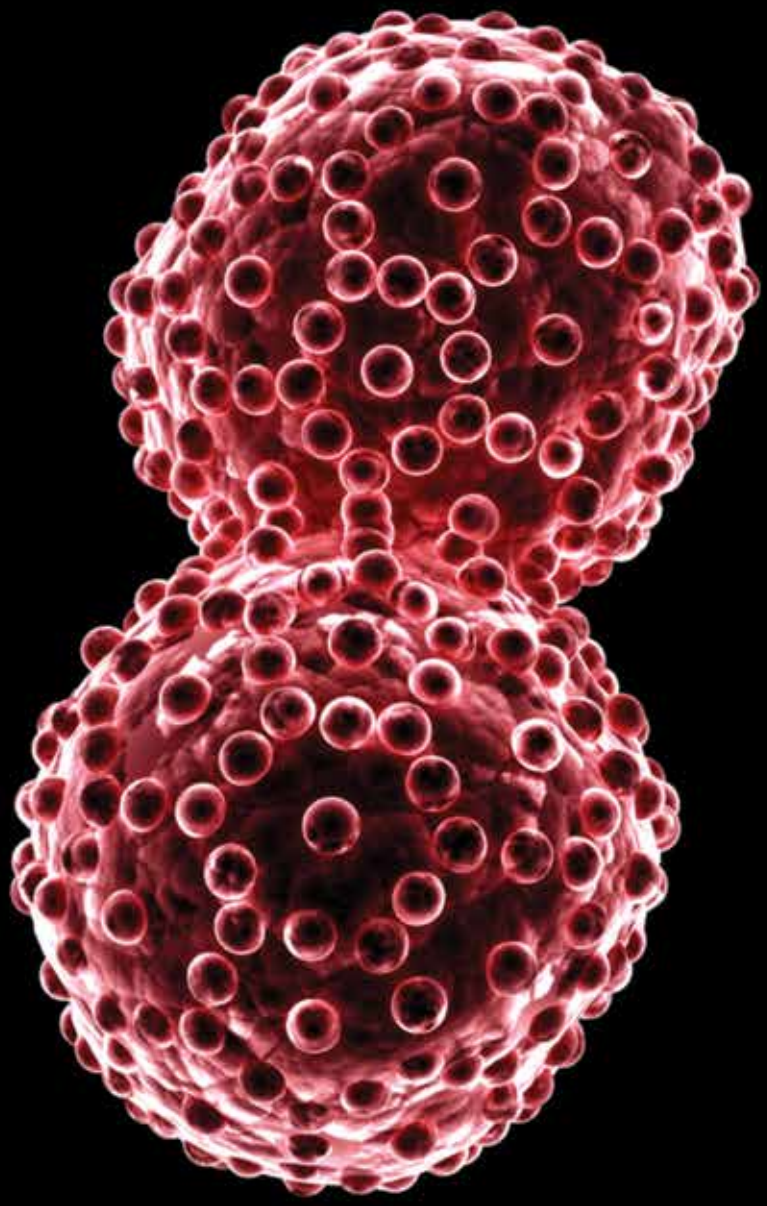
Scientia

INNOVATIONS IN CANCER RESEARCH



EXCLUSIVES:

- The European Cancer Organisation
- The Cancer Research Institute
- The European Association for Cancer Research

HIGHLIGHTS:

- Investigating Breast Cancer at the Molecular Level
- A Synergistic Immunotherapy for Skin Cancer
- Transforming Health Promotion Through Narrative
- Tumours in the Crosshairs: New Tools to Resurrect an Old Strategy for Targeted Cancer Therapy

Issue: #108

WELCOME...

Cancer survival rates continue to increase year after year. In the US, the 5-year survival rate for all cancer types increased from 50% in 1975 to a whopping 68% in 2007, according to the National Institutes of Health. Similarly, Cancer Research UK estimates that 10-year survival rates for all cancers have more than doubled in the past 40 years, from 24% in 1970 to 50% in 2010, here in the UK.

A significant factor driving our improving prognoses is the advances made in cancer research over the past few decades. These innovations have enormously increased our understanding of how this disease arises and progresses, in addition to improving our diagnostics and offering us a vast array of new therapies.

In this edition of Scientia, we applaud the scientists behind these huge leaps and bounds, by showcasing cancer research from across the globe. To open the issue, we have had the pleasure of speaking with Professor Peter Naredi, the President of the European Cancer Organisation (ECCO), who tells us about how the organisation collaborates with its member societies to accelerate cancer research, and to ensure that cancer remains at the top of the political agenda in Europe. Next, we emphasise the importance of health promotion and cancer awareness, by featuring an article describing the work of Drs Sheila Murphy and Lourdes Baezconde-Garbanati. The pair cleverly employ the power of storytelling to successfully encourage Latina and African American women to undergo regular cervical screening, thus reducing the cancer disparities that exist in the US.

From here, we feature a wide variety of different research projects, ranging from unravelling the molecular pathways that lead to cancer, to investigating the latest immunotherapies, drug combinations and novel nanotechnologies to combat cancer. The heroic efforts of the scientists behind these studies are bringing us one-step closer to eradicating the terrible blight that is cancer.

