the secretion dynamics of the same cells at different time periods. Although the group has previously shown that cells from different cancer cell lines can be successfully isolated and expanded, further research is needed to determine whether those cells deemed high producers maintain their secretion levels over an extended time period. However, the success of these experiments is largely dependent on the ability to maintain optimal sterile culture conditions.

For the secretion dynamics experiments detailed here, the antibody production of each cell was measured at 8-hourly time intervals, using a fresh membrane for each time point. The data demonstrated that the amount of antibody secreted by the cells remained relatively steady, and that cells initially characterised as high producers remained so throughout the culture period, even after cell division. Prof Terstappen and his colleagues are actively conducting experiments to establish whether cells that produce high levels continue to do so after isolation and expansion. Importantly, this proposed new technology could reduce the cell selection process from 21 days to a matter of hours, with the possibility of screening >30,000 cells per day.

The Microwell Cell Selection Printer

The pinnacle of the group's research thus far is the creation and ongoing advancement of the Microwell cell Selection PRinter (McSPRinter, NWO grant #15327), a ground-breaking technology combining the described techniques which will no doubt have a huge impactf on the discovery of targeted therapeutic agents.

The concept of the McSPRinter has been met with enthusiasm and has received support and funding from several high-profile collaborators.

Predicted uses of this technology, and the focus of future work for Prof Terstappen and his team, include the screening of rare cells for the development of therapeutic antibodies, and the discovery of new treatment targets. The experiments summarised in this article have the potential to be adapted for the screening of many different cell types, including circulating tumour cells, B-cells encoded to produce antibodies against desired targets and T-cells for the detection of various molecules of interest, including cytokines, which may prove invaluable in the development of highly effective targeted drug regimes.

Meet the researcher

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Prof Leon WMM Terstappen obtained his PhD in Biophysics from the University of Twente in the Netherlands, and in 2007 he founded the Department of Medical Biophysics at the same institution. Having held various research positions around the globe, his research is now primarily focused on circulating tumour cells (CTC), and he remains intrigued by solving medical problems using ground-breaking technologies. Indeed, Prof Terstappen was instrumental in developing the FDA-cleared CellSearch system for the quantification of CTC in blood. His more recent work has resulted in the invention of the McSPRinter, a tool designed to determine the secretions produced by individual cells, with the ultimate aim of extending bespoke medical care to all cancer patients. The recipient of several prestigious awards, Prof Terstappen is an internationally lauded expert in the field of cytometry and the detection of rare cells.

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VyCAP BV, Enschede, Netherlands

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UNIVERSITY OF TWENTE.

DISCOVERING UNMAPPED MOLECULAR TARGETS FOR NOVEL COVALENT DRUGS

Covalent drugs are molecules that irreversibly bind to specific, targeted sites in the body. They work to inhibit the disease-causing functions of certain proteins by preventing them from interacting with other substances. This is a highly promising field of drug development and the focus of **Dr Mikail Abbasov** from Cornell University, New York, USA. By creating and utilising new technologies and through collaborative research, Dr Abbasov has mapped novel molecular targets for potential covalent drugs to treat ailments ranging from cancer to autoimmune diseases.

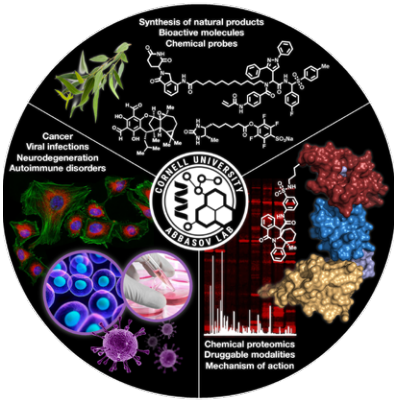
Covalent Drugs: Creating Irreversible Bonds

Therapeutic drugs come in numerous forms for an endless number of diseases. Depending on the ailment in question, a drug will target certain biochemical reactions in the body to reduce its negative impacts. This involves the drug in question binding to a target protein in cells. Whereas some therapeutics form bonds that are reversible, others form permanent bonds to create a long-lasting effect. For example, covalent drugs form strong, irreversible covalent bonds with proteins. Well-known examples of this type of drug include the anti-inflammatory, Aspirin, the antibiotic, Penicillin, and the cancer drug, Ibrutinib.

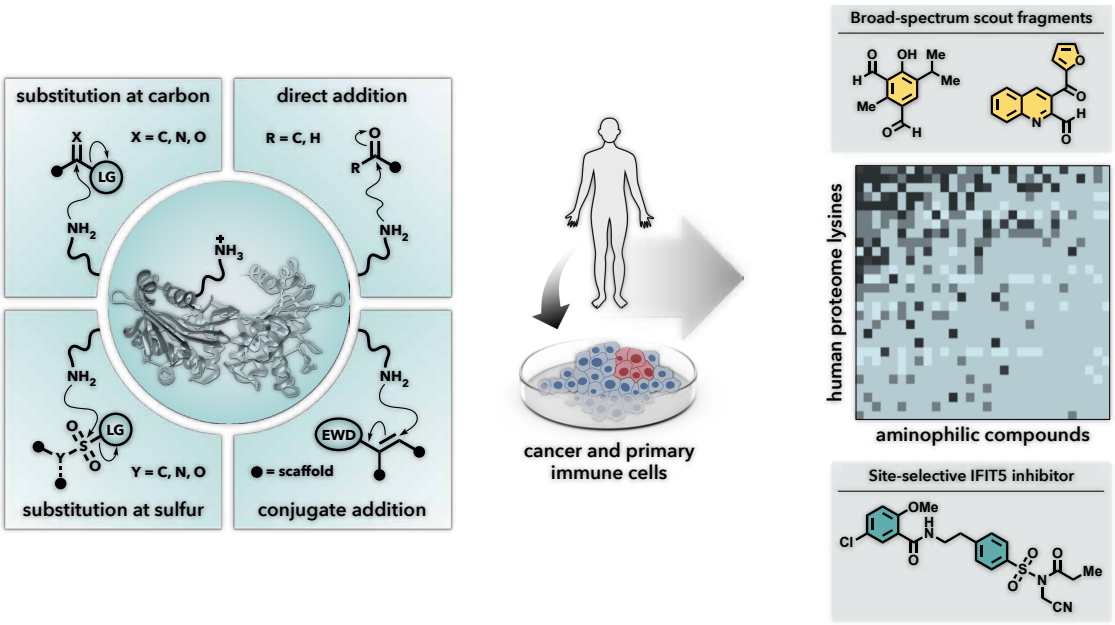
In the past, there have been safety concerns surrounding covalent drugs, with many scientists viewing their irreversible binding actions as dangerous. Some studies showed that these drugs could be metabolised to create highly reactive intermediates that, if accidentally targeted the wrong protein, could become toxic to a patient. More recently, a resurgence in interest in covalent drugs has exploited their compelling benefits whilst fine-tuning how they react in the body to ensure their safety.

These new drugs are called targeted covalent inhibitors and they work by first being guided towards and weakly bonding to the desired protein through a small pocket. This initial interaction facilitates the subsequent formation of the irreversible covalent bond with a nearby amino acid. Consequently, the function of that protein is inhibited.

Covalent inhibitors target nucleophilic amino acids – molecules on proteins that form bonds with an electrophile by donating an electron pair. They can be designed to target very specific nucleophiles on a protein so that other proteins within the same family are not impacted when the drug is administered. This clever technique is being utilised in a number of drugs that are currently in late-stage development. Many of them are targeting different types of cancer and some are even being tested for COVID-19 treatments, such as Paxlovid.



Although the field of covalent drugs is highly promising, there is a distinct lack of research as of yet and much more work is needed to make full use of its potential. Dr Mikail Abbasov from the Department of Chemistry and Chemical Biology at Cornell University in New York, USA dedicates his research to improving our knowledge and



Discovering druggable lysines. Credit Mikail Abbasov.

understanding of the targets of covalent drugs and how they could be used to create ground-breaking therapies.

Working alongside his colleagues, Dr Abbasov explains, 'Our highly interdisciplinary research program spans the fields of chemistry, biology and physics with an emphasis on the development of innovative chemical proteomic tools and technologies that modulate protein function and interrogate pathophysiological signalling pathways associated with human diseases, such as cancer, viral infections, autoimmune and neurodegenerative disorders.'

Discovering Simple Molecules with Drug Potential

Historically, to understand the relationships between the structure and activity of a protein, total synthesis followed by structural derivatisation has been performed. Total synthesis is the process of using chemistry to form a complete, complex molecule using commercially-available ingredients. These complex molecules are usually natural products, which are substances that are created by living organisms and plants, meaning they are already found in nature. Structural derivatisation refers to chemically transforming a chemical compound into a different, yet structurally similar one, known as a derivative.

However, Dr Abbasov saw that these synthetic techniques to create natural products were not usually guided by hypotheses that took into consideration the structural features of the compounds that are needed for bioactivity. Therefore, Dr Abbasov, in collaboration with Dr Daniel Romo at Baylor University in Texas, developed a new strategy for natural product synthesis which he called 'pharmacophore-directed retrosynthesis'. A pharmacophore is the section of a molecule that is necessary for its biological and pharmacological activity.

Retrosynthesis is a method used to understand molecule synthesis by breaking down the final product into more simple precursors. They are continually simplified until the resulting molecules are commercially available or easy to use in a synthetic reaction for the target molecule. Dr Abbasov performed pharmacophore-directed retrosynthesis with a pre-determined or a hypothetical pharmacophore of a natural product in mind. As a result, the intermediates that were synthesised in the process could each be tested for their bioactivity during the total synthesis of the natural product.

Putting this innovative new method into practice, Dr Abbasov investigated gracilin A, a natural product isolated from the Mediterranean sponge Spongionella gracilis. The biological role of the gracilin A prior to this study was largely unexplored.

For this reason, Dr Abbasov endeavoured to use his pharmacophore-directed retrosynthetic approach to create structurally simplified derivatives of gracillin A that can be accessed in the early stages of the synthesis. He aimed to identify derivatives with selective affinity (attraction) towards cyclophilin A and cyclophilin D, proteins associated with immunosuppressive and neuroprotective activities, respectively. Consequently, Dr Abbasov identified simplified, ultrapotent derivatives that exhibited activity greater than that of gracilin A.

This is an important finding because it demonstrates that structurally simplified intermediates can possess greater biological activity than that of the parent natural product, thus reinforcing the advantages that this approach offers for the rapid discovery of potential therapeutics. In particular, the derivatives that have a high affinity for cyclophilin D over cyclophilin A could be useful as neuroprotective drugs that do not have immunosuppressive side effects.

Discovering and Mapping Druggable Lysines

In a subsequent study, Dr Abbasov and his colleagues conducted new and exciting research on biological targets for covalent drugs. In particular, they focused on covalent targeting of amino acids – the building blocks of proteins. Twenty different amino acids are found in the human body and historically, two of them called cysteine and serine have been the main focus for covalent drugs. However, this limits the possibilities for drug discovery.

Consequently, Dr Abbasov, in collaboration with Dr Benjamin Cravatt at Scripps Research in California, set out to investigate another amino acid called lysine. They also brought in teams from the Massachusetts Institute of Technology, Pfizer and Leiden University for the project. Together, they developed a chemoproteomic technology that allowed them to study how lysines react with small molecules. They aimed to find aminophilic electrophiles – chemical species that form irreversible bonds with nucleophilic amine groups on lysines.

This ground-breaking work led Dr Abbasov to profile and log over 200 of these small molecules. By testing human cancer and immune cells, the team found thousands of reactive lysines. This included functional sites on proteins that were previously seen as too difficult to target. Collating this new data allowed Dr Abbasov and his colleagues to create a comprehensive map of lysines that are now known to be possible drug targets.

By fine-tuning both the recognition portion and the reactive fragment of the small molecules, they were able to identify a wide range of proteins in which these lysines are found. These proteins have a wide range of functions in the human body and are known to be involved in various diseases, including, for example, enzymes and transcription factors (needed for protein synthesis). By inhibiting the function of disease-relevant proteins, these types of small molecules could serve as drugs to prevent the harmful progression of a variety of diseases.

According to Dr Abbasov, 'The findings from this project provide a blueprint for chemical biologists and medicinal chemists to understand the rules that govern lysine-targeting covalent inhibitor design, eventually leading to new treatments and drugs.' To aid this development, after mapping the druggable lysines, Dr Abbasov and his team combined this data with human genetic information and cell activation measurements.

This helped to refine their understanding of how different small molecules can impact the activity of the protein and consequently, the cell. They were able to demonstrate that, through lysine binding, aminophilic compounds disrupted a number of biochemical functions within cells, including those involved in the innate immune response. With such real promise for finding and creating new covalent drugs, Dr Abbasov's work is not stopping there.

'Findings from our laboratory have enriched our understanding of the mechanistic underpinnings of pathological processes and provide valuable leads for the design and development of novel therapeutics' notes Dr Abbasov. And this research is ongoing; only one year after starting his position at Cornell University, Dr Abbasov secured a $2M grant from the National Institute of General Medical Sciences to continue discovering the druggable targets of the human body.

Meet the researcher

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Dr Mikail Abbasov was born in Baku, Azerbaijan, but his family had to flee in 1988 to escape devastating conflict. They moved to a rural community in Rahachow, Belarus, which was part of the Chernobyl exclusion zone. This meant that it was common for research groups to visit and carry out tests at the local hospital near Dr Abbasov's school. He became fascinated by their work and was keen to help them when he could. Now an American citizen, Dr Abbasov values the importance of quality education and scientific research, and employs this understanding in all of his work.

His mother is a classical pianist and composer and his father is a painter and sculptor, both influencing Dr Abbasov to pursue the Arts in many forms. He graduated from the School of Music and Performing Arts and is classically trained as a baritone and in the piano. As a keen artist, Dr Abbasov has had his illustrations featured on the covers of several scientific journals and as an actor, he has played a supporting role in the award-winning independent film, The Heist.

Dr Abbasov completed his BSc in Biochemistry and his MSc in Optical Physics at West Texas A&M University in Canyon, Texas. He went on to achieve a PhD in Organic Synthetic Chemistry at Texas A&M University in College Station, Texas, and completed postdoctoral research at The Scripps Research Institute in California. Currently, Dr Abbasov serves as an Assistant Professor in the Department of Chemistry and Chemical Biology at Cornell University in New York. Here, he carries out his research into the targets of covalent drugs and how they can be utilised to develop effective, novel therapeutics.

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THE IMPORTANCE OF THE P2X7 RECEPTOR IN INFLAMMATION AND PAIN

Physiological responses to inflammation and pain are complex and varied, depending on which cellular structure you focus on. Planted within our cell membranes is a channel that facilitates the transport of ions in response to perceived dangers. Named P2X7 receptor, this channel subtype is activated by adenosine triphosphate (ATP) to open the gate for ions, and is of particular interest to **Dr Fritz Markwardt** at Martin-Luther-University Halle-Wittenberg in Germany. Dr Markwardt studies P2X7 to elucidate its structure, function and ATP activation mechanisms to provide novel avenues for drug development.

The Movement of Ions in Response to Danger

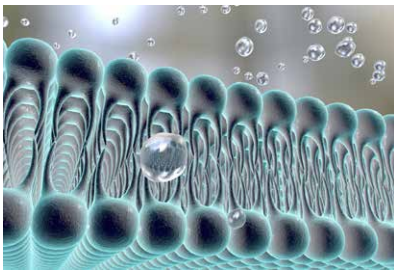
The cells in our bodies have countless, complex structures and mechanisms that help them to carry out their specific roles and keep the whole system running. Ligand-gated ion channels belong to such structures. These are pores in the cell that facilitate the movement of ions like sodium, potassium and calcium, and whether they are opened or closed depends on molecules called ligands. A ligand is just a small molecule that binds to a larger one and in this case, adenosine triphosphate (also known as ATP) binds to channels named P2X receptors to open them.

These P2X receptors are essential for a variety of physiological processes including those involved in the immune system, pain perception and programmed cell death. For this reason, they are found in particularly high numbers in the cells of the immune and inflammatory systems.

Normally, ATP acts as an energy source for the necessary reactions and processes within the cell. However, under pathological conditions such as trauma (cell destruction) and inflammation, ATP is released from cells into the extracellular space. When the outward-facing P2X receptors find and bind the ATP surrounding the cell, they read it as a danger signal. This causes the channel to open rapidly so that sodium, potassium and calcium ions can be transported across the membrane for the necessary response to the danger.

P2X receptors have seven known subtypes and the human form of the seventh subtype is known as hP2X7. This receptor is the focus of Dr Fritz Markwardt's research at the Julius Bernstein Institute of Physiology at Martin Luther University Halle-Wittenberg in Germany. He is a long-serving researcher and professor here and his work in collaboration with other scientists has deepened our understanding of this small yet hugely important component of the body.

The hP2X7 receptor is of particular interest to Dr Markwardt because of the role it plays in inflammation, pain sensation and tumour growth. In describing his research, Dr Markwardt says, 'We are interested in the activation mechanism of the hP2X7 receptor by ATP on the molecular level. Insights into these mechanisms might help to find pharmacological tools which may be helpful in the treatment of inflammation and pain.'

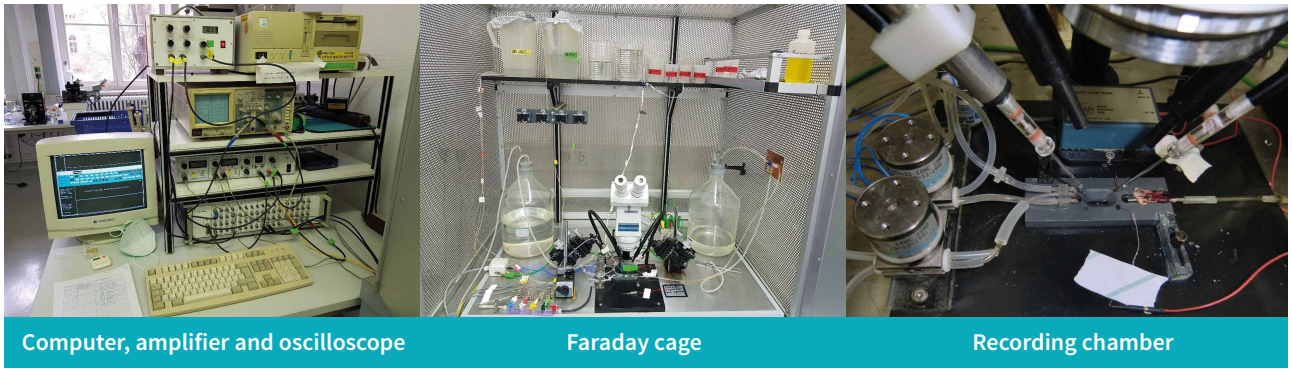


Cell membrane

The Mechanisms of Ion Channel Opening

A large part of Dr Markwardt's investigations has been to understand the activity of ATP and hP2X7 on a molecular level and how they affect ions currents through the channel. The first step of one of his studies involved collaboration with Professor Günther Schmalzing in Aachen, Germany, whose

‘We are interested in the activation mechanism of the hP2X7 receptor by ATP on the molecular level. Insights into these mechanisms might help to find pharmacological tools which may be helpful in the treatment of inflammation and pain.’



Recording setup for two-microelectrode patch clamp measurements
Credit: Fritz Markwardt

group synthesised mRNA constructs of the hP2X7 receptor protein. mRNA is the single-stranded form of DNA that is read by cell machinery to make a desired or necessary protein, including those that make up receptors.

Therefore, when Dr Markwardt introduced this mRNA into the immature eggs (oocytes) of Xenopus frogs, the cells began to produce the human hP2X7 receptors and insert them into their cell membrane. He could then use these cells to measure the ion currents through the hP2X7 receptor ion channels using the voltage clamp technique.

Scientists use this method in electrophysiology to look at how ions move across a specific section of a cell membrane (or a whole cell, or section of tissue). They do this by controlling the voltage across the cell membrane and recording what currents are consequently created. Because Dr Markwardt and his team made these measurements of ion currents of single channels in a patch of the membrane, this is called the patch clamp technique.

Every cell membrane has a natural voltage between -30 and -90 mV, so the team studied how ion currents are impacted by voltages within this range in addition to the concentration of ATP.

Through their results, they were able to describe the mechanism that leads to ATP-mediated activation and opening of the hP2X7 channel on a molecular level.

Characterising the P2X7 Ion Channel Receptor

Dr Markwardt went further to characterise the ion channel by studying which sections of the channel are responsible for selecting the ions that pass through, which is important for regulating normal cell function. He also determined the diameter of the pore for the ions to pass through – or not as the case may be. Cations, positively charged ions missing an electron, are able to pass through the channel, whereas, negatively charged ions with an extra electron, are not. They do, however, have some influence over the opening of the channel once ATP has bound to the receptor. A binding site for anions outside of the cell allows this to take place.

Although the opening of the channels is important, it is also crucial for them to close. Another study by Dr Markwardt found the structures that close the channel when ATP is not bound to P2X7 because it is not present in the extracellular space (outside the cell).

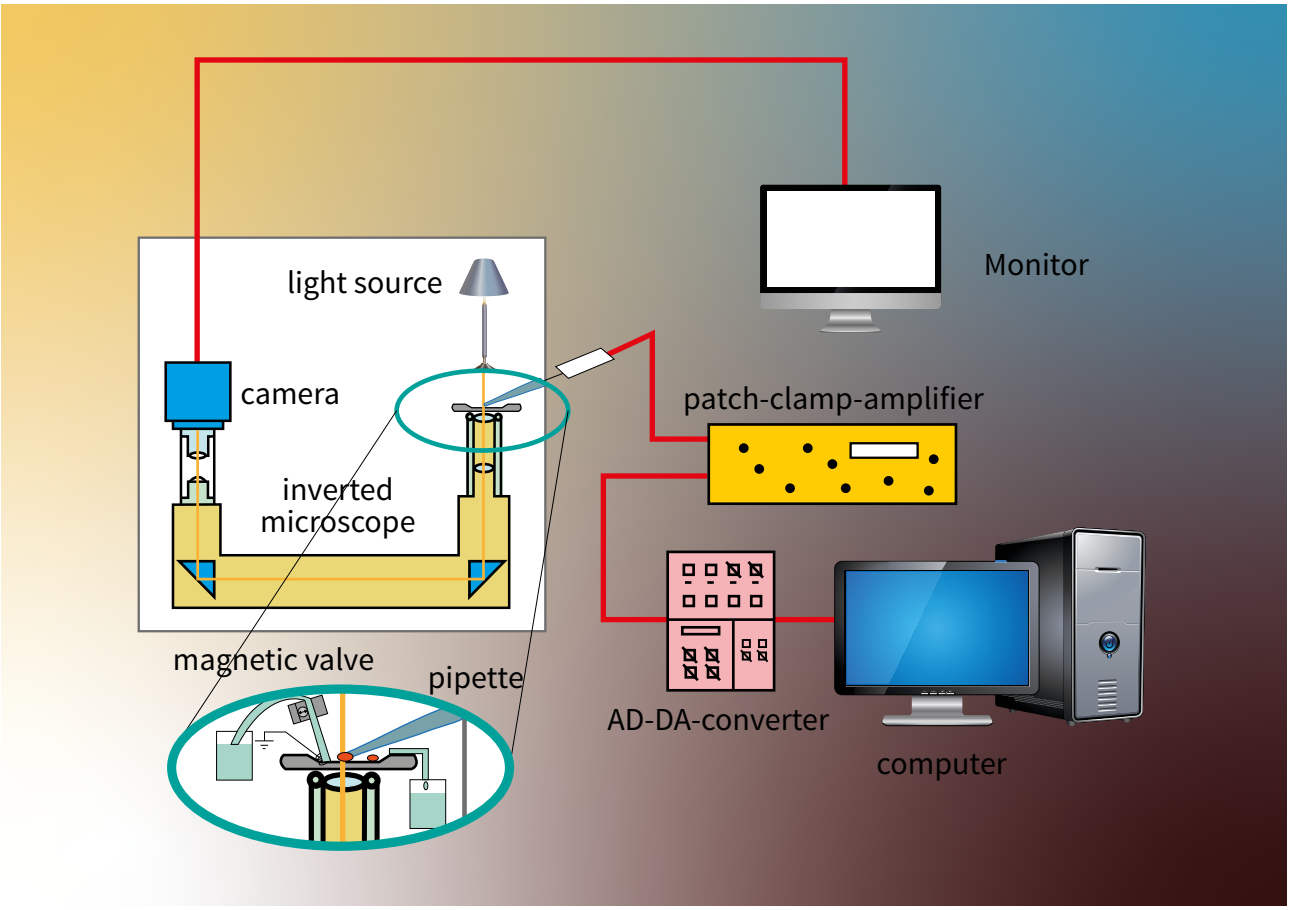
All of these findings helped Dr Markwardt and his fellow researchers to better understand how the P2X7 receptor ion channel and its specific elements function and for what purpose.

Facilitation and Inhibition of P2X7 Activation

One of these elements is called the C-terminal tail, which is the last about 200 amino acids in the chain – the very end of the P2X7 receptor protein. Again using Xenopus frog oocytes, Dr Markwardt carried out experiments to determine the purpose of this tail for the functioning of P2X7 receptors. Uniquely, P2X7 has an extended C-terminal, meaning it is longer than normal, which was of particular interest to the team.

When this tail was removed from the channel protein, it only produced 5% of the normal ion current, revealing the importance of this structure. They found that the extended C-terminal tail is used for regulating the function of the channel and central for optimal activation of the ion channel by ATP attachment.

In a separate study, Dr Markwardt found a factor that inhibits, rather than promotes P2X7 activation. When tissue is inflamed due to an immune or trauma response, the pH of that tissue lowers, i.e., the amount of protons increases in the area. In tests of P2X7



Recording setup for two-microelectrode patch clamp measurements
Credit: Fritz Markwardt

in this environment, he found that the protons bind to the extracellular ATP which means that it then has a lower affinity (attraction) to the binding site on the receptor. In addition to this, protons also bind to the receptor itself which further reduces the effects of ATP at the P2X7 receptor.

As a result, P2X7 activation is dampened during an inflammation response, as ATP finds it much harder to bind to the receptor and to open the channel.

Mechanism of Adenosine Triphosphate Secretion

The way in which cells secrete ATP for P2X7 activation is a vital yet not fully defined aspect of the mechanism of P2X7 receptor activation. Further research by Dr Markwardt and his colleagues discovered an important stretch-activated anion channel involved in the process. Cells need to be able to cope with a variation in volume within their membrane so that they don't burst when it increases or shrivel when it decreases.

When the cell volume increases, the membrane can stretch to accommodate this, creating a mechanical stimulus that puts stretch-dependent anion channels into action (hence the name). The team found that sphingosine-1-phosphate (S1P), a molecule involved in inflammation, can open such an ion channel, facilitating the transport of ATP from the inside to the outside of the cell.

This makes S1P a potential target for inflammatory disorders that go beyond what is necessary for an immune response and create an overactive and damaging one. Inhibiting S1P might prevent the over-release of ATP and the over-activation of P2X7.