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Meet The Team...

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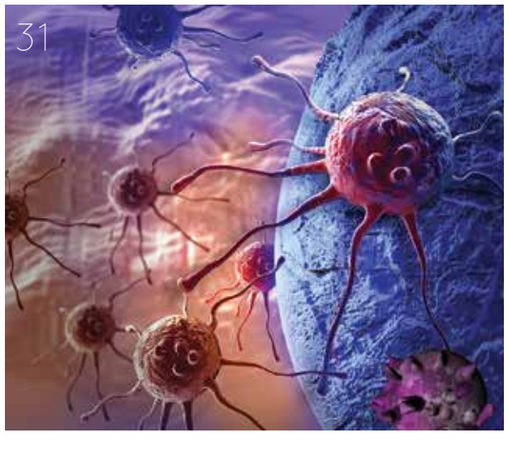
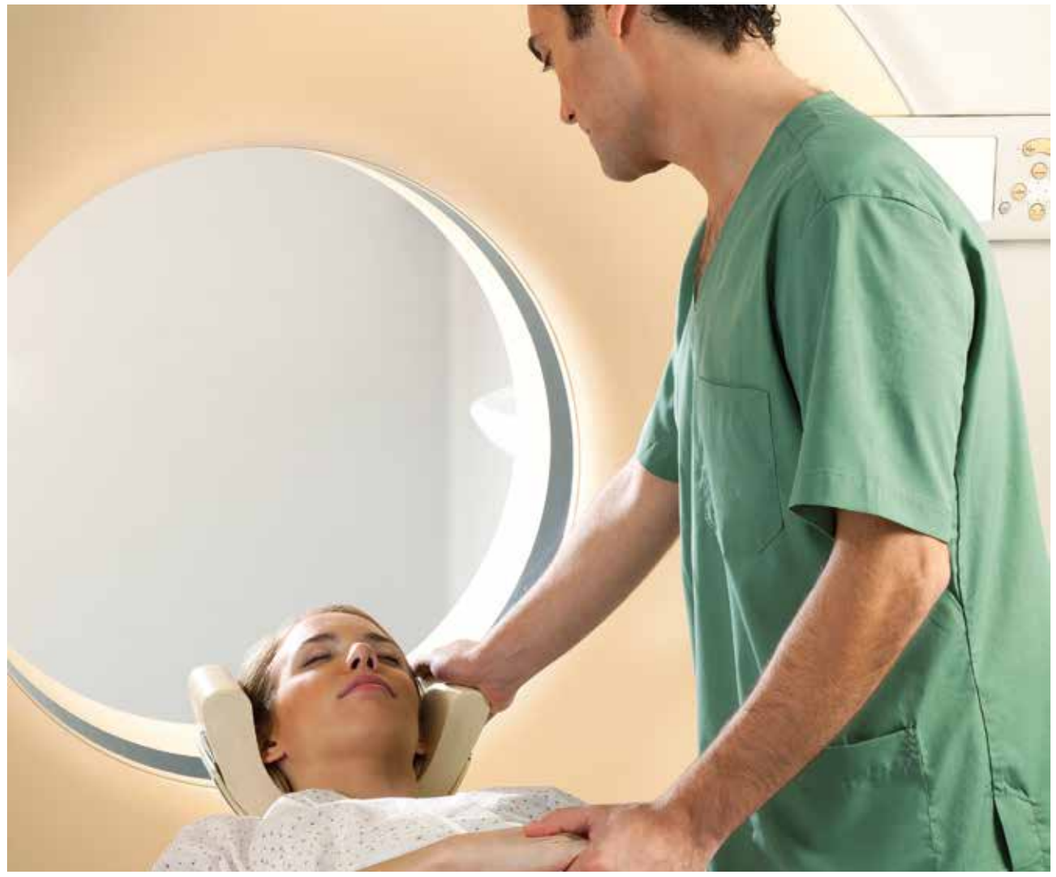
ISSUE : #108



Consisting of 24 Member Societies and representing over 80,000 professionals, ECCO is a not-for-profit organisation that exists to support the right of all European cancer patients to the best possible treatment and care. ECCO achieves this by connecting the European cancer community, creating awareness of patients' needs and encouraging progressive thinking in cancer policy and education. Through the organisation of international multidisciplinary meetings, ECCO actively promotes European cancer research, prevention, diagnosis, treatment and care.

ECCO's 24 Member Societies include the European Association for Cancer Research (EACR), the European Association of Nuclear Medicine (EANM) and the European Association of Neuro-Oncology (EANO). By connecting these societies and organisations, a cohesive multi-disciplinary platform is provided with the aim of working together to improve outcomes for patients, and to be the unified voice of the European cancer community when addressing common policy issues. Here we speak with **Professor Peter Naredi – President of ECCO and Professor of Surgery at the Sahlgrenska Academy, University of Gothenburg** – who tells us about how ECCO works together with its member societies to accelerate cancer research, and to ensure that cancer remains at the top of the political agenda in Europe.



To begin, please briefly introduce ECCO, and tell us a little about its focus and scope.

Since 1981, ECCO has been the only multidisciplinary organisation connecting and responding to all stakeholders in oncology Europe-wide. We now have 24 member societies representing over 80,000 professionals in oncology.

ECCO is a not-for-profit federation that exists to uphold the right of all European cancer patients to the best possible treatment and care, promoting interaction between all organisations involved in cancer at European level.

Please describe some of the other ways that ECCO facilitates cancer research in Europe.

ECCO has a strong focus on cancer research at its congress ECCO2017 (27-30 January 2017, Amsterdam). In this edition of the congress, we have built in a special workshop on the art of designing clinical trials. This workshop is developed by world class experts in clinical trial design and provides unique insights for congress delegates. ECCO aims to help disseminate the latest knowledge and practice in clinical trial design and to foster the education and advancement of young researchers. We have grants allowing such promising young scientists to come to the ECCO2017 congress and learn from the best experts worldwide. We also organise the annual workshop Methods in Clinical Cancer Research with our partners AACR (American Association for Cancer Research), ESMO (European Society of Medical Oncology) and EORTC (European Organisation for Research and Treatment of Cancer). ESMO and EORTC are two of our member societies and AACR is one of our strategic international partners. We believe in fostering the progress of cancer research by building bridges between researchers in Europe and researchers everywhere and by setting global standards of excellence together with our partners.

In what way does ECCO promote co-operation between researchers from different institutions and countries?

ECCO is a powerful multidisciplinary platform – we make it possible for our diverse member societies to connect and unite around common issues. We are dedicated to fostering multidisciplinary cooperation – in the ECCO environment, different oncology disciplines get to meet, learn about each other's areas of expertise and embark on joint projects. Our congress (ECCO2017, 27-30 January 2017, Amsterdam) and its multidisciplinary programme are a perfect embodiment of this philosophy. In addition to each discipline conducting research in its own field, through ECCO, they work closer together and patient outcomes are improved through the close partnership of medical oncologists, surgeons, radiation oncologists, nuclear medicine experts and many others.

Tell us a bit about 'Oncopolicy', and how ECCO helps to ensure that cancer research remains at the top of the EU agenda.

ECCO works with its member societies to identify key areas in oncology that shape and have the potential to undermine the treatment landscape for cancer patients in Europe. We refer to them as time bombs in oncology. They are debated at the ECCO congress in a multi-stakeholder environment and we come out of these discussions with ideas of concrete projects that address these time bombs. We have recently embarked on a project focusing on oncology nursing together with our member the European Society of Nursing Oncology (EONS). This project is a key element of our focus on the oncology workforce in Europe and its sustainable growth and quality. We are also working on a project on essential requirements for quality cancer care (ERQCC) dedicated to two tumour types in 2016: colorectal cancer and bone and soft tissue sarcomas. The outcomes of these projects will be presented at the ECCO congress and the delegates will have an opportunity to participate in their final stage of development. Our congress ECCO2017, which gathers together oncology professionals, patient advocates and policy makers also reviews the most important innovations in cancer research during the year and determines in its multi-stakeholder context which of these discoveries are of greatest consequence to clinical practice.

Describe one or two big challenges that currently face Europe's cancer research agenda?

Over the past few years, we have seen impressive advances in the treatment of certain tumour types, but far from all. We need to focus on those where progress is still elusive and, in order to change this, we need to build on the breakthroughs in immunotherapy and precision medicine.

We also need to systematically evaluate what works to ensure that we invest in the best solutions for patients and that we can afford continued innovation.

What motivates ECCO's approach to education and training?

ECCO places the patient at the heart of all its activities. We work with our ECCO Patient Advisory Committee to ensure that all our projects and the ECCO congress have the patient perspective built in. In this way, we bring together our diverse community of oncology professionals and the advocates representing cancer patient communities. We also aim to work more and more on addressing the issues faced by the growing population of cancer survivors. We believe in the importance of advanced multidisciplinary education of oncology professionals for the benefit of patients and this is the motivation behind the world class educational programme provided by our congress ECCO2017.

Finally, what does the future hold for cancer research in Europe, and what is ECCO's role in that future?

We believe that the future lies in a strong multidisciplinary approach where we all work together to identify the best treatment regimens for each patient. Oncology disciplines will be working closer and closer together conducting joint research projects resulting in further advances for patients. ECCO will be there to foster such cooperation, provide a multidisciplinary review of innovations and facilitate their implementation in clinical practice through the ECCO congress and through our EU policy work.

'We believe that the future lies in a strong multidisciplinary approach where we all work together to identify the best treatment regimens for each patient'

ecco European CanCer Organisation

ECCO2017 EUROPEAN CANCER CONGRESS 27–30 January Amsterdam, The Netherlands

www.ecco-org.eu
www.eccocongress.org

TRANSFORMING HEALTH PROMOTION THROUGH NARRATIVE

Dr Sheila Murphy and her team including **Dr Lourdes Baezconde-Garbanati, Dr Meghan Moran and Dr Lauren Frank** are utilising the potency of narratives in order to change knowledge, attitudes and behaviour and reduce health disparities both in the United States and internationally.