Current Work on the Interactions and Uses of P2X7

With the aid of a grant from the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation), Dr Markwardt and his fellow researchers are continuing to deepen our understanding of the structure and function of the P2X7 receptor. His current investigations seek to elucidate the interactions between P2X7 and P2X4 receptors, a different subtype of the P2X family. Currently, it appears that while the two proteins can interact and even join within cells, they do not impact one another's function.

Dr Markwardt has dedicated a large portion of his scientific career to comprehending the role of P2X7 in the body and its physiological responses. This clarification will help others in the future to innovate and develop novel therapeutics that target the receptor and its related networks. These potential drugs could be incredibly useful for those suffering from inflammation and pain by targeting the mechanisms involved with P2X7 that amplify them – making a positive impact for those that are treated by them.

Meet the researcher

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Dr Fritz Markwardt holds degrees in Physics, Medicine and Physiology in addition to a medical doctorate and a scientific medical doctorate. He is a long-standing researcher and Professor of Electrophysiology at the Julius Bernstein Institute of Physiology at the Martin Luther University Halle-Wittenberg in Germany. Dr Markwardt's work focuses on the mechanism of adenosine triphosphate (ATP) secretion from cells and its action on ATP-gated P2X receptor ion channels. In particular, his work on the structure and function of the P2X7 receptors may help developing novel therapeutics for inflammation and pain.

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UNCOVERING THE MECHANISMS BEHIND NEPHROTIC SYNDROME TO DEVELOP NOVEL THERAPEUTICS

Kidney disease impacts many people throughout the world and it can sometimes take the severe form of nephrotic syndrome. This condition results in many difficult symptoms but it is best represented by the presence of oedema, which is the severe swelling of the legs and eyelids. Currently, the exact mechanisms underlying oedema formation are poorly understood. **Prof Ferruh Artunc** from the Tübingen University Hospital in Germany is using his expertise in nephrology to uncover these mechanisms. His work has strengthened our comprehension of oedema formation in nephrotic syndrome and it may even lead to novel therapeutics in the future.

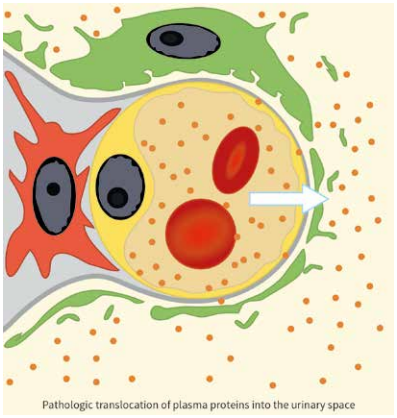
Kidney disease: The Causes and Consequences

Kidney disease is a common disorder that tends to show up in older age. When it is first developing, kidney disease does not usually have any symptoms and so it is often diagnosed from the results of a blood or urine test that has been taken for another reason. There are many reasons a person can develop kidney disease. For example, high blood pressure or diabetes, an inherited condition called polycystic kidney disease, kidney stones and even long-term use of some medicines. These all put increased strain on the kidneys and cause disruption to their normal functioning.

The kidneys are supposed to act as a filtration system for the body, removing waste, extra fluid and acid produced by normal cell function into the bladder. They also maintain a healthy balance of water, salts and minerals like sodium in the blood to keep the muscles, nerves and other tissues working properly. When this system goes wrong and the kidneys fail to excrete sodium, people may experience shortness of breath, weight gain and oedema.

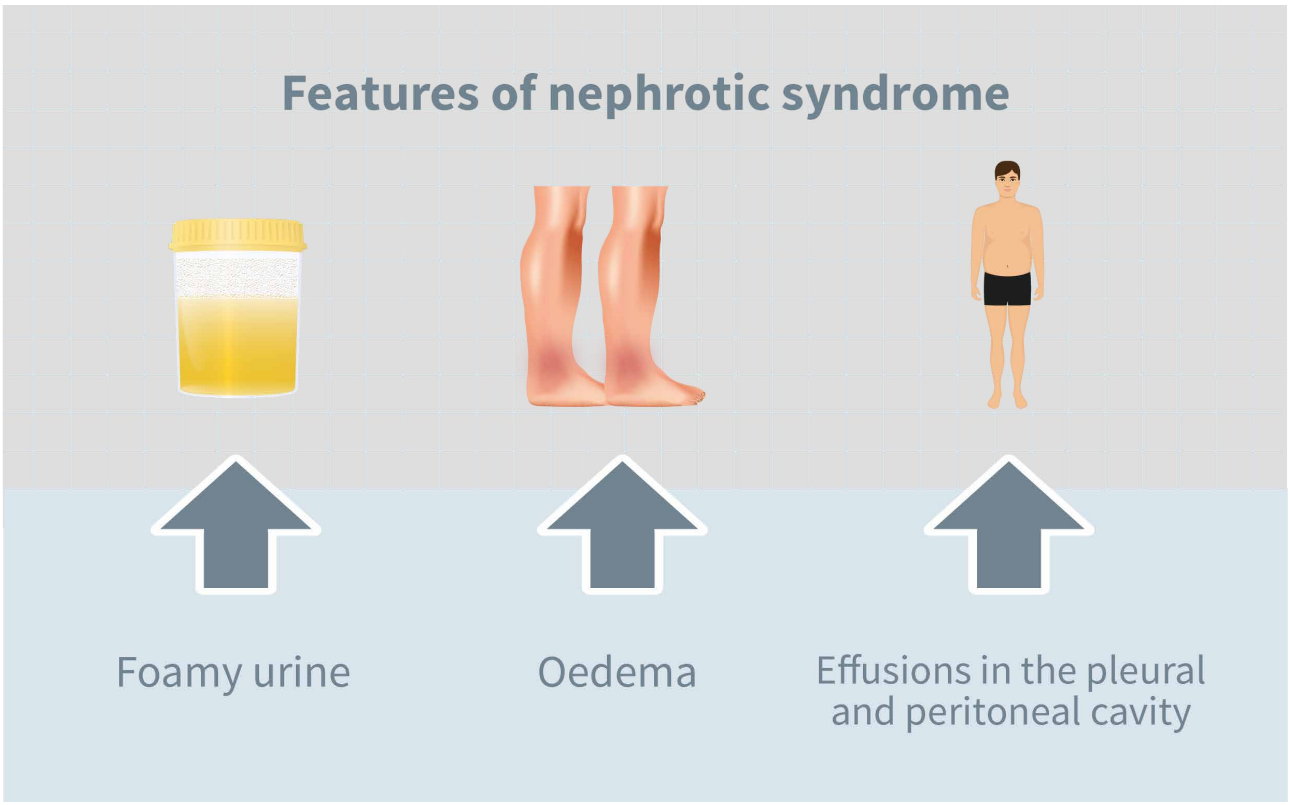
These symptoms should lead to testing for kidney conditions via a blood or urine test and perhaps a scan or biopsy to assess the extent of the kidney damage. Blood and urine tests search for unusually elevated levels of certain substances, such as the waste product creatinine in the blood or proteins in the urine. By estimation of the glomerular filtration rate, the doctor can determine how efficiently the kidneys are filtering the blood.

Although there is no cure for kidney disease, making healthy lifestyle changes can be greatly beneficial, in addition to medicines to control high blood pressure. To ease the swelling symptoms caused by excess fluid, some people are prescribed diuretics. More advanced disease can also be



Credit Ferruh Artunc.

treated with dialysis to clean the blood and sometimes a kidney transplant is necessary. Most people with kidney disease are able to control and live with it for a long time and only a small proportion of people reach an advanced stage of the disease. Nevertheless, it is important to diagnose it earlier rather than later and have regular check-ups because untreated kidney damage can have serious consequences. Kidney failure, hypertension and congestive



Credit Ferruh Artunc.

heart failure with lung oedema can all develop from untreated disease.

Nephrotic Syndrome

One extreme type of kidney disease is known as nephrotic syndrome. Some symptoms are shared with other types of kidney disease, whilst some present as the opposite. For example, many people will experience weight gain as a result of intense fluid retention and swelling. Numerous risk factors are also the same between the two conditions, although nephrotic syndrome is seen more often in children compared to other types of kidney disease. This is as a result of the mysterious condition known as minimal change disease, whereby the kidneys are dysfunctional but tissue samples under a microscope appear completely normal and the cause of the dysfunction cannot be determined.

Although the causes and risk factors leading to nephrotic syndrome vary from person to person, one element is nearly universal. In nephrotic syndrome, there is damage to the glomeruli in the kidneys, which are clusters of tiny blood vessels and filter the blood. Various diseases of the kidney can lead to scarring of the glomeruli and the rest of the kidney tissue.

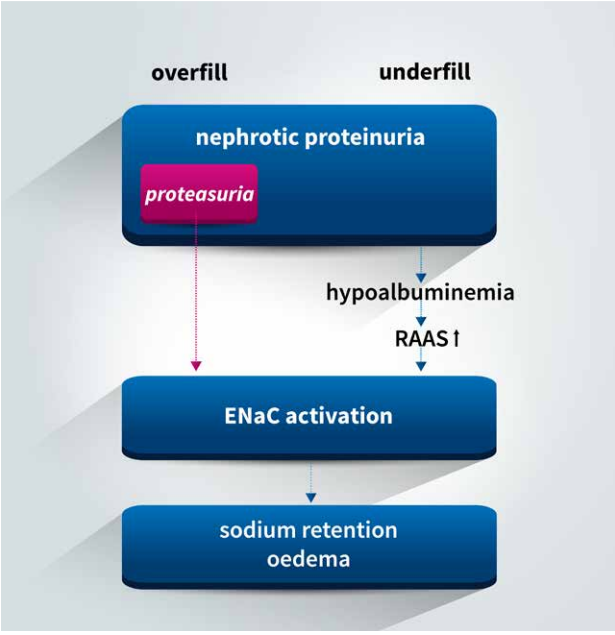
Glomeruli are an essential component of the kidneys and they help to filter waste and to remove excess water from the bloodstream while withholding blood proteins such as albumin. Albumin normally helps to maintain the right amount of fluid within the body but when the filtration barrier of the glomeruli is damaged, it escapes into the urine. For this reason, a hallmark of nephrotic syndrome is oedema alongside an especially high concentration of albumin in urine tests.

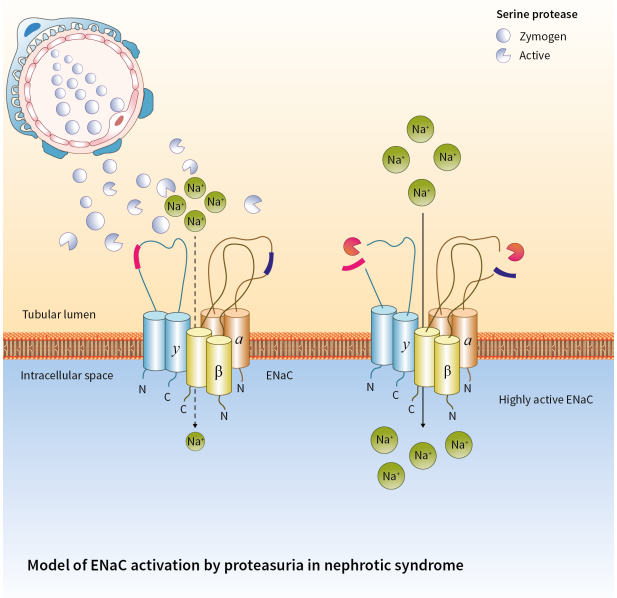
Once there is less plasma albumin available for maintenance of the circulating volume, water and sodium continue to accumulate in different parts of the body such as in the ankles, legs and eyelids after overnight rest. Unfortunately, nephrotic syndrome can result in other complications like blood clots, malnutrition and infections. But as with other kidney diseases, it can be partly remedied through treating some symptoms and ultimately by treating the underlying conditions.

Clearly, nephrotic syndrome is a well-documented pattern of kidney disease, but not all bases are covered when it comes to understanding it on a deeper level. Prof Ferruh Artunc is a medical doctor and researcher at University Hospital Tübingen in Germany and he is using his expertise to unravel the hidden mechanisms behind why oedema forms as a result of nephrotic syndrome. He hopes this may lead to novel and more effective approaches to treating oedema in the near future.

Uncovering the Mechanisms Behind Nephrotic Syndrome

Although oedema in nephrotic syndrome is relatively uncommon, it is not well understood. Two notable theories – the underfill and overfill theories – seek to explain the processes leading to oedema and the consequent





Credit Ferruh Artunc.

fluid build-up. The underfill theory is around 100 years old and uses the idea that losing albumin in the urine is due to the nephrotic syndrome causing fluid leakage from the blood vessels into the tissues of the body. This results in the activation of water and salt conservation by the kidney. On the other hand, the newer overfill theory suggests that the syndrome itself causes water and salt conservation through other mechanisms and in turn, the vessels overfill with fluid which consequently leads to oedema.

These differing opinions and gaps in the research literature have meant that the exact mechanisms of oedema have been unclear up until now. In order to clarify the processes, Prof Artunc and his team set out to elucidate the unseen causes of oedema in nephrotic syndrome – with interesting results.

Previous data had suggested that oedema is likely to be caused by an activation of the epithelial sodium channel ENaC, a special sodium channel. This sodium channel is a tiny tunnel located at the end of the kidney tubule and determines the final amount of sodium excreted in the urine. Prof Artunc's team used mice that had nephrotic syndrome and prominent oedema to study the activation of this channel further. They discovered that water and salt conservation by the kidneys could be prevented by the use of a drug called aprotinin that stops the activity of serine proteases that enter the kidney tubule during nephrotic syndrome and activate the epithelial sodium channel ENaC inhibitor by cleaving its pore open. As a result, aprotinin treatment prevented oedema formation in nephrotic mice.

According to Prof Artunc, 'this indicates that water and sodium conservation in nephrotic syndrome is caused by excretion of active serine proteases with the urine that are filtered from the blood´. More specifically, the team's experiments showed that urinary serine proteases are decisive for stimulating the activity of the epithelial sodium channel in nephrotic syndrome. The team coined the phrase 'proteasuria' for this phenomenon and they believe it could be a promising new target in developing novel therapeutic drugs for those suffering from nephrotic syndrome. Taking into account the previous theories of oedema formation, Prof Artunc and his team believe that their findings can effectively integrate both theories to explain this one process.

Identifying the Culprit

Leading on from this study, Prof Artunc has identified all of the different serine proteases that are found in the urine of both mice and humans with nephrotic syndrome. He and his colleagues are currently researching how important they are to the development of nephrotic syndrome by using knockout mouse models. These are mice that have been modified to not produce specific proteins interest, in this case, a serine protease. This allows the team to study what effect, if any, each specific serine protease has on oedema. If a missing protease results in no oedema in a nephrotic mouse, it is a strong indicator that it is essential to the disease process.

Once a serine protease has been identified as a main cause of oedema, it will be a promising target for novel pharmacological approaches to treat nephrotic syndrome. Creating drugs that inhibit its function would be an exciting development both for scientists and the people living with the disease. Through his innovative and dedicated research, Prof Artunc is helping to push forward our understanding of the mechanisms underlying nephrotic syndrome oedema, with important clinical and theoretic implications.

Meet the researcher

Prof Dr Ferruh Artunc
University Hospital Tübingen
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Prof Ferruh Artunc received his medical degree from the University of Tübingen in Germany in 2001 then went on to work as a medical doctor in the Department of Internal Medicine at the Tübingen University Hospital. He specialised in internal medicine, and specifically, nephrology, which is the diagnosis and treatment of kidney diseases. In his medical work, Prof Artunc also carries out research as a clinical scientist in this field, publishing numerous papers and already being the recipient of multiple awards throughout his career to date, including the prestigious Nils-Alwall-Award from the German Society of Nephrology in 2019.

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Universitätsklinikum Tübingen

A NOVEL ANTIBODY WITH VACCINE-LIKE PROPERTIES TO TREAT HEART DISEASE IN DIABETES

Patients with diabetes mellitus face an increased risk of developing heart disease. The high levels of sugars, lipid and cholesterol associated with diabetes cause thickening of the blood vessels, increasing the incidence of coronary heart disease and stroke. The pioneering team from the Centre of Molecular Immunology, Cuba, in collaboration with **Dr Spencer Proctor** from the University of Alberta in Canada are testing an antibody that targets the walls of the blood vessels, preventing the binding and accumulation of cholesterol.

An Important Collaboration Between Two Countries

The accumulation of lipids and cholesterol in arterial vessels leads to the formation of obstructive deposits, known as plaques, that limit the vital flow of oxygenated blood to organs and tissues. This condition, known as atherosclerosis, affects millions of individuals each year, particularly those with insulin resistance and diabetes. The damage resulting from atherosclerosis depends on the location of the affected arteries. If the obstructions limit the blood flow to the heart, they may cause chest pain, breathlessness and heart attacks. Similarly, when the plaques form within the carotid arteries, they can cause a reduction or blockage of the blood supply to the brain, leading to a stroke. Atherosclerosis can affect any artery in the body and cause an array of conditions, such as kidney disease, erectile dysfunction and even numbness in the limbs.

Atherosclerosis is traditionally treated by reducing the amount of cholesterol-dense proteins, known as LDL or 'bad cholesterol', in the bloodstream. Dr Spencer Proctor (Canada), Dr Sylvie Marleau (Canada), Dr Ana Maria Vazquez and Dr Yosdel Soto (Cuba) have joined forces in a historical collaboration between the two countries offering a novel approach to treat atherosclerosis.

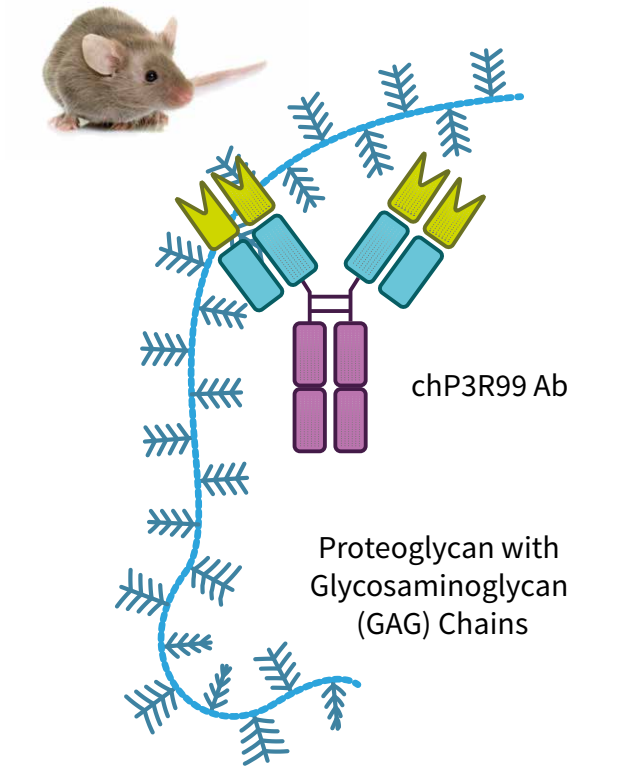
A Radically Different Approach

LDL cholesterol is produced by the liver and is the main target of current cholesterol-lowering medications. Dr Proctor and Dr Soto, however, are leading to also decrease the impact on heart disease by another type of 'bad' cholesterol, known as remnant cholesterol, which is derived from the diet and secreted by the intestine. The accumulation of remnant cholesterol is significantly increased during diabetes. To support this concept, it is worthy of note that the European Union and Canada have begun measuring remnant cholesterol and other non-fasting lipids as a tool to identify groups at risk of developing atherosclerosis.

These clinical outcomes have prompted Dr Proctor and Dr Soto to devise new ways of targeting atherosclerotic plaques. Rather than targeting the production of LDL, Dr Proctor and Dr Soto aim to block cholesterol from all sources from binding to specific anchor-like points in the blood vessels known as glycosaminoglycans (GAGs). GAGs are sugar side chains in the artery walls that act as sticky regions for cholesterol particles. There is currently no drug that specifically targets the GAGs on the blood vessels, preventing both LDL and remnant cholesterol from attaching.

The pioneering efforts of the Centre for Molecular Immunology (CIM) in Cuba have developed an antibody known as chP3R99. The centre has accumulated 25 years of achievements in developing monoclonal antibodies that have proved effective in the treatment of cancer and other chronic diseases. The chP3R99 antibody specifically binds to the sugar side chains, effectively preventing cholesterol particles from all sources from forming plaques on the blood vessels. This mechanism of action is of great relevance to conditions such as insulin resistance and diabetes, where the increased risk of cardiovascular disease is partly due to a greater abundance of GAGs on the arterial wall.

Mouse/human chimeric chP3R99 antibody (IgG1) blocks lipoprotein binding sites on proteoglycans



Pioneering the Development of a Unique Antibody

Scientists at CIM in Cuba first developed chP3R99 in mice and eventually engineered what is known as a 'chimeric' mouse/human antibody, a protein that has been expressed by combining the genetic material from the two different species. The team at CIM in collaboration with Dr Sylvie Marleau (University of Montreal) tested the antibody on arterial tissue from rabbits and mice, demonstrating in several publications that chP3R99 causes a significant decrease in the formation of atherosclerotic plaques. The decrease was caused by the preferential binding of the antibody to the sugar chains on the artery walls rather than reducing cholesterol levels.

Notably, the antibody has a dual mechanism: not only does it bind to the GAGs sugar chains, but it does so in a vaccine-like fashion, which means that it does not need to be injected repeatedly in successive multiple doses to maintain its effect. Once injected, it will produce a cascade of secondary antibodies, which will continue to exert the protective action against the accumulation of cholesterol. The treatment has shown promising results in preclinical models of atherosclerosis. What sets apart the drug developed at the CIM from other compounds in preclinical studies is that other treatments show benefits in the early stages of atherosclerosis, while the chP3R99 antibody also halts plaque progression and stimulates regression in advanced atherosclerotic lesions.

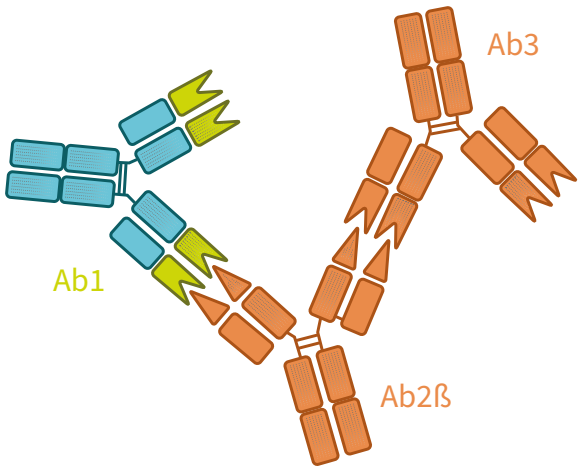
Using the Antibody to Target Remnant Cholesterol

Dr Proctor is an expert in the field of metabolism of lipids in health and disease, particularly in relation to the interaction of lipids with arterial vessels during atherosclerosis and insulin resistance. Dr Proctor's team has previously demonstrated that insulin resistance and diabetes correlate with an increase in the binding of remnant cholesterol to the arterial wall, causing the formation of plaques. The group has contributed to the understanding that diabetic atherosclerosis can accelerate vascular disease. This is due to the fact that insulin resistance causes an extensive re-modelling of the architecture of the arterial wall, leading to an increased production of sugar side chains in GAGs, which in turn will increase the capacity for cholesterol binding. The group have been able to publish their findings by using mice, rats and swine animal models.

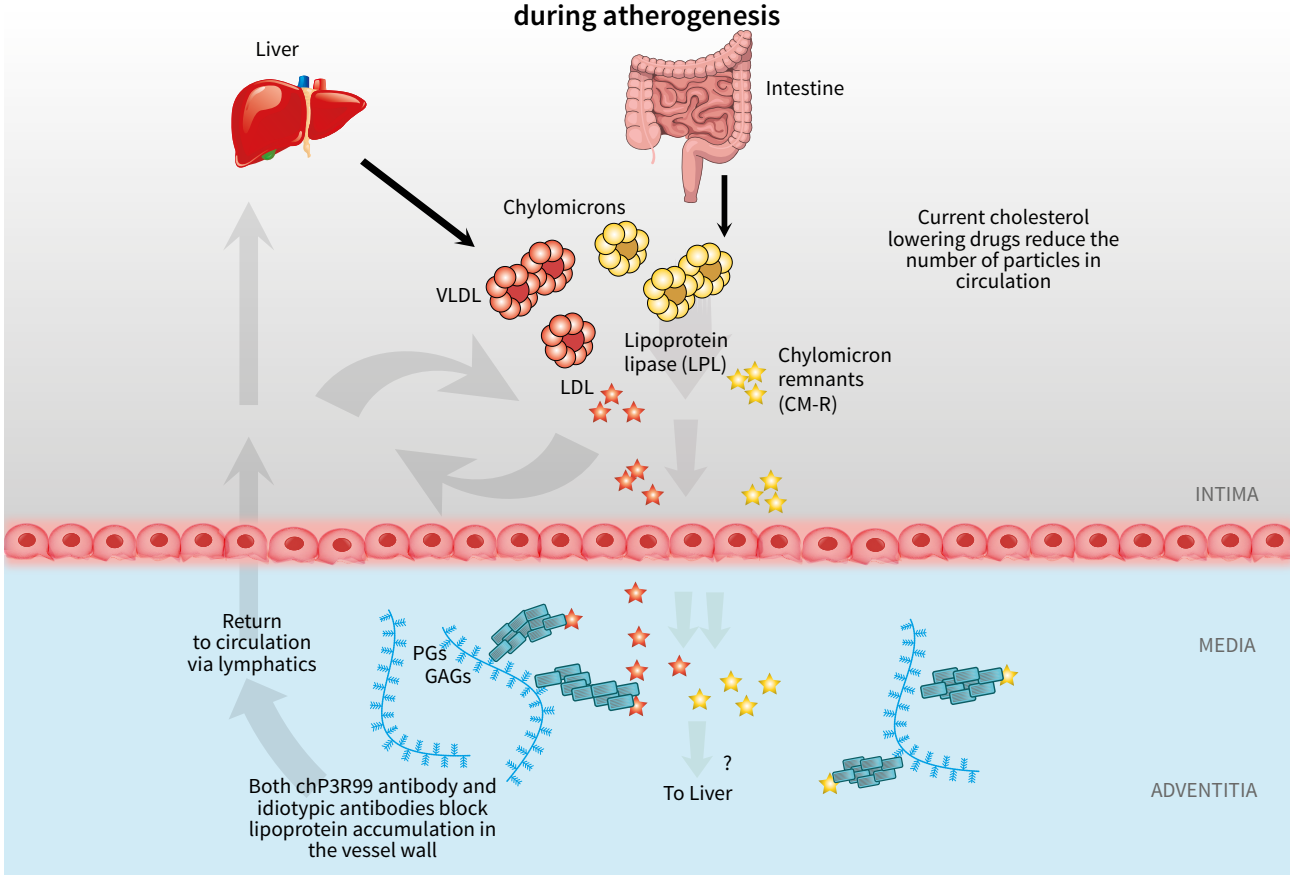
In collaboration with Dr Soto and the team at CIM, Dr Proctor has gathered compelling evidence that shows that the novel antibody is able to inhibit the binding of remnant cholesterol to arterial tissue, proving the significant contribution that all forms of cholesterol and not just LDL have on atherosclerosis in animal models of insulin resistance.