Harnessing the power of storytelling

For thousands of years, human beings have used stories to pass on essential information from generation to generation. In spite of this, Western health professionals often ignore this oral story-telling tradition when giving vital health information to their patients, opting instead to rely on lists of symptoms, facts and figures. Stemming from a prestigious five year grant from the National Institutes of Health, Transforming Cancer Knowledge, Attitudes and Behavior through Narrative, Drs Murphy and Baezconde-Garbanati and their colleagues are challenging this convention by examining the effectiveness of stories or narratives as a way to learn and retain important health information. In a number of studies, the team has empirically tested if narrative forms of health communication produce greater effects on knowledge, attitudes and behaviours than more traditional non-narrative sources of information such as clinic brochures and Public Service Announcements (PSAs).

The eclectic mix of medical researchers, scriptwriters, artists, physicians, psychologists, anthropologists, communication scholars and public health professionals involved in this work understand that one size does not fit all when it comes to health education. Although their research on narrative persuasion has implications for virtually all communication, their recent research has focused on the issue of cervical cancer. According to the Center for Disease Control and Prevention, in the United States Latina and African American women, for example, have far lower uptake of cervical screening and significantly higher levels of cervical cancer than their white counterparts. African American women are also twice as likely to die from cervical cancer as white women. Moreover, prior research has shown that individuals from disadvantaged groups are less likely to benefit from traditional public health interventions that aim to improve knowledge and attitudes and promote prevention through facts and figures. These persistent health disparities underscore an urgent need for culturally sensitive, accessible health information among underserved populations. Could narratives that immerse their audience in a story that includes vital health information and features positive role models with whom these women can identify be part of the solution?

Tamale Lesson

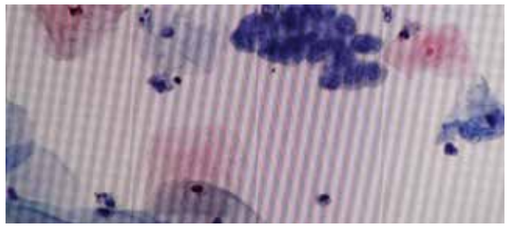
As part of a National Institutes of Health grant, an 11-minute narrative film, Tamale Lesson, was produced which aimed to provide information on the human papillomavirus (HPV), the virus that causes cervical cancer, as well as cervical cancer prevention (via vaccination) and detection (via Pap test screening and new genetic tests). The film focused on Lupita, a young Mexican American woman who has recently been diagnosed with HPV. While preparing tamales for their youngest sister's 15th birthday, Lupita reveals to her sister Connie that she has had an abnormal Pap test and has tested positive for HPV. Lupita then goes on to inform her (and later her mother and mother's friend, Petra) about HPV and its relation to cervical cancer, the importance of Pap tests in early detection and the availability of the HPV vaccine for their younger sister. Lupita also describes and demonstrates the process of a Pap test on the chicken being prepared in order to alleviate the fears of Petra, who has never had a Pap test. The film concludes with Petra going for her first screening.

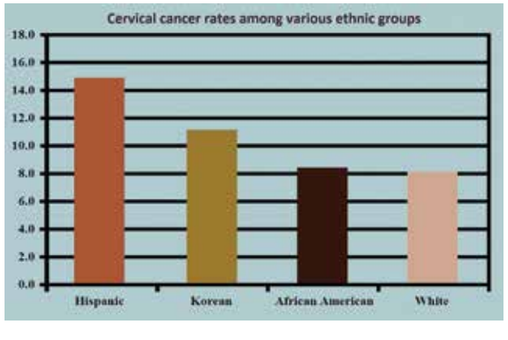
‘This research could radically change how health messages are conveyed across different ethnic groups, generations and modalities’

It’s Time

A second film, It’s Time, was also developed as a non-fictional non-narrative alternative to Tamale Lesson. This film took a more traditional approach and involved doctors and health experts discussing the same key facts surrounding HPV and cervical cancer. It also includes a demonstration of a Pap test on a patient and images of normal and abnormal cervical cells under a microscope.

The film was interspersed with clips of women discussing the barriers to screening and ways in which these obstacles can be tackled. Both pieces targeted and featured primarily Latina women in order to promote their involvement and identification with the film.





To make the comparison between the narrative and non-narrative films fair, both films:

→ Contained the same facts regarding cervical cancer detection, screening and prevention
→ Were the same length (11 minutes)
→ Were created by the same production team from the University of Southern California’s School of Cinematic Arts lead by professors Doe Mayer and Jeremy Kagan resulting in comparable high quality