The project is conducted in the state-of-the-art facilities at the University of Alberta, which hosts research excellence centres such as the Alberta Diabetes Institute and the Group on the Molecular and Cell Biology of Lipids (MCBL). The researchers there have mastered the use of fluorescent imaging techniques and confocal microscopy to trace the interaction of lipids and

chP3R99 (Ab1) induces idiotypic cascade of antibodies



Simplified working model of factors contributing to arterial retention of cholesterol during atherogenesis



cholesterol with the arterial walls. In order to to complete pharmacological and toxicological studies with the chP3R99 antibody, the production process of its latest version is under development to scale up its production at pilot plant (pre-commercial) scale.

In their collaboration, the two scientists and their teams have collected numerous preliminary data that confirm the effectiveness of the antibody against cardiovascular disease in higher-order mammals. They are confident that the treatment will prove to be effective against human arterial tissue.

The Vaccine-like Properties of chP3R99

The preliminary data from the ambitious project by the Canadian and Cuban scientists show that the antibody can be used successfully both as an acute dose treatment and as a series of injections. When administered acutely, the antibody is given as a high dose intravenous injection to insulin-resistant animals with a highly re-modelled arterial wall architecture. When the arterial tissue of the treated animals is analysed ex vivo, it displays significantly reduced binding of both remnant cholesterol and LDL cholesterol to the arterial wall when compared with untreated control animals.

Interestingly, when used in experiments as a vaccine, the drug can be injected just under the skin, in multiple small doses, over several weeks. The animals treated this way are able to develop their own secondary (and tertiary) antibodies against the GAGs of the arterial wall. These 'generational' antibodies are in turn capable of displacing the binding of both remnant and LDL cholesterol, reducing plaque formation when compared to the control group. Although unpublished, these data show proof of principle that the novel compound can successfully be used as a vaccine for the treatment of atherosclerosis in insulin-resistant, higher-order mammals. If confirmed in humans, these exciting results offer a new and effective therapeutic strategy to treat heart disease in diabetic patients.

Future Developments

The collaborating teams hope to corroborate their findings with in vitro studies, to map in detail the mechanism of binding of the novel antibody to GAGs, specifically under conditions of insulin resistance. They aim to confirm that the antibody inhibits both remnant and LDL cholesterol and to ascertain whether there is a competition between the two forms of cholesterol for the binding sites on the arterial wall matrix. They also plan to use discarded donor human blood vessels to measure the protective effect of the antibody against cholesterol retention and atherosclerotic plaque formation in humans.

All the data obtained so far make the two teams confident that the novel antibody will be effective when tested in human-derived specimens. If this is confirmed, the drug will undergo phase 1 clinical trial, offering new hope of long-time protection for millions of individuals with cardiovascular disease.

Meet the researchers

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Director, Metabolic and Cardiovascular Diseases Laboratory
Molecular Cell Biology of Lipids Group, Women & Children's Health Research Institute, Alberta Diabetes and Mazankowski Heart Institutes
University of Alberta
Canada

Dr Spencer Proctor is full professor at the University of Alberta and a principal member of the Alberta Diabetes Institute. Dr Proctor trained as a physiologist and cardiovascular scientist in both Australia and Canada. In 2004, he founded the Metabolic and Cardiovascular Diseases Laboratory (MCVD) at the University of Alberta. Dr Proctor and the MCVD Laboratory are contributing to the link between nutrition and dietary-related chronic disease such as obesity and diabetes to increased risk of cardiovascular disease. Among Dr Proctor's most important findings is the development of a model of over-production of intestinal derived remnant cholesterol particles during insulin resistance/diabetes that is thought to contribute to cardiovascular disease risk.

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Dr Yosdel Soto obtained his PhD in Biological Sciences from the University of Havana, Cuba. Since 2016 he has acted as Head of the Project on Atherosclerosis and is currently the Head of the Department of Immunobiology at the Centre for Molecular Immunology in Cuba. He has authored several publications that demonstrate the therapeutic potential of chP3R99 against atherosclerosis and has been instrumental in the development and characterisation of the latest version of the antibody enriched in arginine residue in a strategic position in the variable region of the antibody, therefore increasing binding effectiveness. In collaboration with Dr Proctor, he is working on the use of the antibody as a vaccine for the treatment of atherosclerosis in diabetes. Dr Soto has received numerous prestigious awards for his work on developing this vaccine and is a Member of the Cuban Academy of Sciences.

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cim
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A THREE-DIMENSIONAL APPROACH TO CONNECTING BIOLOGY & CHEMISTRY

Applying knowledge from one discipline to another is an oft-cited goal for undergraduate students. However, in many universities, science courses are taught essentially in isolation and do not provide adequate opportunities for students to connect their knowledge across disciplines. **Drs Rebecca Matz**, **Sonia Underwood**, and **Kristin Parent**, along with their collaborative research team at Michigan State University (MSU) and Florida International University (FIU), are developing activities that help address this gap.

Integrating Science Disciplines

The approach to teaching science courses in universities is often to compartmentalise disciplines from one another. If a student chooses to enrol in a chemistry degree program, they are immersed in the world of molecular structures, reactions and laboratory experiments. Likewise, a biology student will study evolution, cell structure and molecular biology. Though some broad foundational courses are required (e.g., a biology student will enrol in general chemistry), rarely will students make explicit connections to another discipline within a given course.

Therefore, when a student commits to a particular degree program, they may encounter few opportunities to connect their knowledge across disciplines before graduation. Such compartmentalisation fails to reflect the reality that addressing important scientific challenges requires drawing on knowledge from multiple areas of science (and other fields as well). This poses a dilemma to students – how do they acquire and practise using such knowledge?

As science and technology continue to advance in complexity, it is becoming recognised that a multidisciplinary approach is useful for science learning. Novel discoveries as well as a deep understanding of the fundamentals in biology increasingly require connections with other areas in science. Drs Rebecca Matz, Sonia Underwood, and Kristin Parent, along with their team at MSU and FIU, together share a wealth of expertise in chemistry, biology and science education research. Their two-year National Science Foundation-funded project, 'Creating Assessments for Student Understanding of Core Chemistry Ideas in Introductory Biology' aims to develop activities that assess undergraduate students' abilities to make such connections.

The Three Dimensions of Science Learning

The research team aims to develop these activities aligned with a 'three-dimensional' strategy. Developed by The National Research Council (NRC), the *Framework* for three-dimensional

learning rests on a view of science that 'continually extends, refines and revises knowledge'.

The three dimensions consist of: (1) scientific practices, that is, what we want students to do with their knowledge (e.g., build an argument from evidence); (2) crosscutting concepts,

‘Without assessments to measure students’ abilities to use scientific practices in the context of crosscutting concepts and core ideas, courses will continue to be driven by algorithmic assessments that leave students with inert science knowledge, unconnected between disciplines.’

that is, lenses for understanding problems that are useful across disciplines (e.g., systems thinking); and (3) core ideas, that is, ideas that are both central to a discipline and generative of new ideas (e.g., evolution in biology).

The aim of the *Framework* is to support the development of a future generation of proficient citizens and scientists who are able to draw on knowledge from multiple areas to address modern scientific challenges. ‘In the project, we are designing activities (appropriate for both group and individual work) that ask students to use their knowledge of chemistry to explain a biological phenomenon,’ says Dr Matz.

Project Design

To achieve the project objectives, the team is carrying out a research plan that consists of several tasks.

The first task entailed developing and implementing a survey for instructors

about which areas of connection between chemistry and biology they most valued in their local context. By matching the areas of interest with the instructors’ views, the team aimed to ensure that the assessments would be valid and therefore more likely to be relevant for distribution on a wider scale. The research team then used the results of the survey to prioritise the areas of connection for developing the activities.

Following a simplified method of evidence-centred assessment design, the team determined the goal of each activity as well as the responses students would provide as evidence of their connecting the core chemistry idea with the biological phenomenon. The team designed each activity to incorporate a scientific practice, crosscutting concept and core chemistry idea, and used an instrument called the Three-Dimensional Learning Assessment Protocol (3D-LAP) to verify that the activities reflected each dimension.

Each activity is situated within a biological phenomenon. For example, one activity asks students to explain the connection between the role of energy in the formation and breaking of covalent bonds and a molecular motor that effectively packages DNA into a virus particle using ATP. Students are prompted to recognise that the energy released from ATP hydrolysis must be physically proximate to the virus particle and DNA in order to power the molecular motor.

Each of the activities has been distributed in various ways to students in general chemistry and introductory biology courses at both MSU and FIU. Following pilot administrations and revisions for clarity, the team administered the activities to various populations of students at each institution, collecting approximately 800 student responses in total. Follow-up interviews helped the team to investigate the face validity of the activities, identifying what students were thinking about as they completed parts of the activity and points of confusion.

The team's preliminary analyses indicate a range of responses showing that some students are able to connect across the chemistry and biology concepts, while others continue to demonstrate compartmentalised knowledge; prior course-taking patterns are an important variable to consider in interpreting the student responses. Regardless, students often mentioned to instructors that the activities helped them make connections that were otherwise never explicitly highlighted for them. Collecting feedback from instructors, an external evaluator, and a project advisory board is also ongoing.

Propagation and Future Projects

The research team has endeavoured to share the results of their work with an array of audiences, including the Society for the Advancement of Biology Education Research (SABER), the American Chemical Society (ACS), and the National Association for Research in Science Teaching (NARST). Each audience brings a mix of practitioners and researchers who might benefit from seeing how students are making connections between chemistry and biology using the designed activities.

Upon successful completion of the project, the team aims to expand this work by developing a longer-term project in which specifically designed curricular materials and supports for three-dimensional teaching and learning are provided for larger groups of students. Over a longer time-scale, the researchers hope to build new collaborations that would support designing activities and curricular materials for different combinations of courses, such as chemistry and mathematics or biology and physics.

How pressing is it that universities undertake the large scope of work in order to transform their science curricula? Dr Matz says, 'Without assessments to measure students' abilities to use scientific practices in the context of crosscutting concepts and core ideas, courses will continue to be driven by algorithmic assessments that leave students with inert science knowledge, unconnected between disciplines.'

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Dr Rebecca Matz is an Academic Specialist in the Hub for Innovation in Learning and Technology at Michigan State University. Dr Matz completed her BS in Chemistry at University of Illinois before moving to University of Michigan to complete a PhD in Chemistry and MS in Educational Studies. Dr Matz's research interests lie broadly in assessment and organisational change within STEM. Her work explores the levers and barriers to institutional reform and adoption of new teaching practices, assessment of curricular changes in early mathematics courses, and challenges in making connections across subject areas. In particular, Dr Matz's current work aims to design activities that give undergraduate students the opportunity to use their knowledge of chemistry to explain biological phenomena.

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Dr Sonia Underwood is an Assistant Professor in the Department of Chemistry & Biochemistry and the STEM Transformation Institute at Florida International University (FIU). Dr Underwood joined FIU in 2016 after working as a Research Associate at Michigan State University in chemistry education research. Prior to that, she earned her PhD in Chemistry from Clemson University. Dr Underwood's research interests are focused on developing assessment measures to determine the impact of curriculum transformations, investigating how students use a chemical structure to predict a compound's macroscopic properties, and exploring the connections students make between their chemistry, biology, and physics courses.

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Dr Kristin Parent is an Assistant Professor who joined Michigan State University's Department of Biochemistry and Molecular Biology in 2013. Dr Parent's research focuses on understanding viral entry into host cells, and she uses cryo-electron microscopy to visualise the macromolecular structure of viral machines. Through this research we now better understand how viruses find, attach to, and infect specific hosts. In addition, her research has shed light on viral diversity in the environment. Dr Parent has won several awards and grants for both her research and teaching, including the MSU Outstanding Mentor and Teacher-Scholar Awards, and an NSF CAREER award for her work on virus host interaction and involving undergraduate, high-school and middle-school students in scientific research.

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COMBATTING HUMAN IMMUNODEFICIENCY VIRUS DRUG RESISTANCE

Human immunodeficiency virus (HIV) touches the lives of millions of people all over the world. Successful antiretroviral drugs allow patients to live longer and healthier lives, without the threat of acquired immunodeficiency developing. However, as with all viruses, HIV can mutate and become resistant to once-effective therapies. **Dr Eric Freed** at the USA's National Cancer Institute focuses on elucidating the late stages of HIV replication and how the virus becomes resistant to antiretroviral drugs. His promising results are paving the way for developing new drugs that can combat HIV drug resistance.

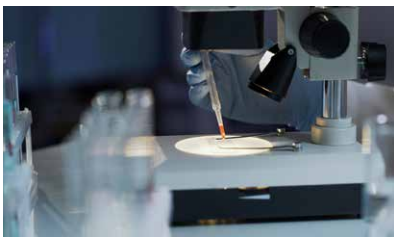
HIV Infection

Over 38 million people are currently living with human immunodeficiency virus (HIV) worldwide and 1.8 million of these are children. Although new infection rates are decreasing, an estimated 1.7 million individuals become infected with HIV each year. HIV is spread through bodily fluids, including breastfeeding, needle sharing, or sexual transmission.

HIV is a retrovirus – a type of pathogen that uses the body's own replication mechanisms to its advantage. It works by attaching to receptors on immune cells called CD4 T cells, before fusing and then entering the cell. Once HIV is inside, it uses its reverse transcriptase enzyme to transform its genetic material from RNA to DNA, which becomes transported to the nucleus. Within the nucleus, another viral enzyme known as integrase inserts the viral DNA into the host's DNA so that it is replicated by the host's mechanisms to create new viral proteins and RNA. These newly formed viral components assemble near the cell surface and combine to form complete HIV particles which burst out of the cell. The newly released virus particle then undergoes maturation, a step that is triggered by using the viral protease enzyme. Mature HIV particles are now free to infect more CD4 cells in the bloodstream and the process of infection continues.

CD4 T cells are an essential part of the immune system for fighting off disease-causing agents like bacteria and viruses. However, when they are hijacked by HIV, the immune system cannot function properly and becomes progressively weaker until AIDS develops. AIDS creates a dramatically augmented risk of infections and opportunistic tumours, almost invariably leading to death if antiretroviral therapy (ART) is not administered.

Fortunately, the development of ART means that people who are living with HIV can live longer and healthier lives, without developing AIDS or passing on HIV to their partner. About 67% of people who are HIV positive are receiving ART around the world. The medication works to reduce the viral



load (the amount of virus present) in a person's blood by disrupting the virus's replication. This can be achieved through nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors, entry inhibitors or integrase inhibitors. Each one of these drugs targets and disrupts a different part of the viral replication process and so prevents HIV from multiplying.

Keeping up with Drug Resistance

Although ART has played a vital role in reducing global HIV numbers, continued research is necessary to keep up with the virus. As with all viruses, HIV can mutate and become resistant to drugs that were once sufficient to keep a person healthy. This means that new drugs or newer versions of established

drugs are needed to ensure that people living with HIV can continue to be treated effectively.

One scientist taking on this challenge is Dr Eric Freed from the USA's National Cancer Institute in Frederick, Maryland. Dr Freed has undertaken multiple projects investigating the molecular mechanisms of HIV maturation and replication and how they may lead to new therapeutic targets. His contributions will provide important insights into how new anti-HIV drugs could work to bypass or avoid resistance.

Solving Mechanisms of HIV Drug Resistance

Gag proteins are the main structural proteins of retroviruses like HIV-1 and are an important element of viral replication by playing the central role in assembly, budding and maturation. During the Gag processing cascade when the virus is exiting its host, Gag's polyprotein precursor known as Pr55Gag is cleaved (cut) by the viral protease enzyme. This step is essential for the maturation and infectivity of the virus. For a number of years, Dr Freed has been working to develop maturation inhibitors (MIs) that target HIV-1 maturation by blocking a specific step in the Gag processing cascade.

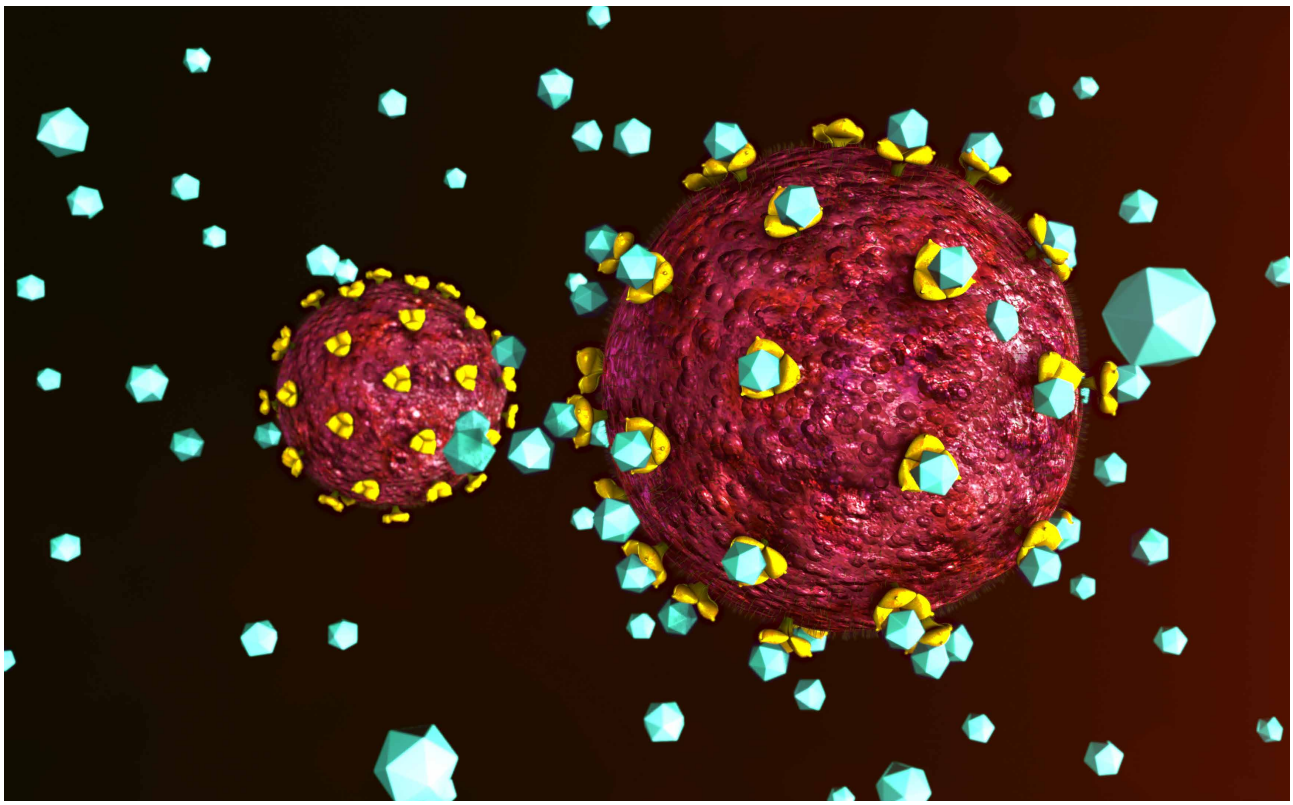
In collaboration with Panacos Pharmaceuticals, Dr Freed characterised how the first-in-class MI known as bevirimat (BVM) works. The collaboration found that BVM works by blocking a late step in the Gag processing pathway, namely, the conversion of CA-SP1 (the capsid-spacer peptide 1) Gag processing intermediate to mature capsid protein. As a result, HIV-1 maturation and therefore replication is inhibited. Finding the molecular target of the compound relied on identifying HIV-1 mutants that are resistant to BVM.

Alongside the studies with resistant mutants, structural studies strengthened the team's understanding of MI activity on a deeper level. At a CA-SP1 junction, an immature lattice structure is made up of a protein formation called a six-helix bundle. MIs stabilise this bundle, which actually prevents CA-SP1 processing and therefore introduces a block in the Gag processing cascade.

However, when a certain resistance mutation occurs in the genetic material of HIV-1, the six-helix bundle is destabilised. This counteracts the inhibitory abilities of MIs and results in resistance of the virus to BVM. Subsequently, Dr Freed and his collaborators are now studying a new generation of BVM analogues that are more potent than their predecessors and can combat drug resistance. Many of them have been identified and clinical trials are already taking place and the results seem promising so far.

Developing New and Effective HIV Drugs

Continuing on from this research, Dr Freed is partnering with structural biology laboratories to determine precisely where MIs bind to the viral Gag protein. They will determine the structure of the CA-SP1 region in the immature Gag lattice, in both the presence and the absence of bound inhibitor.



Uncovering and understanding structures like these involved in the assembly and maturation of HIV-1 are key to developing MIs as antiretroviral drugs. These drugs, together with those against other new targets, may help to provide a solution to drug resistance and the long-term tolerability of currently available drugs. At the moment, other than protease inhibitors, there are no drugs that target this late stage of the replication cycle of HIV-1, so this is an exciting avenue to explore.

More Mechanisms of HIV Replication

Adjacent to this research, Dr Freed is also involved in studies that investigate other steps in HIV-1 assembly and maturation. One interesting molecule in the pathway is inositol hexakisphosphate (IP6) which has unusual abilities. During assembly of the virus, it can promote the formation of the immature Gag lattice and during maturation, it can promote the assembly of the mature capsid lattice. IP6 binds to the six-helix bundle which we've seen is an important element in the maturation process, so the team are studying it in relation to MIs, which also bind to the six-helix bundle.

Another molecule of interest is neutral sphingomyelinase 2 (nSMase2) which is an enzyme used in ceramide biosynthesis. In cells, disruption of nSMase2 prevents maturation by blocking Gag protein processing. Learning about the role of this molecule in viral replication is important to understand how new HIV drugs could be developed.

Additional projects by Dr Freed and his team have revealed the involvement of envelope glycoproteins in HIV-1 drug resistance. The envelope glycoproteins are found on the outer surface (envelope) of the virus and are responsible for the binding of the virus to the receptor on target cells and virus entry into those cells. In cell cultures, envelope glycoproteins that contained mutations to enhance cell-to-cell transfer could counteract the replication inhibition effects of antiretrovirals. This is another previously unknown path of resistance that is a high priority for further study.

Dr Freed's novel research is paving the way for better understanding mechanisms of drug resistance in HIV-1 and as a result, significantly improving HIV therapies. His work will undoubtedly make a real difference to the lives of people living with HIV in the years to come.

Meet the researcher

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Dr Eric Freed received his Bachelor of Science in Molecular and Cell Biology from Pennsylvania State University. Afterwards, he went on to complete a PhD in Cellular and Molecular Biology at the University of Wisconsin. Having received numerous honours and fulfilled many roles, Dr Freed's primary work is at the National Cancer Institute in Frederick, Maryland. Here, he is a senior biomedical research scientist and the Director of the HIV Dynamics and Replication Program. His research focusses on HIV and the mechanisms by which it becomes resistant to drugs, so that new and effective antiretroviral therapies can be developed for the future.

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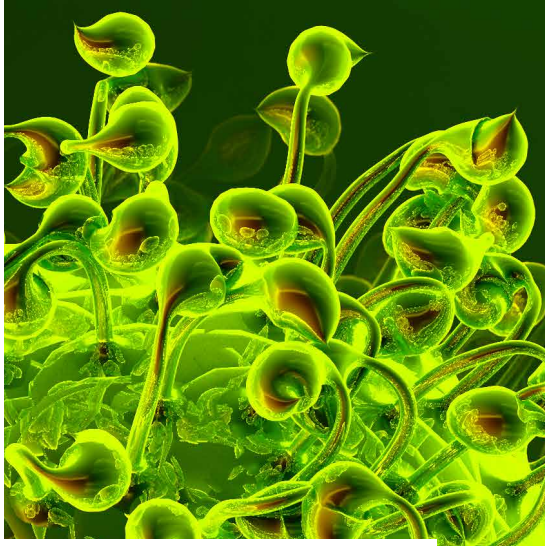
REMDESIVIR: HALTING THE VIRAL REPLICATION OF SARS-CoV-2

The design of effective antivirals is a key priority in the global effort to curb the pandemic caused by the novel coronavirus SARS-CoV-2. The FDA-approved drug Remdesivir (RDV) acts by interfering with the SARS-CoV-2 viral replication mechanism. **Dr Jin Yu** and her team from the University of California, Irvine, conducted a computational study to elucidate how the RNA-dependent RNA polymerase (RdRp), responsible for SARS-CoV-2 genomic replication, is inhibited by RDV. Excitingly, they showed that RDV binds tightly to RdRp, stabilising a closed conformation of the active site for successful incorporation to effectively halt viral replication.



COVID-19: A Rapidly Evolving Disease

Almost two years on from the start of the COVID-19 pandemic, there have been more than 240 million confirmed cases of the disease and almost 5 million deaths linked to COVID-19 worldwide. According to the World Health Organization, COVID-19 remains a rapidly evolving disease that continues to push healthcare systems beyond their current capacities in most countries across the globe.

As the SARS-CoV-2 virus continues to circulate, more variants will continue to emerge. To date, four variants have been designated specific variants of concern, all of which are even more transmissible than the ancestral strain of SARS-CoV-2. Currently, a further five variants of interest are being closely monitored and evaluated. Alongside the implementation of a global vaccination effort, the development of effective antiviral treatments targeting SARS-CoV-2 is a primary focus in the global effort to curb the COVID-19 pandemic.

Using a Nucleotide Analogue to Jam the RNA Synthesis Mechanism

SARS-CoV-2 is a newly emerged member of the family of coronaviruses. Coronaviruses are enveloped, which means they contain an outermost layer that protects their genetic material when travelling between host cells. SARS-CoV-2 is also a single-stranded ribonucleic acid (RNA) virus. RNA is a multifunctional molecule that, among other capabilities, works to convert genetic information into proteins.

The key RNA synthesising engine of the replication-transcription machinery encoded in the genomes of all RNA viruses, including SARS-CoV-2, is the RNA-dependent RNA polymerase (RdRp) enzyme.

Remdesivir (RDV) is the only Food and Drug Administration approved drug available so far for the treatment of COVID-19. Nucleosides are structural subunits of nucleic acids, and nucleoside analogues resemble naturally occurring nucleosides to form an important class of antiviral agents. RDV is one example of an antiviral

compound that was designed to function as a nucleotide analogue, and it can be phosphorylated to closely resemble adenosine triphosphate (ATP), the ubiquitous molecule that carries energy within cells.

RDV was originally developed as an antiviral treatment for the Ebola virus and infections by other coronaviruses, such as Middle East respiratory syndrome and the severe acute respiratory syndrome coronaviruses known as MERS-CoV and SARS-CoV. Critically, RDV works by interfering with viral genome replication and directly competing with substrates of the viral RdRp. But how is this achieved?

Dr Jin Yu from the University of California, Irvine, and her colleagues conducted an elegant computational study to better understand how the nucleotide analogue drug remdesivir (RDV-TP) binds and inserts to the SARS-CoV-2 RdRp active site, in competition with the natural nucleotide substrate ATP.

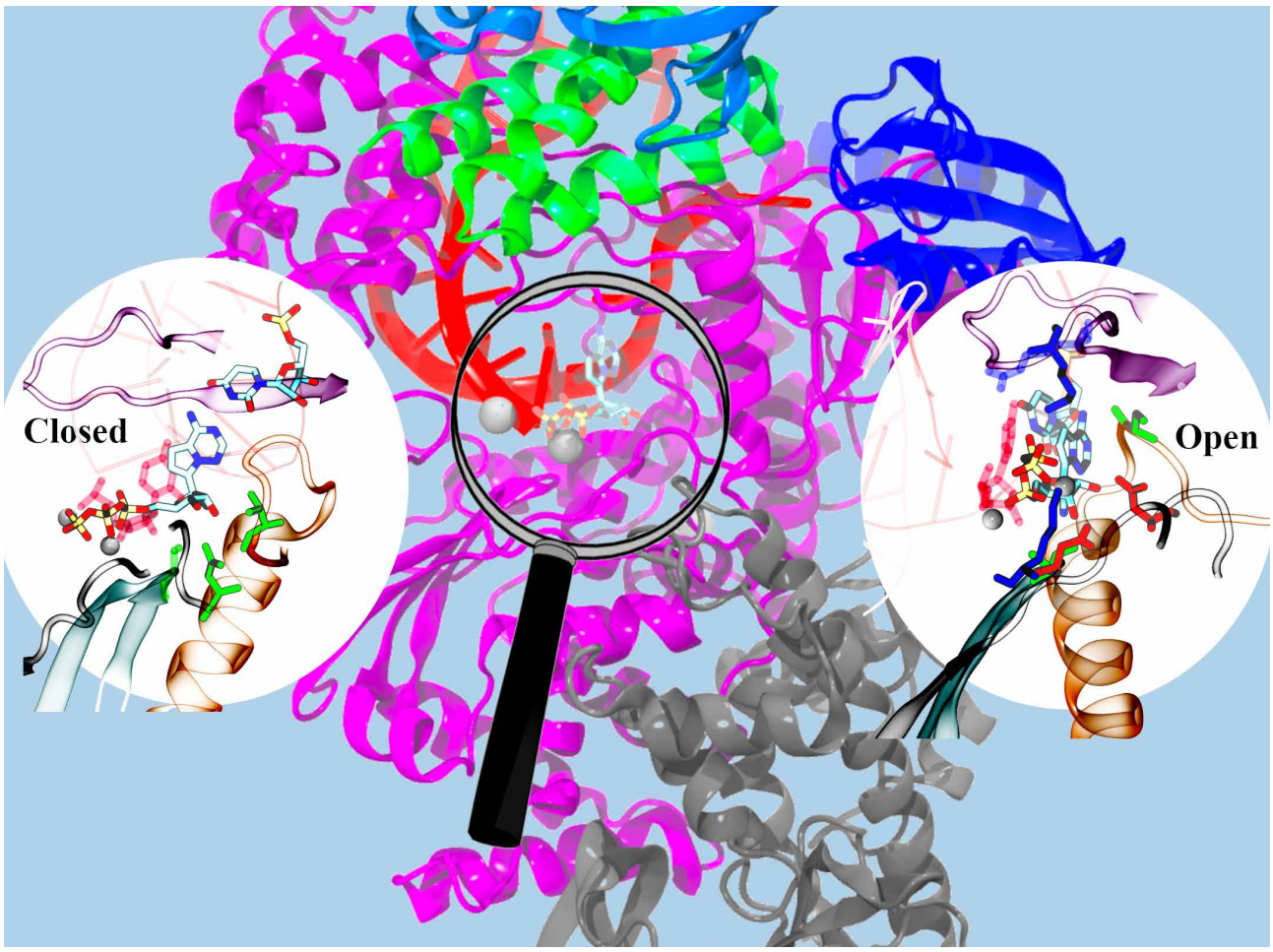
In order to achieve this, the team first constructed atomic structural models, based on the high-resolution structures determined recently for SARS-CoV-2 RdRp. The study, published in September 2021 in Molecular Systems Design & Engineering by the Royal Society of Chemistry (and featured on the back cover of the issue), shows that RDV is first metabolised into its active form, an adenosine analogue, to interfere with SARS-Cov-2 RdRp. The researchers showed that once in the active site, RDV forms base stacking with RNA template nucleotide and then binds tightly to the enzyme, so that it can be incorporated into the synthesising RNA chain and later on, hinder the RNA synthesis functions of the SARS-CoV-2 RdRp, effectively halting the mechanism of viral replication.

The Advantages of *In Silico* Investigations Over *In Vitro* Enzyme Assays

In their 2021 study, Dr Yu and her team simulated the insertion of triphosphate from RDV-TP into the SARS-CoV-2 RdRp active site. The team employed a high-performance computing approach that enabled them to develop a computational microscope to explore the active site of the RNA polymerase. The technique allowed Dr Yu and her team to reveal how natural nucleotides and nucleotide analogue drug candidates incorporate into the RNA chain during viral replication.

The rationale for simulating the nucleotide analogue binding and insertion, rather than monitoring the activity of RdRp *in vitro*, in the presence of RDV, is that the synthesis of the active nucleotide analogue compound requires considerable time and cannot zoom into molecular dynamics details, to some extent hindering rapid experimental testing. *In silico* modelling and simulation make it comparatively straightforward to provide insights on the structural and energetics details of the RdRp directed nucleotide incorporation in viral RNA synthesis. Combined with experimental studies, the computational investigations are expected to reveal the physical mechanisms of viral RdRp function, providing the basis for future collaborative efforts on developing drug therapeutics for the treatment of COVID-19.

During the RNA chain elongation process of replication or transcription, a nucleotide binds to the active site of the polymerase (or nearby), subject to initial screening by selectivity of the polymerase and then by collaborative



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proofreading mechanisms. RdRp recruits ribonucleotides one at a time to the active site and adds the nucleotide to the growing RNA chain. Dr Yu and her colleagues highlighted a complex network of subtle local interactions of amino acids that facilitate the binding and insertion of RDV onto the active site of the viral RdRp. RDV-TP, the nucleotide analogue, disguises itself as a similar ATP substrate for the synthesis of RNA, successfully evading the initial screening, and later, the proofreading step in the RdRp active site.

In fact, the researchers even suggest that the binding and insertion of RDV-TP to the active site of SARS-CoV-2 RdRp can be favoured over that of the natural substrate ATP. The team showed that the RDV-TP initial binding is favoured by forming base stacking with the template RNA nucleotide, and then the RDV-TP insertion is further facilitated and stabilised by hydrogen bonding and salt-bridge interactions between the nucleotide analogue and specific residues around the RdRp active site. Following the successful RDV-TP insertion and incorporation to the viral RNA chain, several nucleotides can be additionally added to the growing RNA chain until termination happens. This is consistent with previous studies that showed that RDV inhibits the Ebola virus and MERS coronavirus via a delayed chain termination mechanism.

Future Developments

The focus of Dr Yu's research is aimed at better understanding the biophysics and biochemistry of living systems obtained through a wide spectrum of molecular modelling and simulation techniques. Dr Yu and her team have access to the world's most powerful high-performance computing resources, the Summit supercomputer from the Oak Ridge National Lab Leadership Computing Facility, that supports COVID-19 research through the COVID-19 High Performance Computing Consortium. This consortium brings together industry, academic and federal agency experts to conduct extensive research in areas like bioinformatics, epidemiology, and molecular modelling to develop strategies to address the threat posed by COVID-19.

The ongoing interdisciplinary studies at the Yu laboratory offer high promise in the development of effective antivirals for the treatment of COVID-19 as well as other diseases. The team's planned *in silico* studies will further clarify the physical mechanism of action of RDV and similar analogues and pave the way for the development of additional antiviral compounds and inhibitors of SARS-CoV-2 RdRp. The team also plans to look further into the RNA synthesis mechanisms in SARS-CoV-2, probing for potential drug resistance of the fast-evolving RNA virus.

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Dr Jin Yu is an Assistant Professor at the Department of Physics & Astronomy at the University of California (UC), Irvine. Dr Yu obtained her PhD in theoretical and computational biophysics in 2007 from the Department of Physics at the University of Illinois at Urbana-Champaign, under the supervision of Professor Klaus Schulten. After completing her postgraduate studies, Dr Yu received the prestigious UC Berkeley Chancellor's postdoctoral fellowship. At UC Berkeley, she conducted research on the physical and mathematical modelling of biomolecular machines, such as the viral phi29 DNA packaging motor, with Prof George Oster and Prof Carlos Bustamante. In 2012, she joined the Beijing Computational Science Research Centre as a Principal Investigator, working on the modelling and simulation of viral T7 RNA polymerase transcription and fidelity control. In 2019 Dr Yu returned to the USA, joining the Department of Physics & Astronomy at UC Irvine, where she continues her interdisciplinary studies on computational biophysics. She currently holds a joint appointment with the Department of Chemistry, and an affiliation to the NSF-Simons Center of Multiscale Cell Fate Research at UC Irvine.

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Moises Romero is a fifth-year graduate student in the Department of Chemistry at UC Irvine. He is the lead author of the current work and has conducted significant modelling and simulation of the SARS-CoV-2 RdRp system. In addition to research, Moises is also heavily involved in diversity, equity and inclusion work, and serves on the board of the Society for Advancement of Chicanos/Hispanics & Native Americans in Science chapter.

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IN SILICO DRUG DISCOVERY FOR COVID-19 USING AN UNSUPERVISED FEATURE EXTRACTION METHOD

In silico drug discovery is useful for screening and identifying large numbers of drug candidate compounds in a way that is not possible using classical experimental approaches. **Dr Y-H Taguchi** at Chuo University, Japan, has developed a computational technique known as 'tensor decomposition-based unsupervised feature extraction'. He has successfully applied this as an *in silico* phenotype-based drug discovery method to repurpose known drugs for severe acute respiratory syndrome coronavirus 2 and has successfully identified various known anti-viral drugs as viable candidates for the successful treatment of COVID-19.

A Mathematical Framework for *In Silico* Drug Discovery

Since January 2020, the COVID-19 pandemic has critically affected communities worldwide, prompting scientists to identify new, effective drugs that could tackle the disease. To repurpose old drugs toward the treatment of COVID-19, we must first understand the mechanism by which SARS-CoV-2 successfully invades human cells, causing the onset of disease. Driven by advances in information technology, a new approach, known as *in silico* experimentation, has generated reports of a large number of candidate drug compounds that may be useful for treating COVID-19. In biomedical research, an *in silico* experiment is one that is conducted with the aid of computer simulations.

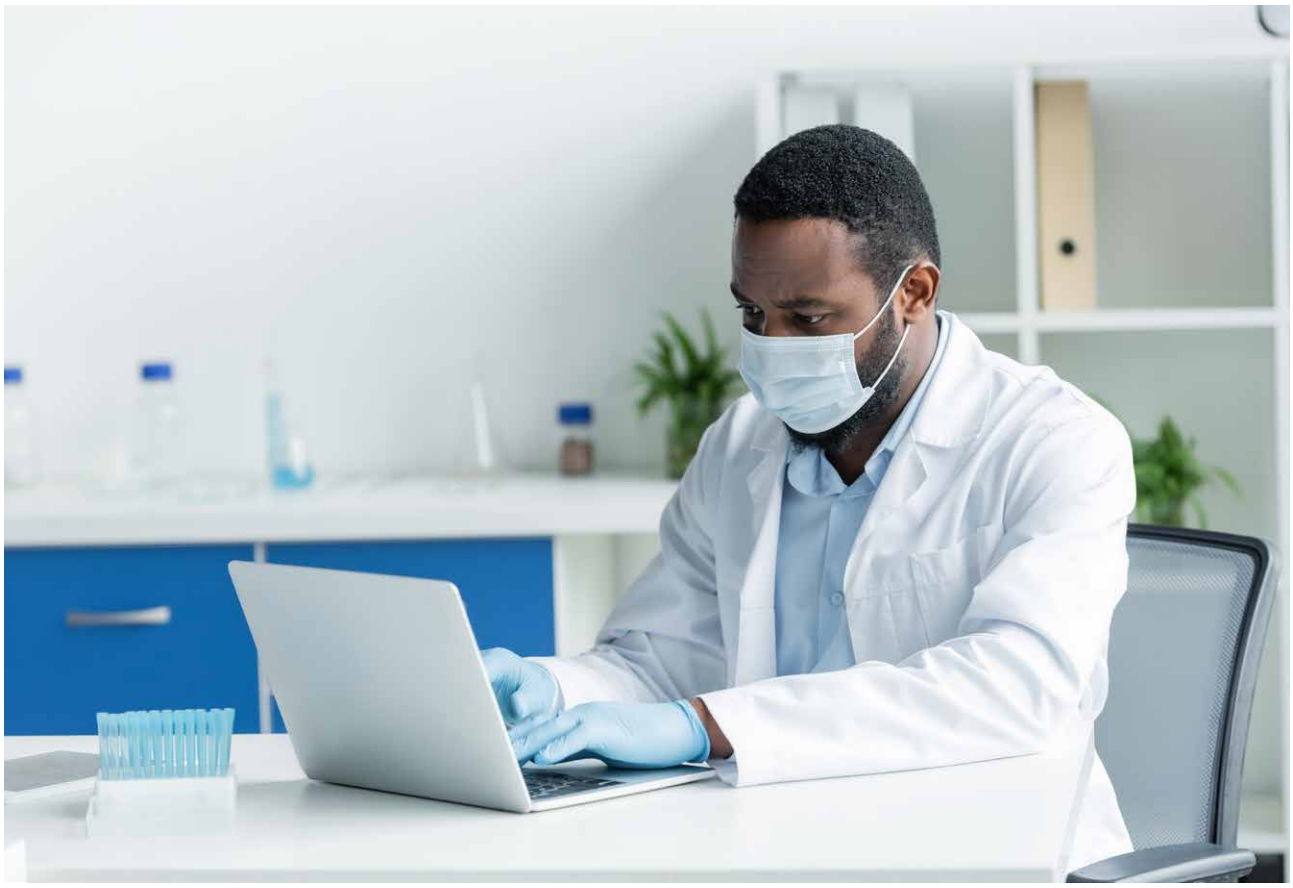
Dr Y-H Taguchi and his team from the Department of Physics, Chuo University, Japan, have developed computational techniques that can support *in silico* experimentation, allowing researchers to predict the function of proteins, discover potential drug-like molecules and identify disease-causing genetic mutations.

Since disease alters gene expression, it is not surprising that there are specific sets of genes for which altered expression patterns can act as biomarkers to identify the presence of disease and estimate disease progression. Dr Taguchi and his collaborators had previously used a mathematical method known as 'tensor decomposition (TD)-based unsupervised feature extraction (FE)' and applied it to a gene expression profile dataset obtained from mouse liver infected with the mouse hepatitis virus, regarded by many as a suitable model of human coronavirus infection. The results of the study were recently published in April 2021.

The main purpose of the methods developed by Dr Taguchi is to perform feature selection, which means selecting a small or limited number of critical variables from a very large number of variables. Feature selection strategies

can be classified into supervised ones and unsupervised ones. Generally, supervised strategies are more popular than unsupervised ones. This is because the purpose of feature selection is usually clear to the user. Despite this, the use of unsupervised feature selections provides a better choice when class labels for large sets of data are unclear or unavailable.

In September 2020, Dr Taguchi's team published the results of the successful application of an unsupervised strategy able to predict anti-COVID-19 drug candidate compounds without prior knowledge of effective known compounds. The team analysed the gene expression profiles of multiple lung cancer cell lines infected with SARS-



CoV-2, in the presence or the absence of several antiviral drugs. All the gene expression profiles were obtained from a public database.

SBDD can find drug candidate compounds in the absence of structural similarity to known drugs and requires massive computational resources for 'docking' simulations between compounds and proteins. Dr Taguchi's TD-based unsupervised FE approach successfully overcame the limitations associated with SBDD, predicting a set of effective drug candidate compounds that are able to treat COVID-19.

Tensor Decomposition as a Feature Extraction Method

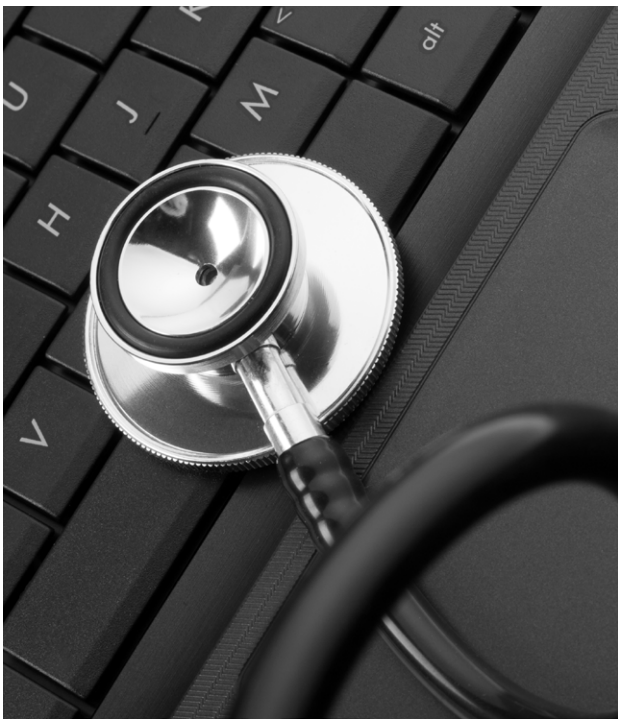
One classic approach used to identify significant variables is to conduct a statistical test. This test would compute the probability that a desired property can appear by chance rather than being associated with a specific feature. For example, if the alteration of a gene, or set of genes, follows the onset of disease, the probability of it happening by chance would be rather small. In scenarios where there are very large numbers of variables and a small number of observations, as in genomic science, this strategy often fails. To perform feature selection in these scenarios, Dr Taguchi has successfully applied a mathematical approach known as tensor decomposition.

Tensors are a feature of linear algebra and are at the top of a hierarchy that includes scalars, vectors and matrices. Scalars are simple numerical values, such as the mass of an object or the price of an item for sale. Vectors are composed of a set of scalars. The elements that make up vectors are represented by adding a suffix to scalars, e.g., x_j, where x is the scalar value and j is a suffix that represents a whole number. This means that the value of the vector depends on both x and j.

As vectors are composed of scalars, matrices, X, are composed of x vectors. Any x vector belonging to a matrix will have to suffixes j and i (x_{ij}). For example, the 'j' component of vectors in a matrix could be an item such as 'Bread', 'Fish', or 'Pork', which can vary in value within certain categories denoted as 'i', with i_1, for example, being 'Mass', i_2 being 'Price', i_3 being 'Calories'.

As vectors are composed of scalars and matrices are composed of vectors, tensors can be composed of matrices. Suppose we have some samples of foods in two different shops. Now, we can define a tensor, X_{ijk}, that describes the j^{th} feature, attributed to the i^{th} food, in the k^{th} shop.

The technique of tensor decomposition can be applied to a large number of experimental conditions. For example, if gene expression is measured for various tissues taken from different patients, gene expression is better represented, not in a matrix, but as a tensor, where patients vs tissues vs genes, are the parameters that define the tensor.



Ivermectin: A Promising COVID-19 Treatment

TD-based unsupervised FE was applied to the gene expression profiles of multiple lung cancer cell lines infected with SARS-CoV-2. Five cell lines underwent two different treatments: one with SARS-CoV-2 and one with a 'mock treatment'. There were 30 samples in the end, as each pair cell line/treatment was analysed in triplicate (5 cell lines x 2 treatments x 3 replicates = 30 samples). Since there is currently a lack of known drugs that are effective in treating SARS-CoV-2, a ligand based drug discovery approach would not be useful because it is based on the known structures of compounds. On the other hand, SBDD requires massive computational resources, like supercomputers, whereas Dr Taguchi's method can be performed with standard computational servers that can be purchased even with reduced budgets.

The researchers identified several candidate compounds that could significantly alter the expression of the 163 genes selected by TD-based unsupervised FE. The 163 selected genes are all responsible for expressing proteins that significantly interact with the proteome of the SARS-CoV virus, which is closely related to SARS-CoV-2. Numerous drugs were successfully identified, especially antiviral drugs, including fluticasone, atorvastatin, gentamicin, among many others. The screening process detected ivermectin as the promising treatment for COVID-19. Ivermectin, which was previously identified as an anti-parasite drug, was recently included in clinical trials for SARS-CoV-2.

Summing up: Remarkable Progress

Dr Taguchi and his collaborators proposed an advanced unsupervised machine learning method for identifying numerous promising drug candidate compounds that could treat COVID-19 infection. When applied to the expression profiles of a pool of genes from lung cancer cell lines infected by SARS-CoV-2, the method identified numerous drug compounds that significantly altered the expression of the genes, indicating a change in the progression of the disease. The study was aimed at consolidating a similar strategy previously employed by Dr Taguchi to understand the infectious process of mouse hepatitis virus, a well-studied model for COVID-19.

In order to confirm the significance of the 163 genes in the context of human disease, Dr Taguchi and his collaborators compared the genes with those identified to be interacting with SARS-CoV-2 in humans. The 163 genes identified in this study turned out to be associated with human genes previously reported to interact with the SARS-CoV-2 proteome, contributing to disease progression.

Although ivermectin was recently reported to inhibit the replication of SARS-CoV-2 *in vitro*, to Dr Taguchi's knowledge, his team was the first to report the *in silico* detection of ivermectin as a possible SARS-CoV-2 drug through an unsupervised feature extraction method. Most *in silico* drug discovery methods are supervised strategies that require known target-drug relations or drug-disease relations, which are currently not available for SARS-CoV-2. Furthermore, as ivermectin was first identified as an anti-parasite drug, no previous supervised *in silico* approach considered it, confirming the remarkable effectiveness of the unsupervised approach devised by Dr Taguchi and his collaborators.

Meet the researcher

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Dr Y-h. Taguchi obtained his PhD in the theory of statistical mechanics of spin systems, from the Tokyo Institute of Technology in 1988. In the same year, he started his academic career as Assistant Professor at the Department of Physics at the Tokyo Institute of Technology. In 1997, he joined the Department of Physics at Chuo University, Tokyo, where he became Full Professor in 2006. Dr Taguchi's most recent research interest revolves around the development of tensor decomposition methods applied to bioinformatics, particularly in relation to proteomics and gene expression patterns.
Dr Taguchi has published a monograph and several peer-reviewed publications. As an outstanding scientist in his field, Dr Taguchki has received numerous prestigious honours and awards for his contributions to bioinformatics.

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THE HOLY GRAIL OF SAFER OPIOIDS: TARGETING MU OPIOID RECEPTOR SPLICE VARIANTS

Despite their numerous side effects, opioid drugs and morphine-like agents have remained a pillar in the medical management of pain. Most clinically used opioid drugs act through mu opioid receptors. **Dr Ying-Xian Pan** and his team from the Rutgers New Jersey Medical School, USA, studies the molecular and cellular mechanisms of mu opioid receptors and aim to develop novel strategies and opioid analgesics for better treating pain without side effects associated with traditional opiates. Efforts to find substitutes for traditional opioid drugs are helping address the opiate abuse crisis that affects many countries around the globe.

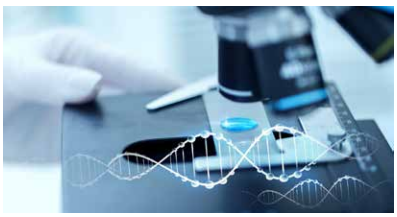
Identifying Multiple Splice Variants of Mu Opioid Receptor Gene

The actions of clinically used opioid drugs are complicated. Although potent in relieving pain, these drugs produce many side effects and their misuse has led to the global opioid epidemic. Dr Ying-Xian Pan, Professor of Anesthesiology at Rutgers New Jersey Medical School, USA, and his team have long investigated the molecular and cellular mechanisms of mu opioid actions that are mediated through mu opioid receptors. This work is providing the foundations to develop novel opioid analgesics that are devoid of unwanted side effects and the potential for abuse.

One of the main findings from Dr Pan's laboratory is that the gene encoding mu opioid receptors, known as OPRM1, is able to generate multiple isoforms or variants of the receptor through 'alternative splicing', a process that allows a single gene to produce more than one protein. Over the past years, Dr Pan's laboratory and others have identified over 65 mu opioid receptor variants from the mouse, rat and human OPRM1 gene. These variants can be categorised into three structurally distinct classes.

The first class of the variants have identical full-length seven transmembrane domains (7TM) sequences, a typical G protein coupled receptor structure, except for an alternative intracellular carboxyl terminal sequence. The second class of the variants are called truncated 6TM variants due to the lack of the first TM. The third class of the variants are truncated single TM variants. Single TM variants only contain the first TM.

Importantly, this 'alternative splicing' of the OPRM1 gene is conserved from rodents to humans, meaning that it is evolutionarily important in its functions. To define the molecular mechanisms of various analgesic drugs acting on the mu opioid receptor splice variants, Dr Pan and his team adopt multidisciplinary *in vitro* and *in vivo* approaches, including molecular biology, biochemistry, pharmacology, and behavioural studies, as well as gene targeting animal models.



Targeting Truncated 6TM variants

Dr Pan and his collaborators have long been involved in researching the Holy Grail of mu opioid analgesics. *In vivo* studies using a genetically engineered exon 11 knockout mouse model generated in Dr Pan's laboratory led to the discovery of the functional

importance of the truncated 6TM variants. In 2009, Dr Pan and his team published an article in the prestigious *Proceedings of the National Academy of Sciences of the United States of America* (PNAS), exploring the role of truncated 6TM variants on the action of heroin, a mu receptor agonist. Disrupting the exon 11 sequence that deleted all 6TM variants in the exon 11 knockout mouse did not affect the pain-relieving action of morphine and methadone. However, it attenuated the pain-relieving action of heroin.

These findings established a role for the exon 11-associated 6TM variants in heroin action and complemented the earlier finding in an exon 1 knockout mouse generated by Dr John Pintar's laboratory, in which all 7TM variants were lost but all 6TM variants were retained. Heroin analgesia was still active while morphine and methadone analgesia were completely lost.

Dr Pan and his collaborators, including Drs Pasternak and Majumdar, further extended their studies to the action of novel opioid analgesic drugs targeting the 6TM variants. In these studies, the researchers identified a novel potent opiate analgesic and published the findings in PNAS in 2011. The novel agonist, 3-iodobenzoyl-6ß-naltrexamide (IBNtxA) developed by Drs Majumdar and Pasternak, acts through the truncated 6TM variants of the murine mu opioid receptor gene (Oprm1). They observed that the analgesic action of IBNtxA was lost in the exon 11 knockout mice, indicating that exon 11-associated 6TM variants mediate IBNtxA analgesic action.