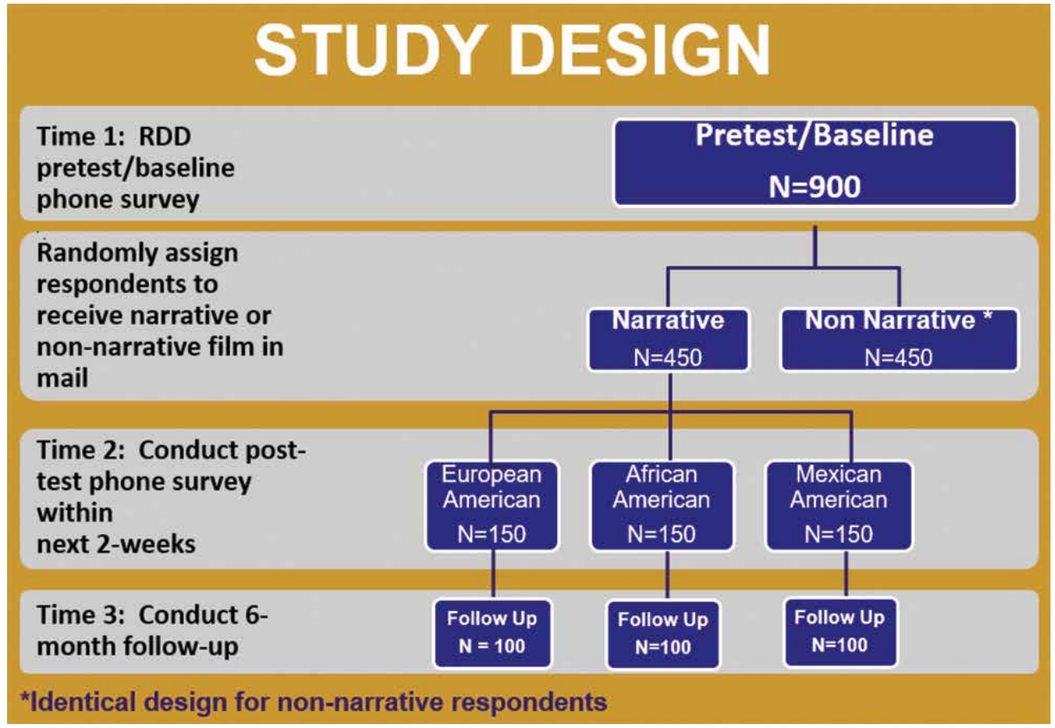
The team’s methods and successful results

A sample of 900 European American, African American and Mexican American women living in Los Angeles were recruited through a Random Digit Dial phone survey (RDD) and we surveyed to assess their baseline level of cervical cancer-related knowledge, attitudes and behaviour. They were then assigned at random to receive either the narrative film on DVD or the non-narrative film in the mail. After viewing the film, women were surveyed again two weeks and six months later.

Both films improved knowledge around cervical cancer, HPV and screening. However, women who viewed Tamale Lesson were able to recall significantly more facts than those who viewed It’s Time. The narrative film also resulted in more supportive attitudes towards Pap tests and strengthened the intentions of Latina and African American women to undergo screening. The narrative film proved particularly useful for those with lower levels of education as it was able to produce positive changes in the women’s perceptions of relevant social norms (namely what percentage of ‘women like them’ had Pap tests) which, in turn, increased their own intention to be screened.

The team’s latest paper, published in the flagship journal of the American Public Health Association, The American Journal of Public Health (Murphy S.T., Frank L.B., Chatterjee J.S., Moran M.B., Zhao N., Amezola de Herrera P. & Baezconde-Garbanati L., American Journal of Public Health, 2015, DOI: 10.2105/AJPH.2014.302332) conclusively demonstrates that narratives can be incredibly effective in reducing inequalities in health. While European American women had more knowledge about cervical cancer and were more compliant with preventative screening than Mexican American women before viewing the films, this disparity had disappeared at the 6 month follow up among those who saw the narrative, Tamale Lesson.

‘Although this research focuses on cervical cancer, the results have clear implications for virtually all health care communication’



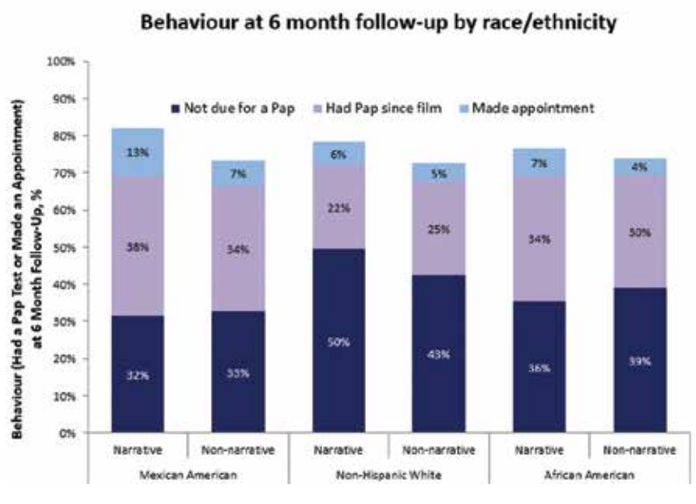
The narrative film also increased the likelihood of participants receiving or scheduling a Pap test. In fact, the Latina women who viewed the narrative film went from having the lowest level of compliance (32%) with screening guidelines to the highest – a whopping 82% – six months after the narrative intervention.

The cultural relevance is clear. While the non-narrative film was effective in motivating women – particularly white women – to be screened for cervical cancer, the narrative film was more motivational among women of colour. Measures of the extent to which they identify with the four main female characters revealed that the European American women were far less likely to identify with the characters in Tamale Lesson. As one participant put it: ‘They’re showing a very Hispanic family and the little girl’s Quinceañera. Anyone can relate to a warm family. That was well done but, quote/unquote it’s… “Hispanic”. You’re not going to be showing it to a bunch of white women in Beverly Hills’. As shown in other research, educational interventions that are culturally sensitive to their audience will garner greater attention and retention. As Dr Murphy explains: ‘Although this research focused on cervical cancer, the results have clear implications for virtually all health care communication’.

Change through identification, transportation and emotion

So why does narrative work? There are at least two key psychological processes – identification with characters and being transported or absorbed into a story.

The first, identification, has roots in social cognitive theory. Social cognitive theory (SCT) posits that one of the ways people learn is through observing others and modelling their own behaviour from these observations. Studies have shown that people are more likely to adopt a behaviour that they have seen others perform, especially if they perceive these individuals as being similar to themselves. Therefore, identification may be positively related to changes in cognition, attitudes, intentions and behaviours.



The team from left to right: Nan Zhao, Lauren Frank, Sheila Murphy, Lourdes Baezconde-Garbanati, Paula Amezola, Joyee Chatterjee and Meghan Moran.

This is particularly relevant to health campaigns that promote behaviour change based upon positive social norms. Often campaigns that attempt to change the public's perception of how frequent or normative a particular behaviour is fail because they lack the contextual detail that makes material applicable and believable to its audience. In narrative persuasion, audiences identify with the characters in the story which, in turn, makes the message conveyed more relevant to their life. In a 2013 study comparing Tamale Lesson with its non-narrative equivalent, Mexican American and African American women identified more strongly with the character of Lupita than their European American counterparts, and this identification with Lupita was significantly associated with more positive attitudes towards Pap tests.

A 2015 paper also shows that identification correlated with the women's perceptions of susceptibility and severity of HPV infection and the effectiveness of the HPV vaccine. For example, women who strongly identified with Lupita, the character with HPV, tended to feel they themselves were at higher risk for HPV, that the vaccine was effective in preventing HPV, and that HPV infection was a relatively manageable condition. In contrast, identification with Connie, her younger sister, deemed HPV infection to be relatively severe. Therefore, identification with characters can be complex and nuanced and narratives may need to include several different positive role models of different ages, ethnicities, and health histories, so that different individuals can find a character they identify with in order to achieve the intended outcomes.

Transportation into the story or narrative is likewise key. Transportation describes a state in which the audience becomes highly engaged or absorbed in a storyline. In a 2011 study looking at a cancer storyline on Desperate Housewives, Dr Murphy and her team found that transportation was the factor most strongly associated with changes in knowledge, attitudes and behaviours surrounding cancer prevention. A subsequent 2015 study likewise revealed that women who found the narrative to be relevant to their lives and were transported into the storyline were more likely to think that HPV infection was serious and that the vaccine was effective. It appears that stories may be particularly well-suited to persuade as audience members tend to suspend disbelief and not counterargue while processing narratives.

Looking to the future

The film Tamale Lesson won the 23rd Annual American Public Health Association's Public Health Education and Health Promotion Award as well as The Top Translational Research Award in Health Communication. Moreover, the research described above recently won the National Institutes of Health Common Fund 10-Year Commemoration Award. Finally, it was largely for this recent work that Sheila Murphy received the 2015 Everett M. Rogers Award given to 'an individual who has made an outstanding contribution to advancing the study and/or practice of public health communication' by the American Public Health Association.

Dr Murphy and her team have begun to expand their research internationally. A Spanish language version of Tamale Lesson has been produced and disseminated not only throughout California but across several countries in the Caribbean and Latin America, including Colombia, Mexico, Panama, Costa Rica and Argentina. Research on the attitudes, knowledge and beliefs surrounding HPV and cervical cancer has also been undertaken in Panama by Dr Lisa Gantz, under the supervision of Drs Murphy and Baezconde-Garbanati. By teaming up with the National Association against Cancer, the National Ministry of Health and the Panamian Association for Family planning, researchers in the University of Southern California and the University of South Florida were able to develop a multimedia toolkit to improve cancer knowledge and attitudes of the Panamian community.

Dr Murphy is also leading another NCI-funded project with Drs Moran, Frank, Baezconde-Garbanati and Dr Sandra Ball Rokeach, that examines barriers to cervical cancer prevention in Hispanic women. Using focus groups and surveys, the team explore the factors that inhibit or facilitate naturally occurring cancer related storytelling amongst women's social networks. In this way, they can determine the individual, interpersonal and community level factors that influence health behaviours and design interventions accordingly.