The most significant finding in the 2011 study was that despite its potent pain-relieving action, IBNtxA lacked the traditional opiate side effects, such as respiratory depression, reward behaviour, significant constipation and physical dependence. To further confirm the role of the 6TM variants in IBNtxA analgesic action, Dr Pan and his team used a gain-of-function approach to see if IBNtxA analgesia can be regained by re-expressing the 6TM variants in a complete Oprm1 knockout mouse, in which all the analgesic actions of mu opioids and IBNtxA were lost. They found that IBNtxA, but not morphine analgesia, was rescued by the lentivirus expressing the 6TM variants in a complete Oprm1 knockout mouse and published the finding in 2015 in the *Journal of Clinical Investigation* and 2018 in Anesthesiology & Analgesia.

Together, these studies demonstrated that truncated 6TM variants can be physiologically and pharmacologically important and act as new therapeutic targets for a novel class of opiate compounds displaying a vastly improved pharmacological profile.

Targeting Specific Carboxylic End of the OPRM1 7TM Variants

While the relevance of the truncated variants has been extensively explored and validated by Dr Pan and others, as described in the examples above, they examined the pharmacological roles of the full-length 7TM variants. Alternative splicing of the mu opioid receptor gene OPRM1 creates multiple full-length 7TM mu receptor variants or isoforms, which only differ in the intracellular carboxylic terminal (C-terminal) portion of the receptor. Many *in vitro* cell line studies conducted by Dr Pan's laboratory and others indicated that although these

full-length 7TM C-terminal variants bound mu opioids equally well because they shared the same binding pocket, they showed marked differences in mu agonist-induced cellular responses.

However, the main question remained regarding their *in vivo* roles in mu opioid actions, which led Dr Pan and his team to generate several C-terminal truncation mouse models to investigate the *in vivo* action of mu opioids. They created three mouse models with truncation of either all the C-termini (mE3M mice), or exon 4-encoded C-termini (mE4M) or exon 7-encoded C-termini (mE7M), and observed divergent roles for the carboxylic termini in morphine-induced behaviours in these mouse models, highlighting the importance of C-terminal variants in the modulation of complex morphine actions.

The results of the investigation, published in 2017 in the Journal of Clinical Investigation, showed that the exon 7 truncation in mE7M-B6 mice diminished morphine tolerance and reward without altering physical dependence, whereas the exon 4 truncation in mE4M-B6 mice facilitated morphine tolerance and reduced morphine dependence with no effect on morphine reward.

It is important to understand that dependence on a drug is different from drug tolerance. The latter occurs when the body needs a higher dose of the same drug to reach the same level of perceived benefits. Dependence, on the other hand, happen when the body experiences a loss of function in the absence of a drug, which can manifest itself as a series of painful withdrawal symptoms. Although tolerance and dependence often go hand-in-hand, they can happen through different biochemical mechanisms, as Dr Pan and his collaborators showed in a 2017 paper.

This paper represented a major advance in the understanding of the functional relevance of mu opioid receptor C-terminal splice variants in mu opioid pharmacology, with several important therapeutic implications. First, the researchers demonstrated that different sequences of the carboxylic terminus can alter drug-induced side effects without affecting the pain-relieving properties of opioids. Second, they showed that targeting specific carboxylic terminus of the mu opioid receptors could be an effective therapeutic strategy in the pursuit of novel drugs with more desirable pharmacological profiles and decreased side effects. Also, it is conceivable that opioid therapies targeting exon 7-associated mu receptor splice variants could decrease the addictive effects of opioids, with important implications for the opioid abuse that affects many countries.

Biased Signalling of Mu Opioids at Multiple OPRM1 Carboxylic Terminal Variants

The concept of biased signalling can be explained by looking at the large body of evidence in the medical literature showing that different agonists can trigger divergent signalling pathways through a single receptor. The co-existence of multiple OPRM1 full-length carboxyl terminal variants raises important questions about the role of these variants on the biased signalling through a single mu agonist.

In the same 2017 paper and a later 2020 paper, Dr Pan and his team demonstrated that a single mu agonist can induce biased signalling through multiple 7TM carboxyl terminal variants in terms of G protein activation and ß-arrestin 2 recruitment, providing a new perspective on biased signalling.

In particular, exon 7-associated 7TM variant, mMOR-1O, displayed greater ß-arrestin 2 bias for most mu agonists than exon 4-associated 7TM variant, mMOR-1, which explained, at least in part, why the similar morphine actions existed between mE7M-B6 and ß-arrestin 2 knockout mice. These studies opened up a new interesting research avenue for the exploration of the roles of other carboxyl-terminal sequences in biased signalling, as there are 22 full-length carboxyl-terminal variants in the mouse OPRM1 gene, 12 in the rat OPRM1 gene and 11 in the human OPRM1 gene that we know of.

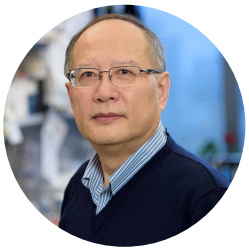
Future Perspectives

Dr Pan and his team will continue to investigate the mechanisms and functions of different mu opioid receptor variants in the action of opioids using several cutting-edge technologies. The proposed studies include mapping mu agonist induced receptor protein interactions for OPRM1 splice variants using proximity biotinylation coupled with proteomics, defining molecular mechanisms of morphine actions using phosphoproteomics and RNA sequencing approaches, and exploring molecular mechanisms and functions of OPRM1 alternative splicing using mini-gene constructs and high-throughput siRNA screening. They believe that these studies will provide potential targets for developing novel therapeutics for the treatment of pain and drug abuse.

The team led by Dr Pan will further examine the *in vivo* role played by individual OPRM1 splice variants in the pharmacological action of mu opioids by using new genetically engineered animal models. These studies will allow the researchers to link each receptor with a particular function. Dr Pan is also aiming to improve the overall pharmacological profiles of opioids by using novel targeting strategies. Evidence in the medical literature suggests that up to 80% of addicts started with prescription drugs. Efforts to find substitutes for opioid drugs would help address the opiate abuse crisis.

Finally, Dr Pan is a co-scientific founder of Sparian Bioscience. One of the most promising drugs at Sparian Bioscience, SBS-1000, is the second generation of IBNtxA that is more potent than morphine but without the many side effects associated with traditional opiates. This compound is expected to be in a Clinical Phase I Trial in early 2022.

Meet the researcher



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Dr Ying-Xian Pan obtained his MD in 1982 from the Shanghai University of Traditional Chinese Medicine, China. In 1986, he obtained his MS Degree in Biochemistry from the Shanghai University of Traditional Chinese Medicine and eventually moved to the USA, where he obtained his PhD in Physiology and Biophysics in 1993 from the University of Cincinnati, Ohio. Dr Pan continued his research career as an Aaron Diamond Foundation Postdoctoral Fellow at the Memorial Sloan-Kettering Cancer Center, New York, where he met his mentor Dr Gavril W. Pasternak, a renowned pharmacologist. After completing his postdoctoral fellowship, he joined the faculty of the Department of Neurology in 1999, where he became a full Member in 2013. Dr Pan is currently Professor of Anaesthesiology at the Rutgers New Jersey Medical School, and a core member at the Rutgers Brain Health Institute. Through studying the mechanisms of opioid actions, Dr Pan aims to develop novel strategies and drugs for treating pain and combating the opioid epidemic. Dr Pan is a co-scientific founder at Sparian Biosciences, a company devoted to the development of safer alternatives to opiate drugs.

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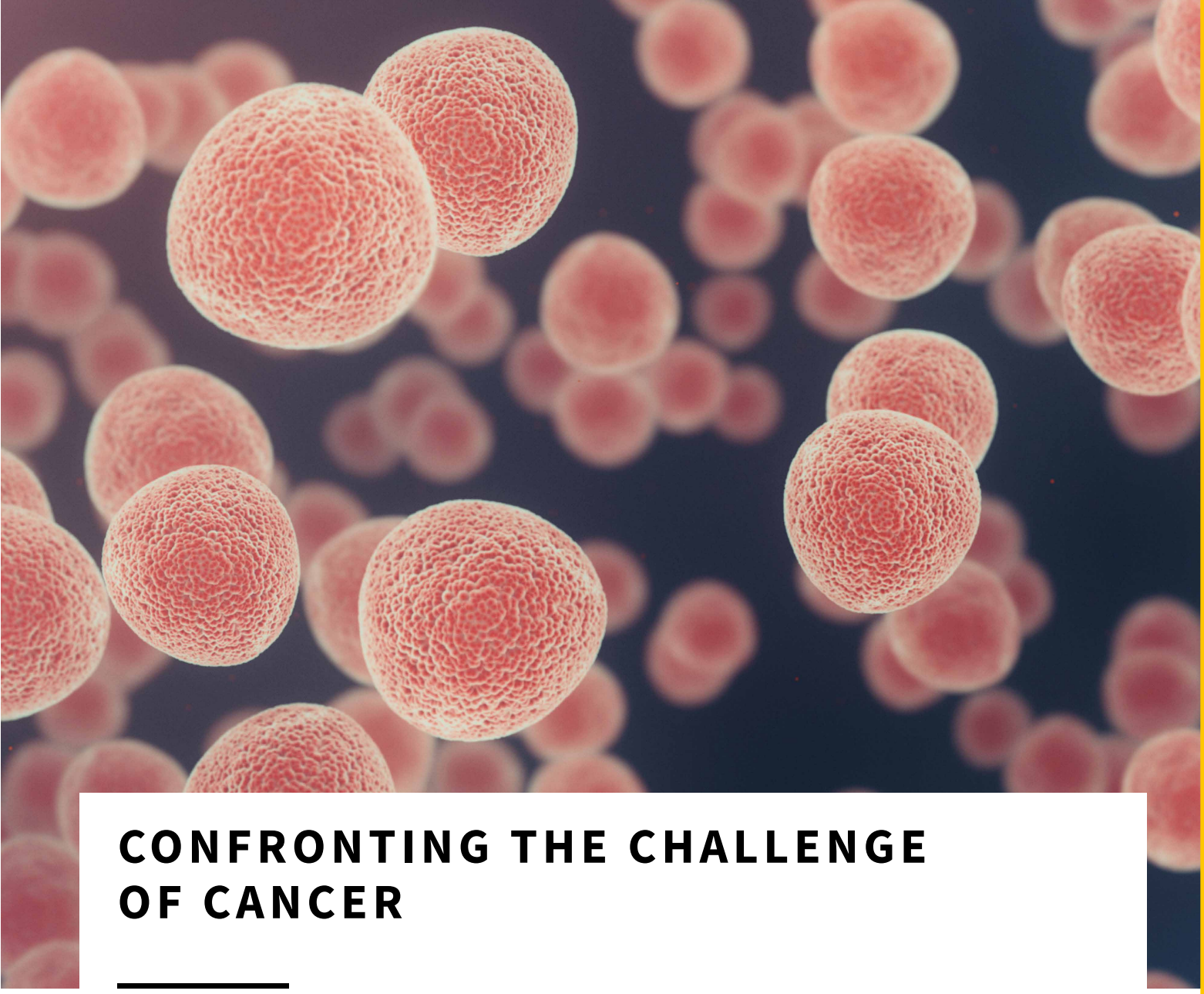
RUTGERS
New Jersey Medical School

CONFRONTING THE CHALLENGE OF CANCER



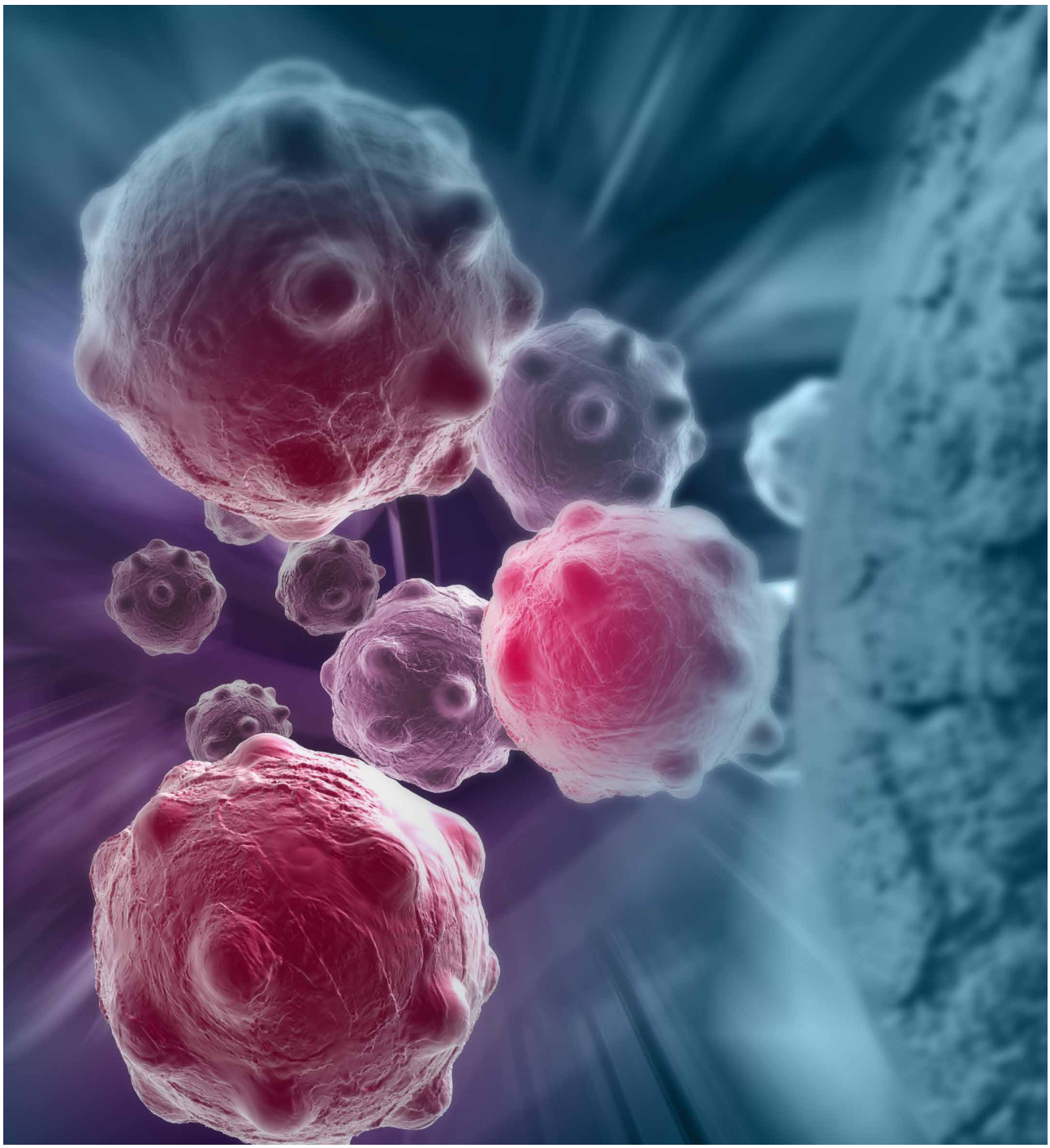
CONFRONTING THE CHALLENGE OF CANCER

Our third and final section of this issue is dedicated to the researchers confronting the global challenge of cancer through scientific endeavour and innovation. According to the World Health Organization, cancer was responsible for nearly ten million deaths across the globe in 2020, affecting people of all ages and all walks of life. Although dating as far back as our earliest medical records, cancer remains a significant and complex challenge to medical science, and a major burden of disease worldwide.

We open this section by meeting Dr Aliccia Bollig-Fischer from Wayne State University School of Medicine in Michigan, USA. Cancer can be caused directly by genetic mutations or by epigenetic alterations (changes to how DNA is processed rather than DNA itself), and the particular focus of Dr Bollig-Fischer is improving understanding of how obesity plays a role in this. She is especially interested in how an imbalance in the body known as oxidative stress arises from obesity and how this may lead to an increased risk of cancer.

Another researcher working to better understand susceptibility to cancer is Dr Joseph Jerry at the University of Massachusetts. In the USA, as many as 1 in every 8 females receives a diagnosis of breast cancer in their lifetime – and it seems that for some females, the hormone oestrogen may be to blame. Exposure to oestrogen occurs naturally typically from puberty through to menopause, and Dr Jerry is working to understand how this exposure interacts with genetic differences to influence breast cancer risk.

How cancer tumours develop is a key question for medical science and the focus of research by Dr Chao Sun at the Institute of Modern Physics, Chinese Academy of Sciences. The mechanisms underlying the effective functioning of our cells are complex but essential for processes such as energy production. We can read how the functioning of mitochondria, the tiny 'powerhouses' of the cell, can go wrong and lead to serious disease, including the development and progression of tumours.



We then to the researchers dedicated to improving cancer treatment. First, we meet Dr Stephen Kry from The University of Texas MD Anderson Cancer Center. Radiotherapy is commonly used and can be very effective in the treatment of cancer but the safety, as well as the efficacy of this approach, depends on getting the right dose. Dr Kry has identified that errors are often made in the calculation of radiotherapy dose and we can read how his research has driven the development of practical, effective solutions to this problem.

Cancer imaging helps clinicians visualise tumours within the body, with important applications not only in diagnosis but also in treatment. In a promising new approach, Professor Matthew Bogyo at Stanford University has developed fluorescent probes that can be injected into patients prior to cancer surgery, allowing surgeons to more clearly identify and accurately remove cancerous tissue and leave healthy tissue intact, optimising patient outcomes.

Finally, it is a sad reality that for many patients with cancer, financial difficulties can be a very serious concern. Dr Arpan Ashok Patel of the University of Rochester School of Medicine and Dentistry has pioneered research identifying the prevalence and impact of financial toxicity for older patients in the advanced stages of cancer. His work highlights, in particular, the need for appropriate tools for clinicians to assess such difficulties and allow signposting to support.

UNDERSTANDING WHY OBESITY IS A RISK FACTOR FOR CANCER

Cancer can be caused by genetic mutations or epigenetic alterations, which are changes to the way DNA is processed, rather than to the DNA itself. These changes can be brought about by obesity, and more specifically, oxidative stress and consequent reactive oxygen species. However, the molecular mechanisms by which this occurs are not well understood. **Dr Aliccia Bollig-Fischer** from Wayne State University School of Medicine in Michigan is studying these processes and paving the way for the development of novel cancer therapeutics.

DNA Methylation and Cancer

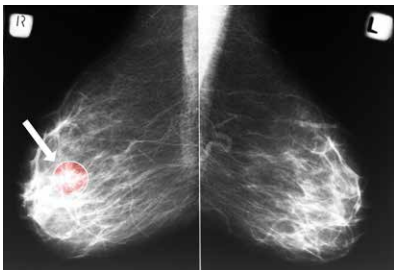
Many people will be familiar with the concept that cancer is caused by DNA mutations. When DNA is damaged and its structure is altered, the process of protein synthesis via transcription and translation is altered. This can either mean that the genes are expressed and the resultant proteins are faulty, or that the wrong amount of protein is produced. Through complex molecular mechanisms, the eventual result can be a tumour.

However, a lesser-known aspect of cancer formation is the field of epigenetics. This is the process by which the way a cell reads the DNA and a gene is expressed is altered, rather than the DNA itself. Whereas DNA mutation is permanent, an epigenetic modification can be reversed. This is a vital part of normal cell function because it helps to control healthy gene expression.

One important example of epigenetics is DNA methylation – the addition of a methyl group onto a base of the DNA. Often, this silences (or represses) the gene, meaning that it is not expressed at all or is expressed at a lower level and less protein is produced from it. This usually occurs on CpG islands, which are areas of DNA where there is a high concentration of cytosine nucleotides followed by guanine nucleotides.

CpG islands are usually found just before the beginning of a gene, at the transcription start site. Therefore, when a CpG island is methylated, the adjacent gene is silenced. Although this is a normal part of protein production, when it goes wrong, it can have serious consequences. If a tumour suppressor gene is silenced due to methylation, or a CpG island is altered and consequently, oncogenes are activated, this can lead to cancer.

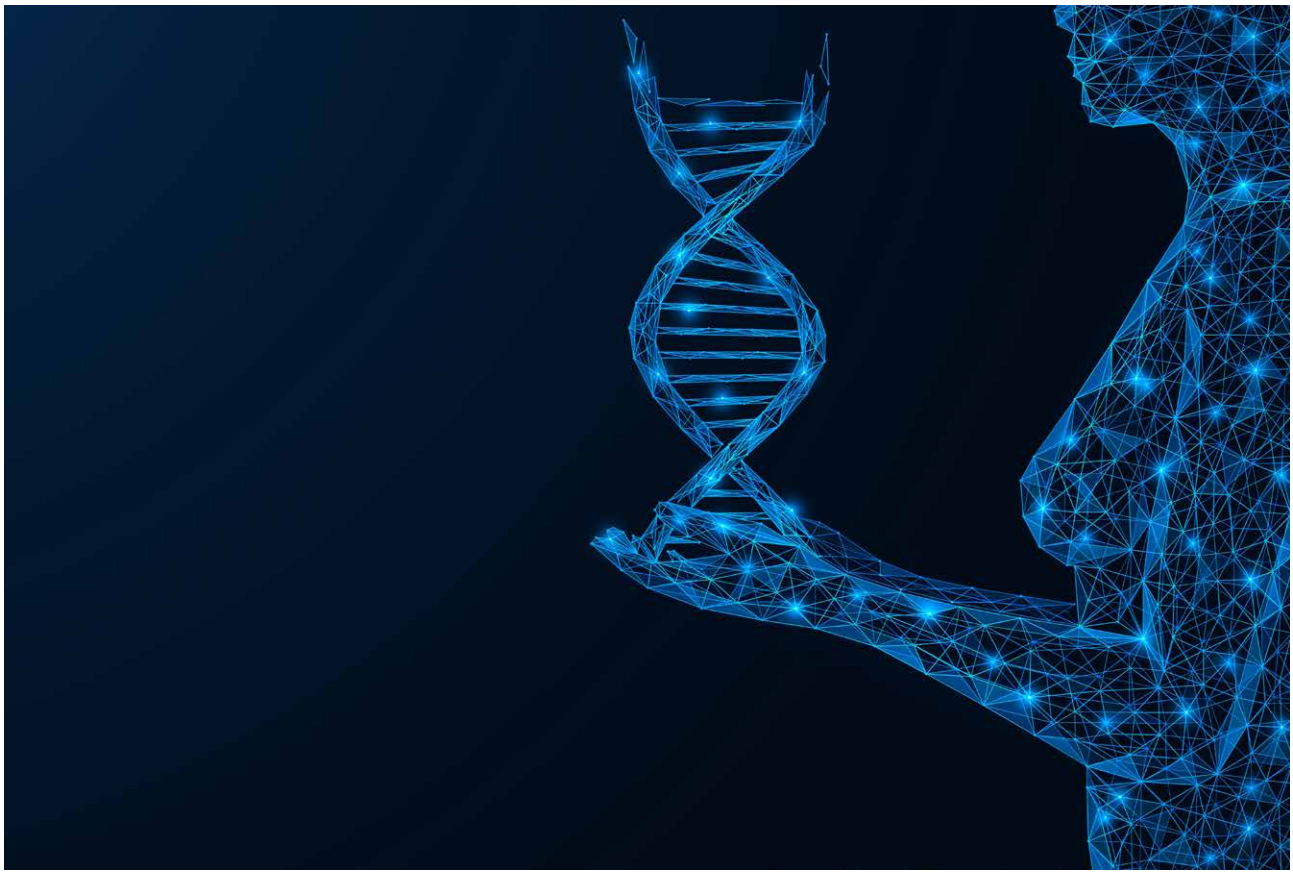
Despite extensive volumes of existing research into cancer epigenetics, there is still much more work to be done. Dr Aliccia Bollig-Fischer from Wayne State University School of Medicine in Detroit, Michigan, carries out dedicated research into the molecular and genetic reasons for how cancer develops. In addition to clarifying these underlying mechanisms, her work could help to give insight into novel therapies for cancer.



Triple Negative Breast Cancer

One specific cancer that Dr Bollig-Fischer is interested in is called triple-negative breast cancer, a type of breast cancer that presents in around 15–20% of new patients. Characterised by lack of expression of the genes for HER2, oestrogen or progesterone receptors, triple-negative breast cancer cannot be treated by standard hormone therapies or anti-HER2 therapies. Specific subtypes of this cancer can, unfortunately, be extremely difficult to treat.

For many types of cancer (including breast cancer), a methylation event known as 5mC is well-researched. This epigenetic modification occurs when a methyl group is added to a cytosine base on its fifth carbon atom.



The slight alteration of the addition of a hydroxy group to the methyl group creates a different molecule called 5hmC. Once thought to be an unimportant oxidation event, new research and improved techniques have shown it to be more interesting. Unlike most methylation occurrences like 5mC, 5hmC is associated with increased gene expression. Therefore, Dr Bollig-Fischer made this the focus of one of her studies.

She and her team scanned the available literature on 5hmC and hypothesised that it regulates the expression of genes that promote cancer stem cell-like cells in triple-negative breast cancer. Cancer stem-like cells are self-renewing cells that drive tumour progression and also lead to metastasis, the spreading of cancer to additional areas of the body. They believed this to be mediated by redox (reduction-oxidation) reactions involving reactive oxygen species (ROS).

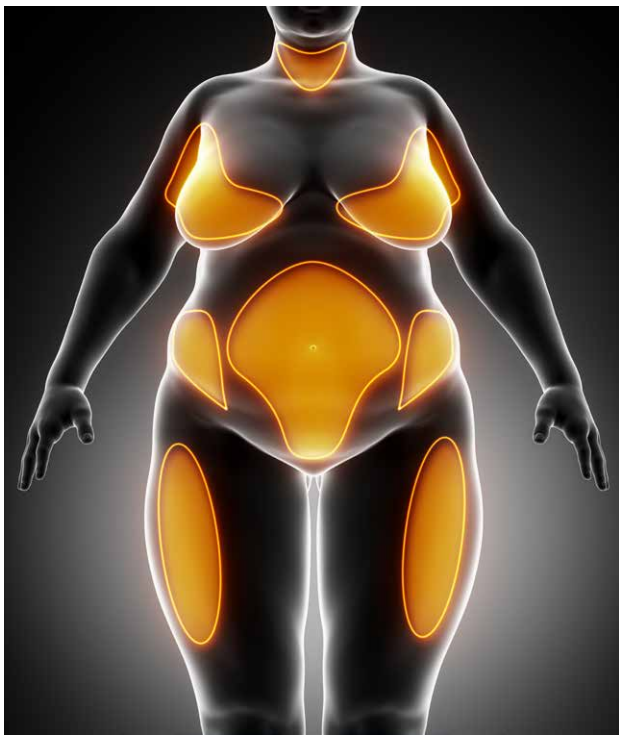
They set out to find the genes that are the target for these processes, looking for those that are regulated by ROS and therefore, sensitive to antioxidants and when 5hmC occurs, associated with the gene expression changes in triple-negative breast cancer. By developing a novel approach for their experiments, Dr Bollig-Fischer and her colleagues discovered a set of genes whereby the 5hmC level was coordinated with the relevant gene expression changes. Critically, these genes could potentially be regulated via a selective or targetted antioxidant treatment.

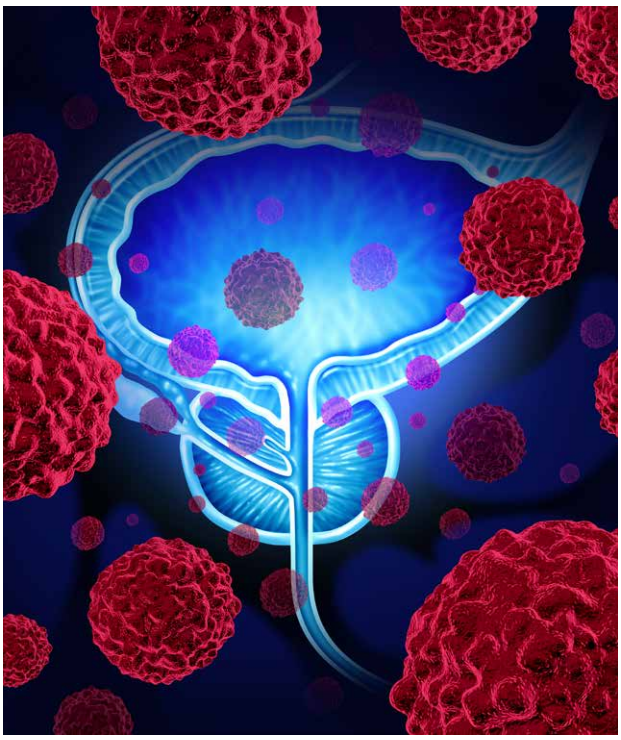
The Role of MBD2-v2 in Breast Cancer

A major risk factor for cancer is obesity and once cancer has formed, it also often leads to poor outcomes. The theory behind this is that the adipose (fat) tissue that builds up results in an immune response in the form of local and system-wide chronic inflammation. One consequence of this is an increase in oxidative stress and ROS which can incite breast cancer, including triple-negative breast cancer. However, the exact underlying mechanisms of this progression are unclear, and Dr Bollig-Fischer wanted to elucidate the issue.

Previous research with her team had found an important epigenetic reader, which is a protein that is vital for the maintenance and replication of cancer stem-like cells in triple-negative breast cancer. It has the rather long name of methyl-CpG-binding domain protein 2, variant 2 (or MBD2_v2 for short). Because of its role in cancer stem-like cell survival, Dr Bollig-Fischer believed it to be a key component in triple-negative breast cancer incidence and recurrence.

She hypothesised that obesity fuels an increase in MBD2_v2 expression which promotes cells to turn into cancer stem-like cells. Tests using obese mice and lean control mice with triple-negative breast cancer tumours confirmed her theory. The tumours of the obese mice had much higher levels of MBD2_v2 in addition to another protein called serine- and arginine-rich splicing factor 2 (SRSF2). Tumours also appeared more frequently in obese mice.





When the genes for SRSF2 were knocked down (removed) from the mice, they could no longer make the protein. The side-effect was that MBD2_v2 expression also decreased and in turn, cancer stem-like cells also diminished within the tumours. Dr Bollig-Fischer says this provides evidence for a new mechanism of cancer progression that is initiated by obesity. A majority of triple-negative breast cancer patients are overweight or obese, which emphasises the need to understand what lifestyle changes will help these patients in addition to what pharmacological interventions could be useful.

Further Proteins Involved in Breast Cancer

Delving further into triple-negative breast cancer, Dr Bollig-Fischer conducted another study linking 5hmC with obesity. The epigenetic event of 5hmC is brought about by an enzyme called tet methylcytosine dioxygenase (TET1). Although it is an important enzyme to maintain embryonic stem cells, it is not well understood how and why it can support cancer stem-like cells. This has led to extensive research in this specific area from Dr Bollig-Fischer and her team.

They have found that hydrogen peroxide, an ROS, regulates the SRSF2 needed for MBD2_v2 activity. This supports the idea that obesity leads to cancer due to inflammation, an increase in ROS and then higher SRSF2 expression. Additionally, another protein of interest called TAR DNA-binding protein was put into the mix after investigations. TET1 upregulates TAR-binding protein via 5hmC and therefore, SRSF2 is upregulated to promote cancer stem-like cells.

Furthermore, in a brand new discovery, they reported that dysregulation within triple-negative breast cancer cells allows hydrogen peroxide to form signals that increase the levels of TET1. The team found this is more severe in obese patients, which further clarifies why obesity is a risk factor for this type of cancer. As with the previous research, this new understanding could be utilised to advance prevention and treatment strategies for triple-negative breast cancer.

Different Risks of Prostate Cancer

In further studies, Dr Bollig-Fischer shifted her focus to prostate cancer. African American men are much more likely to be diagnosed with the disease compared to European American men, and they are also at higher risk of dying from it. She and her team used tissue samples from prostate cancer patients for their research and they made some interesting discoveries.

In the African American patients, the tissue adjacent to their tumours over-expressed a pro-inflammatory signalling molecule called interleukin-6 (IL-6) in comparison to the European American patients. Even though IL-6 is an immune molecule with wide-reaching roles, the team found that it inhibited the expression of the tumour suppressor, p53. The additional IL-6 also promoted cancer cells to self-renew and was associated with prostate cancer cells becoming stem-like.

As with the breast cancers she studied, these cancer stem-like cells were encouraged by MBD2_v2 whose expression had been elevated by the excess IL-6. This continually expanding knowledge of the mechanisms behind cancer incidence and recurrence will help Dr Bollig-Fischer and many other scientists to develop novel, targeted treatments. Hopefully, this will result in more positive outcomes for patients, regardless of gender or ethnicity.

Meet the researcher

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Dr Aliccia Bollig-Fischer received BScs in Biology and Chemistry from St. Cloud State University in Minnesota. She went on to achieve her PhD in Human Physiology at Michigan State University and then completed postgraduate training at the Karmanos Cancer Institute and Wayne State University in Michigan. Currently, Dr Bollig-Fischer is an Assistant Professor in the Department of Oncology at Wayne State University. She has received multiple honours and awards for her research, which is centred around understanding lifestyle factors and the molecular mechanisms that lead to cancer.

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WAYNE STATE UNIVERSITY

VARIATION IN DNA REPAIR MECHANISMS CAN INFLUENCE EFFECTS OF OESTROGEN AND ENVIRONMENTAL CHEMICALS ON BREAST CANCER SUSCEPTIBILITY

All women are exposed to oestrogen from puberty through menopause. Oestrogen is a natural hormone that is important for breast development and the maintenance of tissues in women but is also linked to an increased risk of breast cancer. As many as 1 in 8 women in the USA will be diagnosed with breast cancer over their lifetime, and the majority of these breast cancers are sensitive to oestrogen. **Dr Joseph Jerry** and his collaborators at the University of Massachusetts are studying the environmental exposures and genetic differences that alter the consequences of exposure to oestrogens.

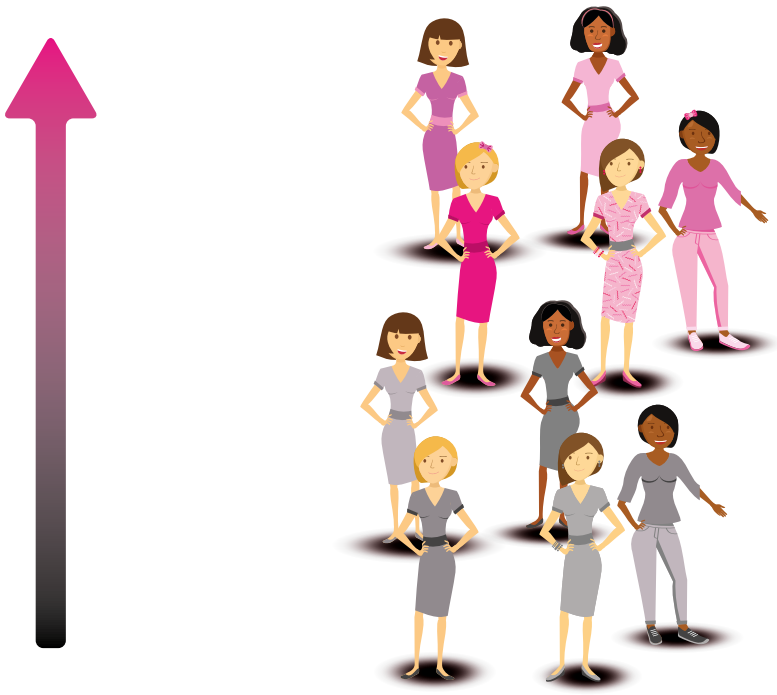
Oestrogen Signalling and the Link with Breast Cancer

At some point in their lifetime, 1 in 8 women in the USA will be diagnosed with breast cancer. Genetic factors and exposure to endogenous (i.e., internal) and environmental hormones can both influence the development of the breast epithelium and affect susceptibility to breast cancer. There is a large body of evidence demonstrating that breast cancers are sensitive to the actions of oestrogen in the majority of cases. Oestrogen is a natural hormone that has an important role in breast development and the maintenance of tissues in women. For a subset of women who are sensitive to endogenous or environmental oestrogen exposures, this may be associated with the subsequent development of breast cancer. Overall, however, the majority of women do not develop detectable breast cancers in their lifetime.

Dr Joseph Jerry, and his collaborators from the Department of Veterinary and Animal Sciences at the University of Massachusetts, the Pioneer Valley Life Sciences Institute and Baystate Medical Center, are working on an ambitious research plan. They are outlining how genetic variation in the general population affects the role played by oestrogen in the early stages of breast cancer. Then, by understanding the molecular basis for premalignancy in the human breast, they aim to develop diagnostic tools that can identify the women who are at high risk. Importantly, this identification of at-risk women would allow the provision of appropriate hormonal therapies at an earlier, more effective timepoint than currently possible.

The Effect of Dietary and Environmental Oestrogen on DNA Damage

Dr Jerry and his colleagues have worked extensively on the hypothesis that environmental chemicals mimicking the effects of oestrogen exacerbate or prolong the damaging effects of oestrogen in women who are sensitive. It has long been observed that exposure to oestrogen-like compounds available in the diet or the formulation of cosmetics can trigger the activation of oestrogen receptors. An abundance of



these receptors in breast cells may make them susceptible to DNA damage by exogenous (i.e., external) oestrogen-like compounds. Critically, DNA damage in epithelial breast tissue can contribute to the formation of malignant tumours.

The team published an article in 2020 reporting on the experimental observations of the effects on the DNA in mice and on human breast cancer cells following treatment with benzophenone-3 (BP-3) and propylparaben (PP), environmental chemicals that can mimic oestrogens referred to as environmental xenoestrogens. BP-3 is an ultraviolet filter used extensively in personal care products (such as sunscreens, cosmetics and lotions), while PP is commonly used as an antimicrobial agent in food and personal care products.

The study concluded that exposure to PP and BP-3 induced DNA damage in mammary glands of mice at concentrations relevant to acute oestrogen exposure in humans. PP and BP-3 also affected the DNA stability of cultured breast cancer cells and could cause DNA damage in the breast tissue of susceptible individuals. According to the researchers, the DNA damage in breast epithelium was caused by the formation of oestrogen receptor-dependent R-loops, a specific type of DNA damage that could in future be used as a sensitive endpoint for the screening for potentially harmful chemicals.

Genetic Variation Affects the Risk of Developing Breast Cancer

While all women are exposed to endogenous and environmental sources of oestrogen, it is important to remember that 7 in 8 do not go on to develop breast cancer and furthermore, there are reports that oestrogen may have a protective effect against breast cancer, for some women, at least. The strikingly different responses to oestrogen exposure among women prompted Dr Jerry's team to investigate if the variation in the responses among individuals could be related to small changes, known technically as polymorphisms, in specific genes. The team analysed human breast tissue samples from female donors undergoing reduction mammoplasty surgery to examine the DNA integrity following exposure to oestrogen.

The results of the study confirmed that responses are highly variable among women, suggesting that genetic polymorphisms could result in significant differences in intracellular signalling pathways among individuals. These differences may be important for identifying groups of patients who might be more at risk of developing breast cancer following prolonged exposure to endogenous and environmental oestrogens.

In another article, Dr Jerry and his co-authors reported that oestrogen signalling appears to be increased in the earliest stages of breast cancer and it is involved in benign or pre-malignant breast lesions known as atypical hyperplasias (AH). Understanding how AH lesions form could provide valuable insights into the molecular changes that cause breast epithelial cells to become malignant. The authors found that some genes can act as a 'signature' that discriminates the histologically normal tissue from AH tissues in 8 out of 10 cases. The genetic profiles associated

with AH breast lesions revealed variations in the oestrogen receptor levels among others. Monitoring the genetic profiles of patients presenting with AH lesions could help identify early changes in the epithelium that could be linked to an increased risk of cancer.

Genetic Polymorphisms Can Undermine DNA Repair Mechanisms

Dr Jerry's team is aiming to understand the cellular mechanisms promoting breast cancer in the oestrogen-sensitive subgroup of women, while at the same time trying to ascertain what factors contribute to cancer resistance in the majority of the population.

The group has recently published a manuscript in which they identify genetic polymorphisms in mice that alter DNA replication and repair pathways. Here, the researchers demonstrated that genetic polymorphisms were responsible for susceptibility to mammary tumours in a strain of rodents and resistance in another. The inherited polymorphisms interfered with DNA damage repair in all tissues, however, the development of tumours occurred most often in the breast epithelium. This suggests that the breast epithelium is especially reliant on DNA damage repair to maintain its genomic integrity.

The team is currently working on unpublished data that provide further evidence on oestrogen-induced DNA damage. They show that the damage is elevated among mice and rats that are susceptible to mammary tumours. Furthermore, the researchers speculate that alterations affecting the DNA repair pathways could exacerbate the risk of breast cancer in humans. Preliminary data confirm that the sensitivity to 'pathogenic actions' of oestrogen is also higher in women with an inherited risk of breast cancer compared to the general population. Given that urinary concentrations of environmental oestrogens suggest that more than 20% of women are exposed to levels that are sufficient to stimulate DNA damage, it is important to identify individuals who might be more susceptible to these adverse effects of environmental oestrogens.

Future Perspectives

Dr Jerry and his collaborators argue that it is vital to understand and map out the cellular mechanisms that control the levels of DNA damage in most women. Once those pathways are identified, they can be used as therapeutic targets for treating or preventing breast cancer in the subset of women who are most at risk. Biomarkers of DNA damage in breast cancers would provide tools to assess the risk of progression and to identify specific therapies. This means that it would be beneficial to observe specific patterns of gene expression among women and whether those would provide the medical profession with suitable biomarkers for the identification of risk among the general population.

Preliminary data from Dr Jerry's current research show that specific molecular probes can identify DNA damage in the nuclei of cells by emitting fluorescence. These probes could be employed for the analysis of tissue obtained from core biopsies. The prevalence of the DNA damage caused by oestrogen be used to define thresholds associated with increased risk of breast cancer.

Dr Jerry and Dr Grace Makari-Judson are Co-Directors of the Rays of Hope Center for Breast Cancer Research, a collaboration between the University of Massachusetts, Baystate Medical Center and the Rays of Hope charity. Through the generosity of women, the Center has worked with advocates, scientists and clinicians to create a unique repository of normal human breast tissues. This resource is allowing researchers to get closer to a breakthrough in this important field of medicine. The Rays of Hope Center has helped lay the groundwork for the research on chemicals in cosmetics and sunscreens that may contribute to the cause of breast cancer for a subset of women.

More research is needed to understand the impact of Dr Jerry's findings. Most critically, we need to better elucidate the health risks posed by chemicals in personal care products and identify individuals for whom the chemicals pose a significant hazard.

Meet the researcher

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Dr D. Joseph Jerry obtained his PhD in Nutrition in 1987 from The Pennsylvania State University. After postdoctoral training in genetics (Jackson Laboratory) and molecular virology (Baylor College of Medicine), he joined the University of Massachusetts Amherst in 1993, where he is currently Professor of Veterinary & Animal Sciences. Since 2011, he has been Co-Director at the Rays of Hope Center for Breast Cancer Research. The Rays of Hope project has established a Breast Research Registry with more than 1,200 individuals enrolled as of June 2020. The resource provides lifestyle data as well as tissue specimens and breast cell cultures from donors. Dr Jerry and the team of collaborators have shown that oestrogen and environmental xenoestrogens stimulate DNA double-strand breaks that are mediated by oestrogen receptors. Their studies using human breast explants indicate that the pathogenic effects of oestrogen may be enriched among individuals who are susceptible to breast cancer.

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Anna Symington, Advocacy Director for the UMass-Baystate Site, Breast Cancer and the Environment Research Program funded by the National Institutes of Health

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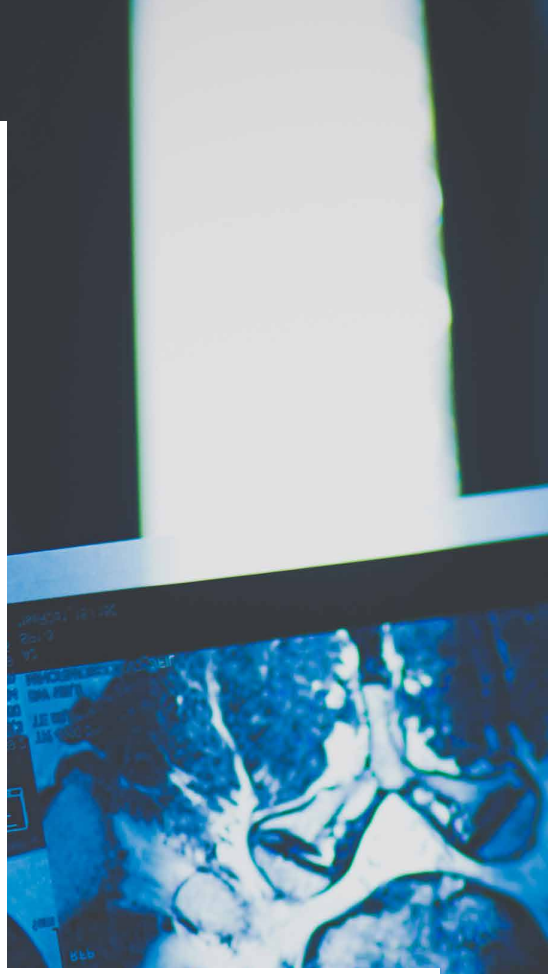
THE COMPLEX RELATIONSHIPS BEHIND TUMOUR PROGRESSION IN CANCER

The processes and metabolic pathways in our cells are complex and extensive, but essential for normal life functions, including cell respiration and energy production via the mitochondria. However, sometimes these processes go wrong resulting in disease, including cancer. **Dr Chao Sun** from the Institute of Modern Physics, Chinese Academy of Sciences, dedicates his research to unravelling the mechanisms behind tumour progression in relation to mitochondrial dysfunction.



Mitochondria: The Powerhouse of the Cell That Can Go Wrong

Within every cell, there are tiny structures that work together to keep it alive and functioning. These structures are known as organelles and the many different types each have their own purpose to fulfil. One such organelle is the mitochondrion, which is commonly referred to as the powerhouse of the cell. The reason for this is because it produces the energy needed to power the processes and biochemical reactions in the cell, in the form of adenosine triphosphate (ATP) molecules. Starting with a glucose molecule, ATP is synthesised through a series of chemical reactions called the Krebs cycle, or the citric acid cycle. The glucose needed for this energy creation comes from the food we eat and this cycle is an important part of aerobic respiration – creating energy with glucose and oxygen.

In addition to creating energy, mitochondria are involved in other important cell processes. They absorb and store calcium for when it is needed and can even generate heat when body temperature is too low. Although they take part in processes necessary for life, they also take part in cell death, known as apoptosis. They help to decide when a cell dies by releasing a chemical that activates the caspase enzyme which breaks down the cell from the inside.

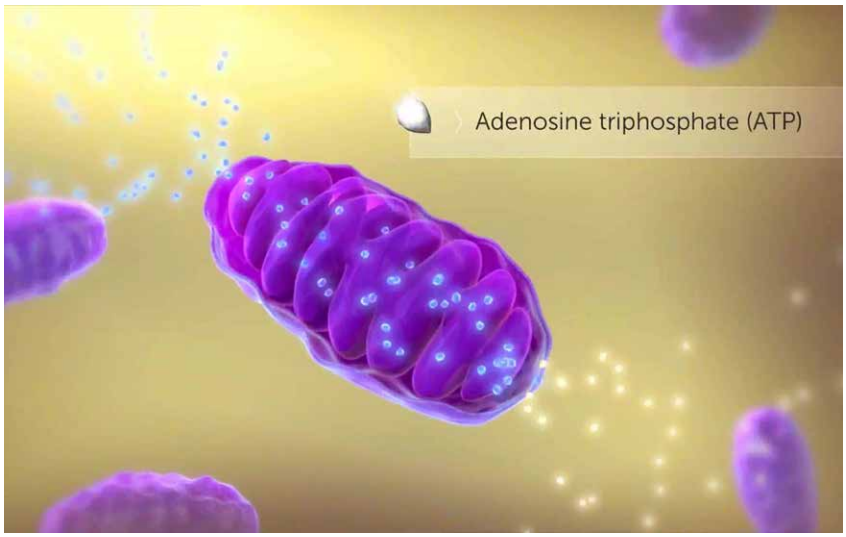
The unique structure of the mitochondria, and the smaller structures within, help them to carry out their jobs effectively. Like only two other organelles, the mitochondria have two membranes surrounding them and the folds of the inner membrane, called cristae, increase its surface area to allow more space for ATP creation. Sized between 0.75 and 3 micrometres, mitochondria can be found in varying numbers depending on the cell type. Mature red blood cells have none at all, whereas liver cells have more than 2,000. Usually, cells that require more energy have a higher number of mitochondria.

In human body cells, only two organelles hold DNA – most are in the nucleus but a small amount is stored in the mitochondria. However, it is more susceptible to damage and consequent

Mitochondrion

disease here due to molecules called reactive oxygen species, or free radicals, that are a by-product of ATP synthesis. Mitochondrial diseases are also caused by spontaneous or inherited mutations in nuclear DNA that result in faulty proteins within the mitochondria. If this means that it cannot function and create sufficient energy for the cell, it may cause a variety of different symptoms, depending on which cell is affected.

Muscle weakness, vision and hearing issues, neurological and gastrointestinal problems can all arise thanks to mitochondrial dysfunction. Additionally, there is evidence that Parkinson's disease, chronic fatigue syndrome and even cancer may involve malfunctioning mitochondria.



Dr Sun and his team at the National Institutes for Quantum and Radiological Science and Technology, Japan

Hypoxia and Disease

Cancer in relation to mitochondrial dysfunction is the focus of Dr Chao Sun's research at the Institute of Modern Physics, Chinese Academy of Sciences in Lanzhou, China. He also links these processes to hypoxia and molecules called hypoxia-inducible factors (HIFs). These are a type of transcription factor, which are proteins that control the process of DNA being decoded to eventually create new proteins. HIFs respond to a decrease in oxygen within the cell, known as hypoxia.

This environment can be a result of a variety of issues like anaemia and heart and lung diseases preventing oxygen from getting around the body properly. However, hypoxia can also be caused by structural and functional problems of the cell and organelles. When the mitochondria are malfunctioning, respiration decreases because less oxygen is available and other important pathways to maintain a healthy cell are disrupted.

When HIFs are activated by hypoxia, they participate in reactions related to metabolism, proliferation and angiogenesis. This means they take part in biochemical reactions for cell life, cell multiplication and the formation of new blood vessels. These are all important factors that allow cancer, the abnormal and uncontrolled growth of cells, to develop. 'We systematically discuss the crosstalk between HIFs and mitochondrial dysfunctions in cancer development', says Dr Sun, in consideration of the interesting results arising from his studies.

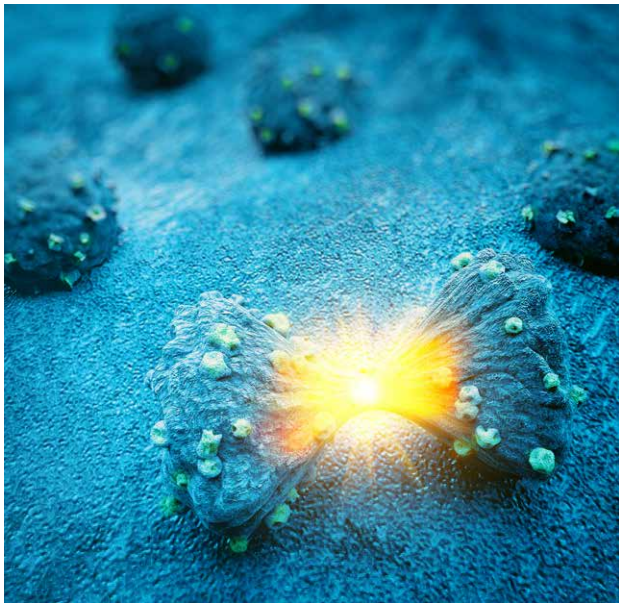
Proton Displacement in Adenosine Triphosphate Synthesis

In order for ATP synthesis to be possible, protons (positively-charged hydrogen atoms) are pumped into the space between the membranes of the mitochondria, called the intermembrane space. This creates a strong electrochemical gradient (mitochondrial membrane potential, MMP) across the inner membrane. A constant MMP means that protons pass back through via the enzyme ATP synthase which consequently makes energy. Normal physiological functioning and sufficient energy supply for the cell requires a maintained MMP.

An early study by Dr Sun investigated how an anti-oxidant drug called MitoQ interacts with this process. The purpose of MitoQ is to reduce the number of reactive oxygen species in the mitochondria and it is being tested as a therapy for a number of diseases from Parkinson's to heart disease. A section of the MitoQ molecule is positively charged and it remains in the intermembrane space, making the mitochondria act as though this space is full of protons.

Dr Sun found that to keep the MMP balanced, the organelle reacts by producing fewer protons and decreasing the proton pumping rate. However, this disrupts the normal ATP production and supply, so he dubbed the phenomenon pseudo-MMP (PMMP). With the mitochondria no longer able to cope with cell energy and respiratory demand, the cell begins to degrade itself in a process called autophagy.

These were exciting results from Dr Sun, because they were the first to talk about PMMP, the mechanisms by which it occurs and how autophagy takes place when protons are displaced by other positive charges. This gives insight into why the drug may be effective for killing cancer cells.



Mitochondrial Transplantation as a Cancer Therapy

A subsequent study by Dr Sun and his colleagues looked into how mitochondrial dysfunction and consequent disturbances to energy metabolism are associated with cancer. These links can be seen in cancer cells with mitochondrial dysfunction which causes an increase in glucose breakdown for energy (glycolysis), decreased cell death and resistance to radiotherapy.