Meet the researchers

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Dr Sheila Murphy is a Full Professor at the Annenberg School for Communication and Journalism at the University of Southern California. Her work primarily focuses on health-related decisions and on the role of narrative or storytelling in shaping the public's knowledge, attitudes and practices. Understanding ethnic and cultural diversity is a second major theme that characterizes her work. Dr Murphy uses an unusually wide variety of methodological tools including experiments, large-scale surveys, focus groups, content analysis, social network analysis, multilevel analysis and field observation in order to paint a more complete picture of a particular problem. Dr Murphy has received the American Public Health Association's Public Health Education Award, The Top Translational Research Award in Health Communication and the National Institutes of Health Common Fund Award. For her work on narrative Dr Murphy recently received the 2015 Everett M. Rogers Award given to 'an individual who has made an outstanding contribution to advancing the study and/or practice of public health communication' by the American Public Health Association. Dr Murphy also works internationally with GirlEffect increasing the value of girls in various African countries, and with the BBC integrating health-related information into entertainment programming in developing countries.

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Dr Lourdes Baezconde-Garbanati, PhD, MPH, is a tenured Full Professor in the Department of Preventive Medicine at the Keck School of Medicine of the University of Southern California. She is also the founding Director of the Center for Health Equity in the Americas and Co-director of the Global Health Tract in the MPH program. She is a member of the directorate of the Institute for Health Promotion & Disease Prevention Research at USC and the USC/Norris Comprehensive Cancer Center, with oversight of the Patient Education and Community Outreach Center. Her work focuses on community-based research and public health initiatives that explore the role of culture in health behaviours, with an emphasis on the elimination of health disparities. Her research focuses on providing an evidence base and culturally grounded strategies for best practices to modify cultural and lifestyle risk factors for cancer and tobacco control at the community level. This includes the award winning Tamale Lesson, a video to increase cervical cancer screening among Hispanic women, and Es Tiempo, an outdoor media and clinic intervention to reduce cervical cancer disparities among Hispanics. She has received multiple awards and recognition for her work, and is well published in a variety of topics. She has a strong record of extramural funding from NIH and the California Department of Public Health, and has published widely in a variety of peer reviewed journals and community based outlets.