The team transplanted mitochondria from healthy cells into brain cancer cells and starved them of glucose. They found that this slowed down the rate of glycolysis and decreased the number of protons available in the cells. The transplant also enhanced gene and protein expression related to the Krebs cycle, meaning more of the proteins necessary for the pathway were created, so respiration increased. It also reactivated the process in the mitochondria that encourages cell death, whilst inhibiting unwanted multiplication of the cancer cell.

In tests with mice, Dr Sun discovered that when healthy mitochondria were injected into tumours, they successfully entered the cancer cells through endocytosis. As a result, the healthy mitochondria inhibited the growth of the tumour and he believes they could make tumours more sensitive to the destructive effects of radiotherapy. These are exciting findings because they are the first to demonstrate mitochondrial transplantation as a potential therapy for tumours, including those that are resistant to radiotherapy.

Dr Sun proposes that mitochondria could be taken from healthy cells of a cancer patient, processed outside of the body and then reintroduced to their tumour. If successful, this could be a promising and fascinating intervention as it would be highly specific to and effective for the patient, without the risk of immune rejection.

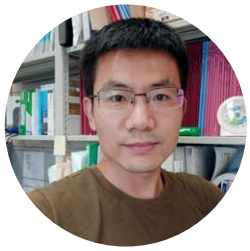
A Complex Relationship

Shifting his focus to HIFs in relation to cancer, Dr Sun and his team conducted a systematic review of the evidence for a relationship between HIFs and mitochondrial dysfunction in cancer development. They described how cancer cells are reliant on changes to normal metabolism that preserve energy, support cell growth and produce molecules that encourage tumours to thrive. Understanding how these adaptations come about is essential to discover weaknesses in pathways that could be exploited with treatments.

As previously mentioned, there is extensive evidence that HIFs have a role in regulating pathways in cancer and Dr Sun paired this understanding with the Warburg effect. This is a hallmark of tumours whereby metabolic adaptations, such as high glucose uptake through glycolysis, help cancer cells to survive in hypoxia. A product of this high glucose uptake, lactate, induces the production of HIFs and the hypoxic conditions activate the molecules. Both HIFs and mitochondrial dysfunction cause energy metabolism to be altered and are, therefore, very likely connected. Dr Sun explains that ‘activation of HIFs can lead to mitochondrial dysfunction by affecting multiple mitochondrial functions including mitochondrial oxidative capacity, biogenesis, apoptosis, fission, and autophagy’.

As a whole, Dr Sun’s review demonstrates that tumours developing and growing is in part due to an ‘extensive and cooperative network’ of mitochondrial dysfunctions and HIFs. Looking to the future, he believes that further elucidating these complex relationships through additional research will aid the progression of diagnostics and therapeutics for cancer patients worldwide.

Meet the researcher



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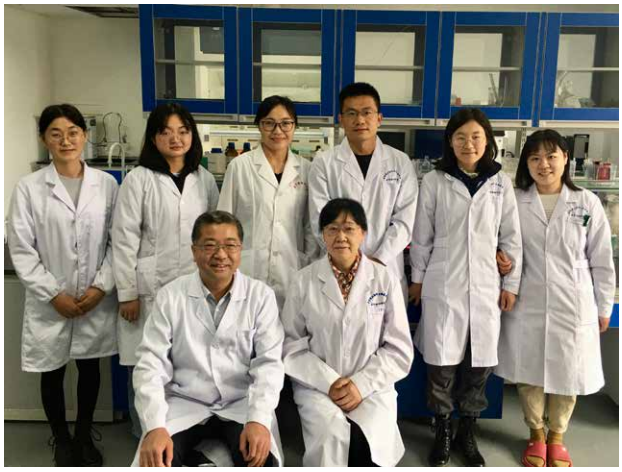

Dr Sun and his team at the Chinese Academy of Science, China

Dr Chao Sun received his undergraduate degree in Pharmacology and his Master of Medicine from Shihezi University in Xinjiang, China. He then achieved a PhD in Biophysics from the Institute of Modern Physics, Chinese Academy of Sciences in Lanzhou, China. Currently, this is where Dr Sun is a senior professor and also where he carries out his research. His work involves investigating mitochondrial dysfunction and how it can be involved in cancer development. In addition to his teaching and research duties, Dr Sun is also a guest associate editor for the scientific journal, Frontiers in Public Health. At present, Dr Sun is also a collaborative researcher of National Institutes for Quantum and Radiological Science and Technology, Japan, and serves as a visiting professor at Yantai University, China.

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IMPROVING THE QUALITY AND ACCURACY OF RADIOTHERAPY THROUGH RESEARCH

Radiation therapy is an effective and widely used method of treating cancer, and as with any treatment, it is essential to get the right dose. However, **Dr Stephen Kry** from The University of Texas MD Anderson Cancer Center has found widespread errors in the systems that calculate the doses patients receive. Through his research, he has helped to identify where these errors occur, how common they are, and provide possible solutions. He hopes that his work will go on to improve the quality and efficacy of radiotherapy for many cancer patients.

Cancer Prevalence and Treatments

Cancer is a disease that touches almost everyone in some way, at some point in their lives. In fact, half of all people will develop it. Characterised by the overproduction of cells in a specific part of the body, some cancers have the ability to cause destruction where they grow and spread to other tissues. As the human body has over 200 types of cells, there are over 200 types of cancer, but among the most common are those occurring in the head and neck, breast, prostate, lung and bowel.

Thankfully, cancer research has made incredible progress over the last few decades and many types of cancer now have effective treatments. If the cancer has produced a solid tumour, surgery is often the first step to begin fighting back. Some patients receive chemotherapy, which targets cells that multiply more often than the body's normal cells. Because chemotherapy drugs are circulated through the bloodstream, they can reach cancer cells in most parts of the body. Other patients may instead, or in addition, receive radiotherapy.

Radiotherapy: Is It safe, Is It Effective?

About 50% of people with cancer receive radiotherapy as part of their treatment. This may be with the aim to eradicate the cancer, make a tumour smaller to facilitate its removal (called neoadjuvant treatment), or just to relieve and control symptoms.

Most often this is done as external radiotherapy, where a machine known as a linear accelerator is used to direct ionising radiation or high-energy electromagnetic waves (i.e., X-rays) at a tumour. Internal radiotherapy involves swallowing or injecting a radioactive liquid or inserting a radioactive object near cancerous cells. The radiation from both procedures creates damage to the DNA within the cells, which prevents them from growing and dividing. Eventually, if the treatment is successful, the cancer cells die and the tumour stops growing. It is important that a sufficient dose of radiation is delivered to kill the tumour. Healthy cells around the tumour can also be damaged by radiotherapy, but they usually recover after some time.

Like all cancer therapies, radiotherapy has side effects, and too much radiation dose increases the risk and severity of these side effects. Two of the most common consequences are general fatigue in the following weeks and sore and red skin where the radiation had been targeted. Depending on where the patient has been treated, they may also experience decreased salivary production (dry mouth) for patients treated in the head and neck, shortness of breath for patients treated in the lung, or diarrhoea or rectal bleeding for patients treated in the pelvis. If the goal is to cure the cancer, not just lessen symptoms, radiotherapy is often given five days a week for one to seven weeks. The result is that 40% of people who receive radiotherapy as part of their treatment are cured.

External radiation therapy, in particular, is very technologically complex. There are more than 100 simultaneously moving parts on a linear accelerator shaping the radiation dose in three dimensions to ensure the radiation is limited, as much as possible, to just the tumour. This complex delivered dose must accurately match the intended dose, which is calculated in 3D using specialised computer systems. It is necessary that the intended and delivered dose agree to ensure that the patient has received the optimal dose. Disagreements could lead to not enough dose being delivered and a reduced chance of killing the tumour, or too much dose being delivered and excess side effects.

Phantom Heads

The term 'head and neck phantom' may conjure up images more in line with Halloween than cancer research. However, they are in fact a vital part of Dr Stephen Kry's studies into radiation oncology at The MD Anderson Cancer Centre. Head and neck phantoms are anatomically realistic models of a human head. Their shape, proportions and density mirror the real thing and inserts include simulated tumours as well as organs at risk. Devices called dosimeters within the phantoms measure the amount of radiation a specific location in the head has received. Using these phantoms, the accuracy of radiation therapy can be tested.

This phantom was developed by the Imaging and Radiation Oncology Core (IROC) in 2001. IROC provides quality assurance support for clinical trials by the USA's National Cancer Institute, ensuring that all institutions provide consistent and accurate radiotherapy doses. IROC monitors more than 2,000 radiotherapy clinics and hospitals worldwide, and the head and neck phantom is irradiated by around 200 institutions every year.

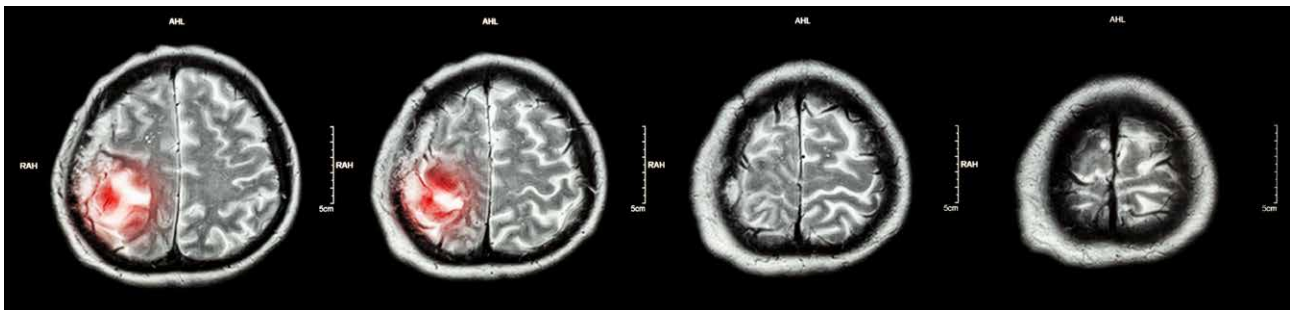
Finding the Errors in Radiotherapy Delivery

While this phantom was originally developed to credential institutions who wish to partake in clinical trials, it is also broadly used by institutions who want to assure themselves that they are able to deliver radiotherapy correctly. The phantom tests the simple but critical objective of whether the institution can deliver the dose they intend to deliver. This practice has a number of benefits as it not only reduces variability across the studies but it also helps institutions identify errors within their own processes and make improvements for the future.

To pass, institutions have to give the head and neck phantom a delivered dose within 7% of what was planned, within 4 mm of the planned spot. These criteria are loose compared to the dose accuracy needed biologically and compared to what should be achievable technically – institutions should be able to deliver the dose within 2–3%. When this testing started in 2001, pass rates started at 66%. Although they are now around 90%, this is still not quite good enough for Dr Kry, given that it means ~10% of institutions still fail to deliver radiation doses that are appropriately accurate. There were no studies that investigated the reasons why this was happening until recently, when Dr Kry and his team set out to find answers.

Using a thermoluminescent dosimeter, they determined the accuracy of radiation delivery on head and neck phantoms across hundreds of institutions. The team found that most failures to pass the phantom test were due to incorrect doses being delivered – usually too little radiation. Small inaccuracies and errors in the institution's configuration of the dose calculation software were identified as primary culprits.

Dr Kry and his colleagues were concerned about the high levels of inaccurate radiation dose delivery across the sites IROC worked with. This issue could directly and negatively affect cancer patients: unexpectedly under-dosing patients may lessen their risk of side effects, but it would also lessen the chance that their cancer was cured. Dr Kry saw that resolving inaccuracy was of the utmost importance. To resolve these dose discrepancies, it was necessary to understand where errors were originating from so that institutions could address these issues.



Head and neck phantom. Credit Stephen Kry.

Revealing Dose Errors

Dr Kry investigated several aspects of the way in which institutions predict radiation dose using their specialised computer system. Because the radiation delivery is so complex, the computer system is customised at each institution to describe each unique radiation beam. This process of modelling the physical radiation beam within the computer system is a complex process with ample opportunities for errors.

The first evaluation was of basic characteristics and calculations from the computer. IROC compared measured and delivered doses for more than 1,000 radiation machines at institutions that participated heavily in clinical trials. The institutions selected for evaluation focussed on those that involved higher numbers of patients in clinical trials. These establishments usually had no particular concerns about their dosing. A focus of these visits was to evaluate radiation dosimetry – how well the dose calculated by the treatment planning system matched that actually given. This was done for simple radiation fields (e.g., a square beam of radiation, instead of a complex-shaped field that would be used to treat a patient).

The team discovered that even for these simple cases, dosing inaccuracies were relatively common and came down to errors in how the institution had modelled the radiation in their computer system.

For more complex and realistic patient treatments, Dr Kry dug further into the head and neck phantom program. Dr Kry and his team developed a system to recalculate 259 head and neck phantom irradiations to search for and identify calculation errors in institutions' treatment planning systems. Using this system, the team evaluated the doses to the head and neck phantom that were predicted by hundreds of cancer centers, each using their own clinical treatment planning system. Dr Kry's team found a concerning number of failures in the ability of institutions to calculated doses accurately. If an institution had failed the head and neck phantom test originally, the team revealed that 68% of them were due to calculation inaccuracies. Overall, a concerning one in five institutions showed errors in their treatment planning systems for radiotherapy

Using this new evaluation system, Dr Kry and his colleagues can now inform an organisation when their treatment planning system is inaccurate. They want to emphasise the importance of creating accurate beam models in the treatment planning system so that patients are given the best possible care.

To aid institutions in improving the accuracy of their treatment planning system calculations, Dr Kry's team has worked on providing guidance on how to better develop beam models. For example, in 2019, they created a dataset that could be used as a reference by people testing their treatment planning systems. This helps them to assign the correct parameter values to the systems so that the consequent beam models calculate the correct radiation dose for the patient. Enabling an institution to double-check their calculations and detect anomalies in this way allows them to confidently treat their patients, knowing the dose they are giving is as accurate as possible.

Work for the Future

Dr Kry hopes that his team's research will aid the continuing improvement of the quality and accuracy of radiation therapy. He has identified the magnitude and origin of dosing errors that are unfortunately common in radiation oncology. Based on the underlying causes of dosing errors, he has developed practical solutions that can be used in real-world treatment centres. As clinical physics develops and evolves, his work will undoubtedly be useful for a long time to come.

Meet the researcher

Dr Stephen Kry
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Dr Stephen Kry hails from Calgary, Canada. He completed his Bachelor of Science (Honours) in Physics in 1999 at the University of British Columbia in Vancouver. He then went on to receive both his MSc in Medical Physics and his PhD in Medical Physics from The University of Texas MD Anderson Cancer Center in Houston. Whilst taking on many appointments and responsibilities throughout the years as part of various professional and scientific societies, he has also fulfilled numerous roles at MD Anderson. Currently, Dr Kry is a tenured Associate Professor in the Department of Radiation physics. He is the director of MD Anderson's Accredited Dosimetry Calibration Laboratory, Principal Investigator of the Imaging and Radiation Oncology Core (IROC), and Director of the Houston office of IROC. He is a member of the Graduate School of Biomedical Sciences at The University of Texas Health Science Center where he maintains a laboratory with several graduate students. His work has earned him multiple honours and awards such as the Highest Commendation from the Graduate School of Biomedical Sciences at his university. Dr Kry's research focuses on improving the safety and quality of radiotherapy treatments for cancer patients.

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FUNDING

National Cancer Institute

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THE UNIVERSITY OF TEXAS
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Making Cancer History®

IMPROVING CANCER SURGERY THROUGH ENZYME-ACTIVATED FLUORESCENT PROBES

Cancer is the second cause of death worldwide, despite continuous research efforts in the pursuit of better treatments. One of the most promising developments is that of cancer imaging, which aims to help clinicians visualize tumors within the body. **Professor Matthew Bogyo** and his team from Stanford University have developed fluorescent probes that can be injected into patients prior to cancer surgery. The probes emit fluorescence once in the tumor microenvironment, helping surgeons to distinguish cancer tissue from the surrounding healthy tissue to enable complete removal of the cancer and ultimately, improve patient outcomes.

Visualizing the Enemy

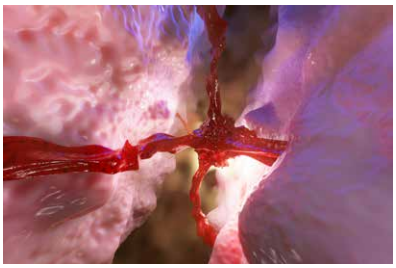
With a global death toll exceeding 9 million a year, cancer is the second leading cause of death worldwide. The World Health Organization estimates that globally, 1 in 6 deaths are due to cancer. This results in significant economic impact on healthcare budgets around the world, and of course, hugely detrimental impacts on the social and emotional wellbeing of patients and their families.

Thanks to advances in research, several treatment approaches are available in the effort to manage cancer, including chemotherapy, immunotherapy, and other forms of targeted drug treatments. Although any of these could serve as a primary cancer intervention, surgery is currently the most common for virtually all types of solid tumors.

The overall success of surgical intervention is dictated by the extent to which all cancer cells can be effectively removed from the affected organ while sparing as much of the surrounding healthy tissue as possible. Excess removal of tissue often leads to significant complications, while incomplete removal leads to increased recurrence rates (in the region of 30–65%) and the need for repeated surgeries (20–50% in breast cancers), causing considerable stress and anguish among patients.

Cancer imaging aims to help clinicians visualize tumors within the body, enabling both selective and sensitive detection of the tumor boundaries through the use of imaging contrast agents which have the potential to positively impact surgical treatment outcomes of many types of cancer.

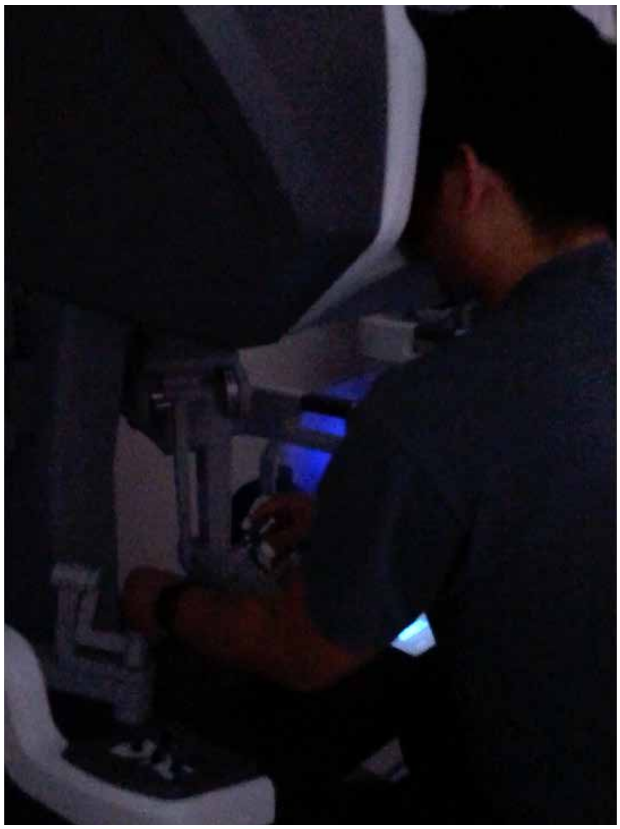
Professor Matthew Bogyo and his colleagues at Stanford University have successfully developed and optimized the use of molecular probes that allow the real-time visualization of the tumor margins, enabling surgeons to achieve the complete removal of cancer tissue, thus minimizing the risks of cancer reoccurrence and metastasis.



A Growing Cancer Tumor Spreading.

Enhancing the Selectivity of Contrast Agents

Despite the many advantages of fluorescence-guided surgeries (FGS), there are currently no Food and Drug Administration (FDA) approved optical contrast agents. The current challenge to this is achieving sufficient selectivity of contrast agents, as many of the



Optical Imaging During Surgery. Credit Matthew Bogyo.

established cancer targets are expressed in both healthy and tumor tissues.

To selectively target cancer cells, Professor Bogyo and his team have focused on developing contrast agents that produce fluorescence signals only within the tumor microenvironment. They achieved this by engineering smart probes that can only emit fluorescence when processed and cleaved by proteases that are highly expressed in tumor tissues.

Proteases are enzymes responsible for breaking down proteins into smaller molecules. Increased levels of one family of proteases, the cysteine cathepsins, can be found in tumors and are associated with growth and metastasis. This family of enzymes is also present in other pathologies such as inflammation and arthritis. Therefore, the contrast agents being developed in the Bogyo Lab could also be used for the detection and treatment of other diseases.

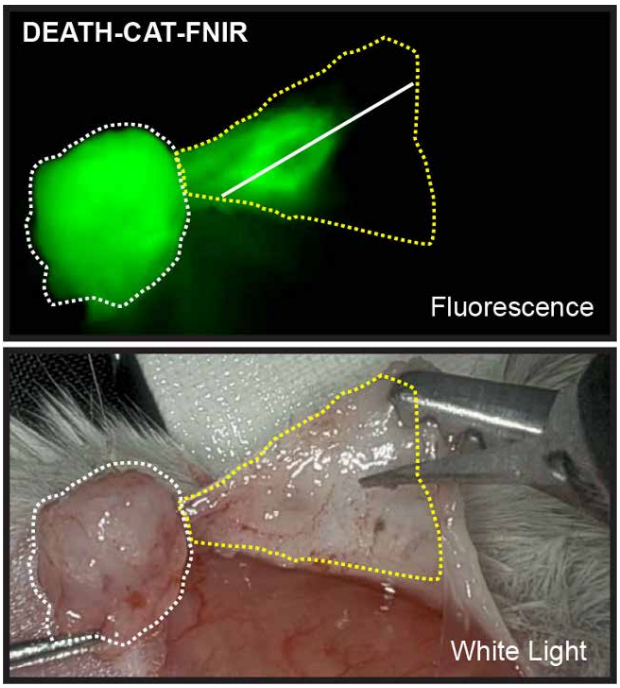
In 2005, Professor Bogyo and his team demonstrated for the first time that cathepsin-targeted fluorescent probes are effective in mouse models of cancer using non-invasive imaging methods. Then in 2015, they further reported on the development and optimization of a series of cathepsin-fluorogenic probes compatible with existing clinical instrumentation for use in FGS, demonstrating that they are suitable for the detection of diverse cancer types including breast, colon, and lung tumors using surgical robots.

Coming up with the optimal smart probes was not an easy task. Professor Bogyo and his team tested various molecular structures to facilitate the enzyme-assisted cleaving of the fluorophore. After numerous attempts, they developed a final, optimized probe, named 6QC-NIR, which was administered in a mouse model of breast cancer. The probe provided sufficient contrast to differentiate the tumor from normal surrounding tissues in less than 1 hour after administration. This facilitated the surgical removal of both primary and secondary tumors, which were buried deep within the tumor bed.

Having established the 6QC-NIR probe, Professor Bogyo's team continued to look for ways to improve the contrast agents and their detection capabilities. In a paper published in 2017, Professor Bogyo's team reported on the contrast capabilities of another fluorophore, indocyanine green (ICG), which they used in the development of the 6QC-ICG smart probe to better suit the capabilities of clinical imaging systems. Their studies in mice showed that the 6QC-ICG probe resulted in an overall brighter signal compared to the one generated by the previously developed 6QC-NIR probe.

AND-gate Contrast Agents

The single greatest challenge for the use of protease substrates as smart probes is optimizing their selectivity. The problem is there is a need to make sure the probes are only processed by the intended protease but unfortunately, many proteases are found both in healthy tissue and within the tumor



Highlighting Residual Tumor Cells After Resection. Credit Matthew Bogyo.

microenvironment. This makes it challenging to generate a signal only in the tumor tissue and not in the surrounding normal healthy tissues.

To solve this problem, Professor Bogyo and his team further developed imaging probes using an 'AND-gate' strategy in which multiple protease activities must be present to activate the probe. The term 'AND-gate' is a Boolean logic element commonly used in computer science. It represents a system that requires at least two inputs to produce a single output – in this specific case, the generation of fluorescence.

AND-gate probes represent a significant advance because they produce an optical signal only when multiple reporters are processed within the tumor microenvironment, rather than a single proteolytic event by a single protease. This results in greatly increased contrast over healthy tissue when compared with single-parameter probes.

The first AND-gate probe, named DEATH-CAT-1, used substrates that are cleaved by cysteine cathepsins and caspases proteases that regulate cell death – thus the name DEATH-CAT. Although all tissues contain some level of active cathepsins, caspases are activated only during the late stage of apoptosis, a process of programmed cell death, which typically does not take place in healthy tissues. However, cell death is triggered within tumors as the result of events such as nutrient and oxygen starvation. This 'two-step enzyme verification' reduces background signals in the healthy normal tissues.

Professor Bogyo and his team changed the probe's linker to resist cleavage by proteases other than the two primary target enzymes in the tumor microenvironment. They also replaced the ICG dye with FNIR dye to prevent aggregation and increase water solubility. The new DEATH-CAT-FNIR probe had fast activation, an overall high signal, good stability, and was able to locate cancer lesions with a diameter of less than 1 mm.

Overall, the data presented by the team confirm the value of the AND-gate approach, which significantly improves the specificity and sensitivity of optical probes. Critically, AND-gate probes will enable surgeons to visualize metastatic lesions in real time during FGS, improving patient outcomes and preventing removal of healthy tissue, while at the same time decreasing the risk of relapse due to incomplete removal of tumor tissue.

Future Directions

Professor Bogyo and his colleagues continue to expand their work, fine-tuning probes to make them more widely applicable. In their latest research, they have shown that it is possible to accelerate and enhance the process of probe design by the direct screening of diverse substrate libraries obtained directly from diseased tissue extracts.

Professor Bogyo and his team have recently screened libraries of protease substrates directly in mouse breast cancer tissues to identify the optimal peptide sequences to incorporate into the design of next generation optical probes. This approach has resulted in new probes with increased overall signals in tumor tissues. These probes are not only brighter but are able to distinguish human breast cancer from adjacent mammary tissue. Critically, this suggests that it should be possible to use tissue from relevant animal models or even human biopsy tissue to build optimal smart-optical probes for use in specific types of human cancers.

In principle, this approach should work for the identification of optimal substrates for any enzyme that is active during given pathological processes. Therefore, this strategy could be applied to any diseases involving inflammation, such as fibrosis, arthritis, and osteoporosis, where contrast agents could benefit disease management. A similar strategy could also be used to target pathogen-derived enzymes for imaging infectious disease. In fact, the Bogyo Lab is currently developing new types of protease substrates that can be used for diagnostics and imaging of important human infections such as Mycobacterium tuberculosis.

The current cancer imaging probes are now entering human clinical trials with the help of several companies. Professor Bogyo aims to obtain FDA approval for the smart fluorescent probes so they can be authorized for common use in cancer surgery. By integrating the probes with surgical camera systems, cancer can now be visualized in real time during surgery at the flick of a switch, enabling clinicians to dramatically improve treatment outcomes.

Meet the researcher

Professor Matthew Bogyo
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Professor Matthew Bogyo received his PhD in Chemistry from Massachusetts Institute of Technology in 1997. He established his career as a Faculty Fellow at the University of California, San Francisco. Later, he directed the Chemical Proteomics Department with a focus on applying small molecule probes to the field of drug discovery. In July 2003, Professor Bogyo joined the Department of Pathology at Stanford University and in 2013 was promoted to professor. His laboratory works on the development of new chemical probe technologies that are applied to studies of proteases and their roles in complex biological pathways associated with human disease. During his career, Professor Bogyo has published over 250 primary research publications and took on the role of the President of the International Proteolysis Society. He has received numerous awards including the Searle Scholar Award and the Terman Fellowship. He is the co-founder of Akrotome Imaging, an innovative start-up company developing imaging contrast agents for the detection of surgical margins.

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UNDERSTANDING FATIGUE: THE DEBILITATING SIDE-EFFECT OF CANCER TREATMENT

Cancer comes with many symptoms, but its life-saving treatments also cause negative side effects. One of these is radiation-induced fatigue, a clinical subtype of cancer-related fatigue and a debilitating issue that causes extreme lack of energy in those receiving radiation therapy. While this is a well-known effect of the treatment, exactly why it happens is unclear. **Dr Chao-Pin Hsiao** at Frances Payne Bolton School of Nursing in Case Western Reserve University, Ohio, is studying the molecular and genetic-level mechanisms that drive fatigue induced by cancer/cancer treatments. Her work on finding fatigue biomarkers provides promise for future combative therapies.

Cancer-related Fatigue

A common and challenging side-effect of cancer and its treatments is fatigue. Known as cancer-related fatigue, this feeling of extreme lack of energy and tiredness can be acute and last a month or less, or chronic, creating a long-lasting impact. Whereas fatigue in a healthy person is caused by activity and relieved by rest, cancer-related fatigue does not follow these patterns and its persistence is emotionally draining.

This health issue can affect all aspects of a person's life, as even the seemingly most simple of activities become difficult. Walking short distances, minor physical actions, thinking clearly and memory can all be negatively impacted, resulting in a diminished quality of life. Even after cancer treatment has ended, the fatigue and its consequences may remain.

One frequently used approach is radiation therapy, and this varies depending on the type, location and size of a tumour. It works by sending high doses of either X-ray or gamma radiation directly to the tumour, with the aim of killing the cancer cells and shrinking the tumour. The radiation damages the DNA inside the cancer cells beyond repair, which prevents them from repairing themselves and dividing. Eventually, the cells die, are broken down and removed by the body.

Although this is a life-saving technique, it has numerous side effects – many of these are shared across patients, but some are dependent on the location of the tumour and radiotherapy. Nausea, tenderness at the target of radiation, hair loss, skin changes, fertility issues and others can occur during and after treatment. However, one of the most common and severe implications of radiotherapy is radiation-induced fatigue, a type of cancer-related fatigue.

Even though the symptoms of fatigue in cancer are well-documented, it is not entirely clear why they occur. Investigating the molecular and genetic-level mechanisms of radiation-induced fatigue in prostate cancer patients is the work of Dr Chao-Pin Hsiao. She carries out her studies at the Frances Payne Bolton School of Nursing in Case Western Reserve University, Cleveland, Ohio, where she is also an Associate Professor.

Radiation therapy

The Role of the Mitochondria

In order to understand Dr Hsiao's research, first we need to get to grips with a key component of the cell known as the mitochondria. Often called the 'powerhouse' of the cell, these organelles (structures with a function in a cell) are bound by two membranes. These membranes are a vital component in the role of the mitochondria – to make energy in the form of adenosine triphosphate (ATP) from the food we eat.

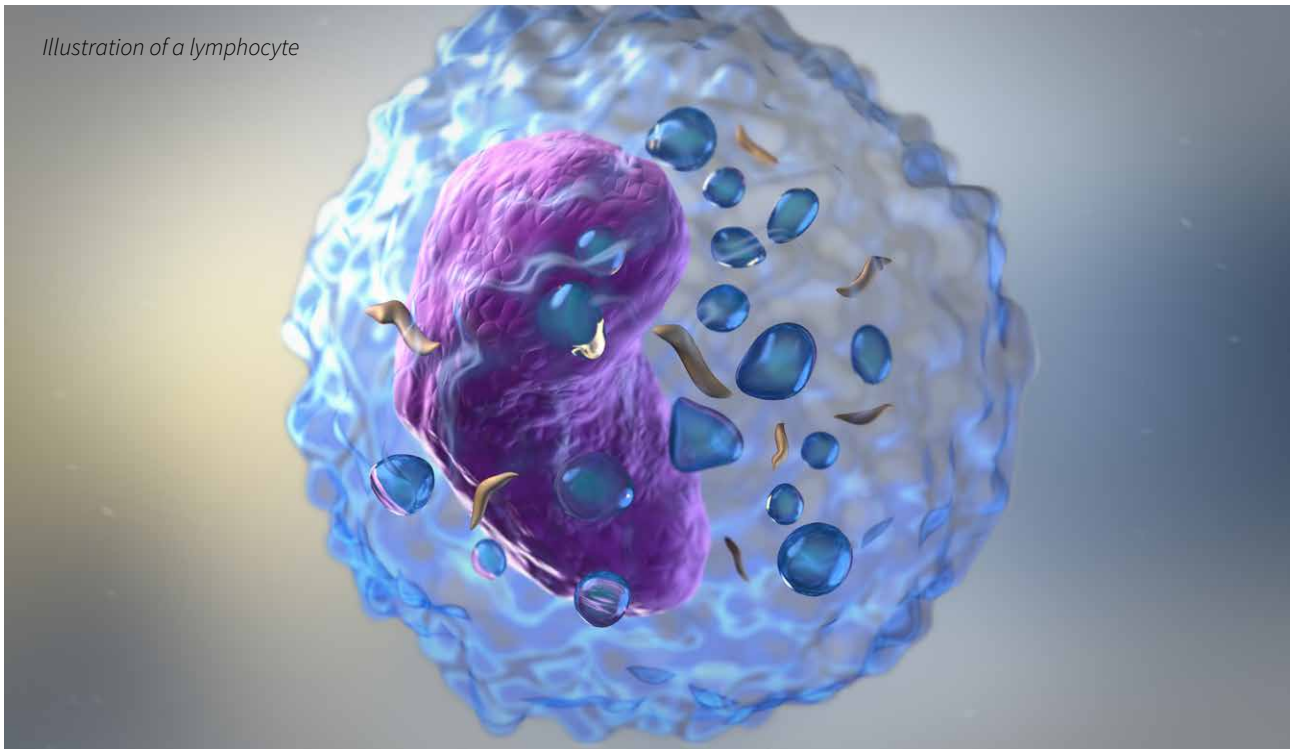


Illustration of a lymphocyte

This conversion occurs via a series of biochemical reactions called the Krebs cycle, or the citric acid cycle. The whole process is known as oxidative phosphorylation and once it is complete, the ATP that is produced can be used to power the body or be stored for later in times of energy deficit. In addition to its function of energy production, mitochondria also play a part in normal cell death, calcium storage and heat production.

Dr Hsiao focuses much of her work on the role of the mitochondria in radiation-induced fatigue and she is uncovering how its molecular and genetic mechanisms are involved. Because the radiation causes planned damage to cells, the result is tumour shrinkage, but this is in addition to other damage that results in the side-effects.

Mitochondrial Genes and Fatigue

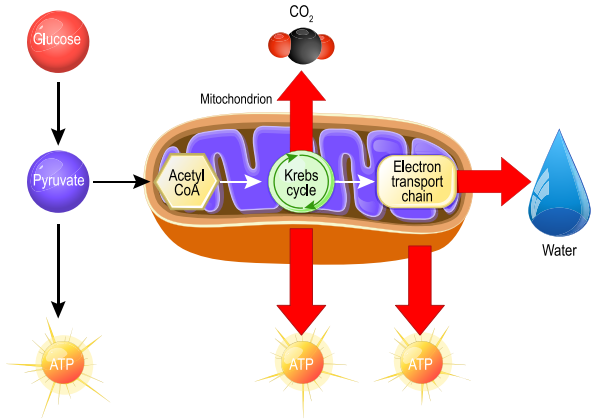
Some of this damage results in the instability of the genome (the complete set of DNA) and inflammation which results in the production of reactive oxygen species that cause further DNA damage. The radiation also impacts the normal jobs of the mitochondria and inhibits its respiratory chain that facilitates energy production.

In an early study, Dr Hsiao studied these biological markers in relation to radiation-induced fatigue in men with prostate cancer receiving a type of radiotherapy called external beam radiation therapy (EBRT). She looked into the genes that are involved in creating new mitochondria (mitochondrial biogenesis) and energy production (bioenergetics). After taking blood samples from the participants, she could decipher which genes were expressed (activated) in ways different than normal during fatigue.

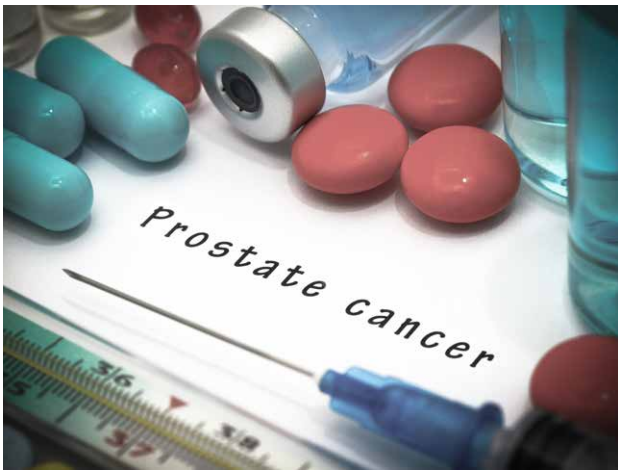
Interestingly, she discovered that specific 14 genes involved in mitochondrial biogenesis and bioenergetics were differentially expressed during radiation therapy and of these, four were significantly related to fatigue. One of these is called BC1 (ubiquinol-cytochrome c reductase) synthesis-like (*BCS1L*) and Dr Hsiao is now delving deeper into the 'how and why' this takes place with the goal of eventually developing effective therapies for managing fatigue. Additional research from her team looked further into the role of incorrectly functioning mitochondria in radiation-induced fatigue. More specifically, they looked at faulty mitochondrial bioenergetics. In a similar format to the previous study, prostate cancer patients were scored on their fatigue level and blood samples were taken at three points during their radiation treatment.

Again, Dr Hsiao honed in on the *BCS1L* gene and these results helped to form a new hypothesis. This is that reduced *BCS1L* (gene/protein) impacts the respiratory chain in the mitochondria. The series of reactions in energy production requires certain proteins in an electron transport chain and when *BCS1L* is in short supply, one of these proteins called complex III is affected. The Rieske iron-sulfur protein is unable to be incorporated into complex III as normal and this results in diminished oxidative phosphorylation and ATP production. Thus, reduced ATP means less energy and more fatigue.

Dr Hsiao believes these findings have the potential to identify novel targets for pharmacological therapeutics but may also be useful for nutritional therapies, known as nutraceutical interventions, to eventually treat fatigue-induced by cancer.



Aerobic Respiration



Improving Techniques and Understanding

Working with Dr Leorey Saligan at the National Institute of Nursing Research during her fellowship, Dr Hsiao studied another group of prostate cancer patients, 20 of whom were receiving EBRT and 20 who were on active surveillance who acted as controls between 2010 and 2012. All men were tested for their level of fatigue using a system called Functional Assessment of Cancer Therapy-Fatigue (FACT-F). Those receiving radiation therapy were assessed before, during and at the end of their treatment.

At each of these time points, the participants also had blood taken to test for the expression of genes of interest. The FACT-F scores of radiotherapy patients and controls were no different at the start, however, they decreased at the midpoint for those on treatment and even further at completion. This reducing score indicated worsening fatigue over the treatment time. The blood samples revealed 42 genes that had altered expression during fatigue. Most significantly, a gene called MS4A1 was downregulated, meaning its function was minimised.

This gene is involved in the formation of B-cells – important components of the immune response. Therefore, these results indicate that fatigue during radiotherapy may be related to the downregulation of MSA41 and the consequent impairment of the B-cell immune response.

In order to better understand these processes, Dr Hsiao and her team set out to improve the method of obtaining and analysing B-cells from the blood. B-cells are a type of white blood cell called a lymphocyte which is in the family of peripheral mononuclear cells (blood cells with a round nucleus). And like other cells, mononuclear cells rely on the mitochondria for energy. Their new protocol allowed them to look at integrative mitochondrial function, or specific dysfunction, and the individual complex activity of its electron transport chain during oxidative phosphorylation.

Using this improved technique, Dr Hsiao took peripheral mononuclear cells at three time points from a group of patients with prostate cancer, some receiving radiation therapy and others on active surveillance. She used these cells to determine their mitochondrial oxidative phosphorylation (energy production), electron-transport chain complexes activity, and expression level of *BCS1L* gene and protein. At the same time points, she measured the patients' fatigue on the Piper Fatigue Scale.

In line with her previous studies, Dr Hsiao found significantly worse fatigue in those receiving radiation therapy and related downregulation of *BCS1L*, reduced complex III activity and reduced oxidative phosphorylation. The more severe the downregulation of *BCS1L*, the more a patient suffered from radiation-induced fatigue.

This interesting result suggests that *BCS1L* and complex III in mononuclear cells could be important biomarkers, meaning they indicate how the body responds to a disease or treatment. Therefore, they could be promising targets for future remedies against radiation-induced fatigue.

Mitochondrial Bioenergetic Markers of Cancer-related Fatigue

In one of her most recent studies, Dr Hsiao continued to search for evidence of bioenergetic biomarkers for cancer-related acute and chronic fatigue. Again, she focused on the relationships between a messenger RNA (mRNA) and the protein of *BCS1L*, mitochondrial bioenergetics (oxidative phosphorylation, complex III activity, ATP and reactive oxygen species generation), and fatigue symptoms induced by cancer and cancer treatments. This work led Dr Hsiao to believe that m*BCS1L* and complex III activity are indeed bioenergetic markers for radiation-induced fatigue and therefore, may also be therapeutic agents.

Dr Hsiao's dedicated research into the debilitating fatigue that often goes hand-in-hand with cancer and its treatments shows how understanding its molecular and genetic-level mechanisms is vital. As a result of her work, we now have promising new avenues to explore in the development of potential therapies to ease the suffering of patients undergoing life-saving cancer treatment.

Meet the researcher

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Dr Chao-Pin Hsiao received her undergraduate and master's degrees in nursing from the National Taiwan University and then achieved a PhD in oncology nursing from the University of Arizona in the USA. She went on to complete postdoctoral and research fellow positions at the Symptoms Biology Unit of the National Institute of Nursing Research, the National Institutes of Health in Maryland. Dr Hsiao has held several research and teaching positions and now serves as an Associate Professor at Frances Payne Bolton School of Nursing, Case Western Reserve University in Cleveland, Ohio. Here, she carries out her research into the molecular and genetic mechanisms of mitochondrial bioenergetics in cancer-related fatigue.

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FINANCIAL TOXICITY: IMPACT ON OLDER ADULTS WITH ADVANCED CANCER

Dr Arpan Ashok Patel of the University of Rochester School of Medicine and Dentistry is dedicated to better understanding the prevalence and impact of financial toxicity amongst older, more advanced cancer patients. His recent work in this field is the first to analyse real conversations between patients and oncologists in this high-risk group. His findings show that those struggling with financial stability following cancer treatment are more likely to have a lower health-related quality of life, highlighting the need for better screening tools to identify at-risk patients to allow appropriate signposting to support.

Receiving a cancer diagnosis is a devastating blow. Although patients should be focusing on their treatment, their thoughts often turn to finances, especially if they are without insurance or are not eligible for government support. In 2011, the Centers for Disease Control and Prevention found that nearly one-third of families in the USA reported having financial burdens related to healthcare costs. Moreover, around 10% had medical bills that they were unable to pay.

Cancer is one of the most expensive diseases to treat. Treatment costs are much more likely to be patient-funded than for other chronic diseases. After receiving a diagnosis, out of pocket medical expenses can increase exponentially up to tens of thousands of dollars. Even those with Medicare, an American government health care plan for the elderly, are likely to experience high out of pocket expenses for cancer treatment, especially if they do not have any supplementary insurance. A recent study revealed that one in every ten cancer patients with Medicare pays expenses that equate to 60% of their household income.

Patients with private insurance may also experience additional costs due to being underinsured or exceeding the cover threshold.

The intensive and lengthy nature of treatments such as chemotherapy may lead to cancer patients losing their employment. Without a regular income, patients may have to resort to withdrawing retirement funds, selling their homes or even filing for bankruptcy. Sometimes, patients question if they will be able to afford to continue treatment. This adds extreme emotional stress on top of the physical stresses endured throughout their treatment journey.

What is Financial Toxicity?

Financial toxicity refers to the monetary burden of paying for cancer care costs and the negative impact of this on patient financial stability. Although the impact of financial toxicity upon cancer survivors has not been widely researched, in 2014, a large-scale study reported that almost half of cancer patients taking part were having difficulties living on their household

income. Furthermore, those with a high financial burden were likely to be experiencing a poorer quality of life.

Dr Arpan Patel from the University of Rochester School of Medicine and Dentistry, works in the field of oncology and believes we should not overlook the impact of the financial strain upon cancer patients. He has witnessed first-hand the relationships between patient distress, financial toxicities and quality of life when treating complex illness. Recently, Dr Patel has been involved in research focusing on the impact of financial toxicity upon older adults with advanced cancer.

Older and More Advanced Patients

Older adults are likely to face a different set of pressures than those of younger

patients. Their main source of income is typically either from the government or from private retirement savings (which may be in the form of a pension). In the USA, all over 65s are entitled to Medicare. However, this government financial assistance does not cover all healthcare expenditures, meaning patients have to make up potential shortfalls out of their own pockets. Cancer is also more prevalent in this age group, and the rate of prevalence in older adults is growing. Indeed, the worldwide cancer diagnosis amongst over 65s is predicted to double by 2035.

Previous research has identified two key and important themes in relation to financial toxicity amongst older adults. This first is that patients who report financial toxicity are more likely to have a lower health-related quality of life. The second is that patients often wish to discuss treatment expenses with their oncologists but these conversations rarely take place due to healthcare providers' discomfort.

Dr Patel and his colleagues wanted to focus their research on the experiences of older and more at-risk cancer patients. They aimed to assess the prevalence of financial toxicity in older advanced cancer patients and to examine the relationship between financial toxicity and health-related quality of life. In addition, they wanted to understand more about the financial conversations between these patients and their oncologists.

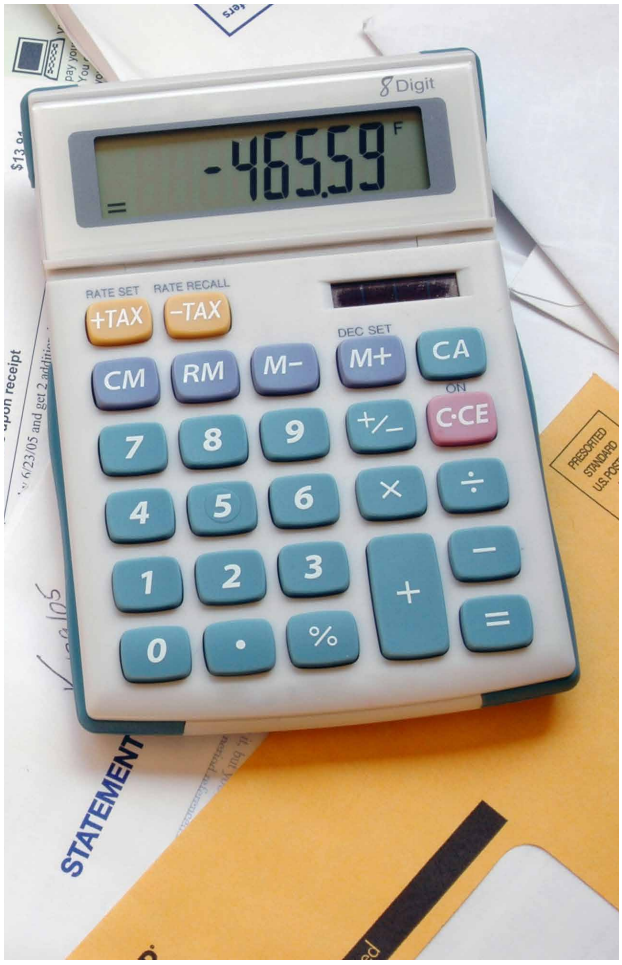
Ascertaining the Prevalence of Financial Toxicity

Dr Patel asked questions about financial hardship to ascertain whether patients met the criteria for financial toxicity. The main questions asked about treatment delays due to financial issues and levels of income for food and housing, as well as for clothing, medicine, repairs to the house and transport. The researchers found that almost 20% of patients over the age of 70 with advanced cancer had experienced financial toxicity. The data also suggested this was more likely to be experienced by those who were female, Black/African American, single, had a lower average household income, had a lower level of education, were not employed and whose health costs were covered by Medicare alone.

It is important that healthcare providers use screening tools to assess if patients are at risk of financial toxicity. Dr Patel and his colleagues believe that if validated, even the simple financial hardship questions used in this study could be an effective way of identifying the most at-risk patients.

Assessing Health-Related Quality of Life

As part of the study, patients also undertook various assessments to measure their health-related quality of life. These assessments examined how a persons' physical, mental, emotional and social functioning affected their health, comfort and ability to participate in or enjoy life events. Dr Patel and his colleagues found that patients with financial toxicity were likely to report higher levels of mental health issues such as depression, anxiety and distress. In line with previous research, they found that in this high-risk group, those with financial toxicity were more likely to have a poorer health-related quality of life.



The Importance of Cost Conversations

A unique part of the study was that at least one clinic visit between the oncologist and the patient was audio recorded. These recordings revealed that only 50% of patients experiencing financial toxicity had a conversation about costs or finances. Previous research has suggested that around 80% of patients wish to have this kind of conversation, indicating a gap between what the patient desires and practices. Why might this disparity exist? Dr Patel notes that both parties may be reluctant to bring up the subject of cost. For patients, there is the potential embarrassment of acknowledging they cannot afford treatment while oncologists may not feel comfortable about discussing this topic.

When the researchers examined the content of these conversations, four main themes emerged. The first was the cost of care, which was mainly initiated by the patient or caregiver. The second was about the patients' ability to continue to work or provide for their household in relation to these costs. The third was typically initiated by the oncologist and focused on the patient ability to afford care. The final theme was about costs that were not treatment related.

Shockingly, almost 12% of oncologists dismissed cost conversations when patients or caregivers raised them. However, in the majority of cases, oncologists did offer interventions or signpost to other resources that would be able to help the patient. Dr Patel and his colleagues are keen to emphasise that direct discussions around cost can help prevent financial toxicity. These discussions can allow oncologists to share knowledge about external resources that can provide support, such as social workers, financial advisors, support groups, charities and co-pay assistance.

Addressing Financial Toxicity at the Policy Level

The findings from Dr Patel and his colleagues shine an important light on the extent to which older and more advanced cancer patients are experiencing financial toxicity and the negative impact this can have. Importantly, they identify that there is a clear need for proper screening tools that can identify patients who are at high risk of financial toxicity. Furthermore, they note that there is currently no routine training for healthcare providers and no standardised approach between organisations. As a result, Dr Patel believes that policy change is needed to ensure interventions relating to financial toxicity occur at a national level. Critically, the lack of adequate assessment, particularly in older people with advanced cancer, means that they cannot receive appropriate referrals to support.

Meet the researcher

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Dr Arpan Ashok Patel is a clinician who specialises in haematology and oncology. Currently, he is Assistant Professor in Thoracic-Oncology in the Department of Hematology Oncology at the University of Rochester's Wilmot Cancer Center, where he also serves as Chief Quality Officer for the Division of Hematology Oncology and Associate Director for Informatics for Department of Medicine. Dr Patel gained his MD at the Medical University of Lublin in Poland in 2012 and then completed training in internal medicine at SUNY Upstate Medical University in New York, staying on for an additional year as Chief Resident. Dr Patel then undertook his fellowship training in haematology oncology with the University of Florida in Gainesville, winning the Chief Fellow Award in 2019. His research interests include thoracic-oncology, quality improvement, financial toxicity, patient distress and education.

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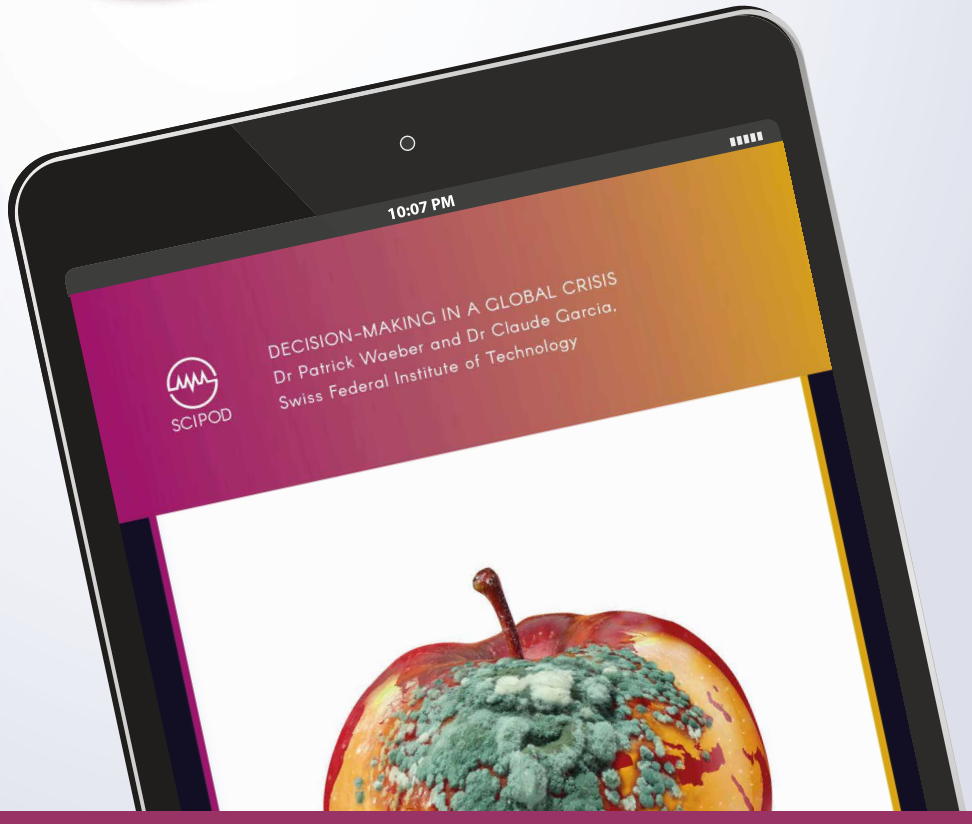

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