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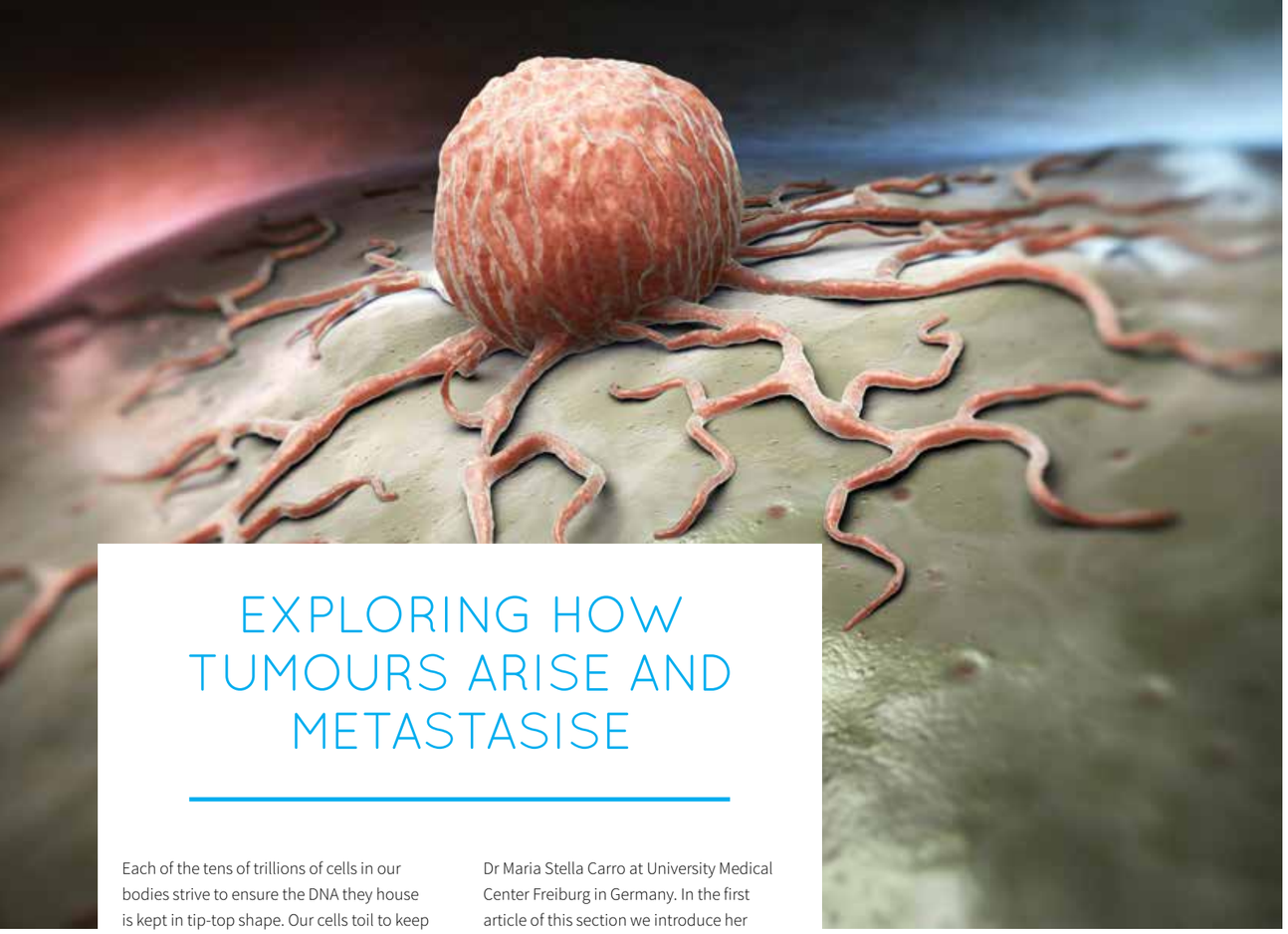
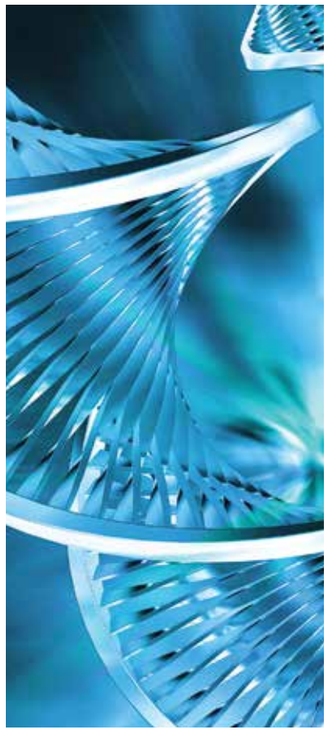
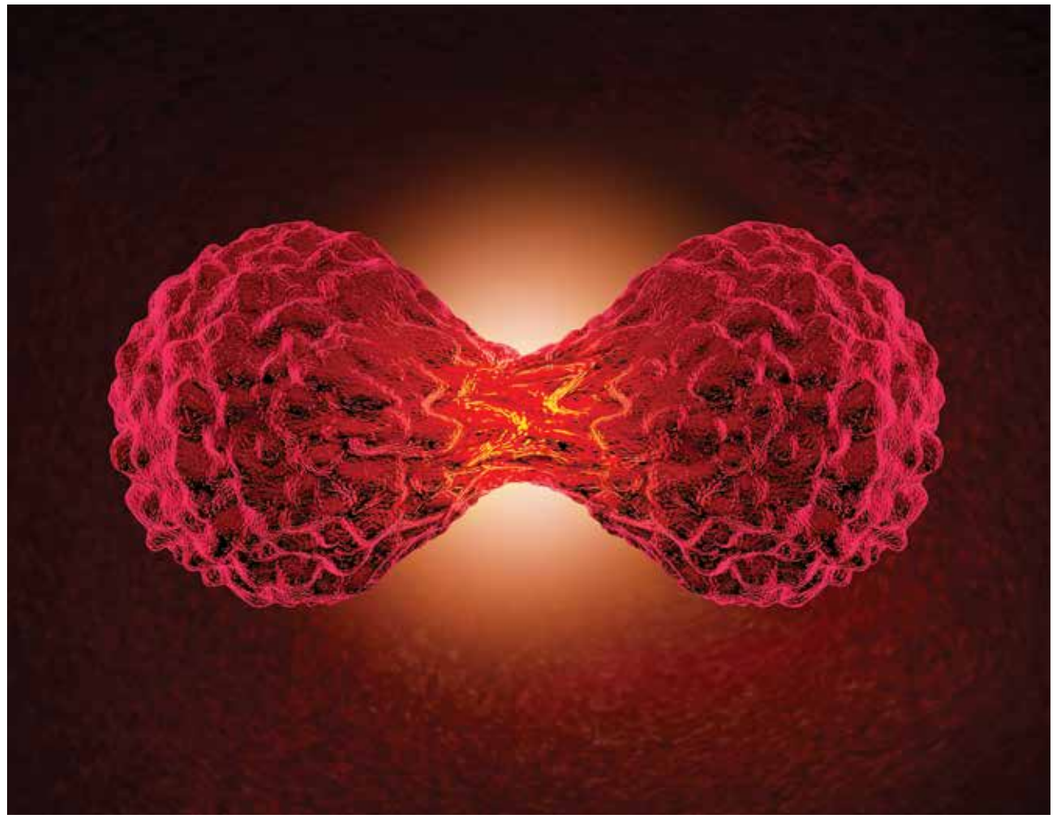


EXPLORING HOW TUMOURS ARISE AND METASTASISE

Each of the tens of trillions of cells in our bodies strive to ensure the DNA they house is kept in tip-top shape. Our cells toil to keep this double-helical structure error-free by making sure it's repaired when damaged and replicated perfectly during mitosis. However, due to the sheer number of cells in our bodies, mistakes inevitably arise that cannot be adequately repaired by our cells. This is not cause for huge concern, as tumour suppressor genes code for proteins that come to our aid and instruct the damaged cell to commit suicide – in a process known as apoptosis. This removes the threat to the rest of the body and we can carry on as normal. But what happens when DNA damage occurs in the tumour suppressor genes themselves? This can cause their deactivation, so that the complex sequence of events that leads to tumour development can begin – in a process known as carcinogenesis.

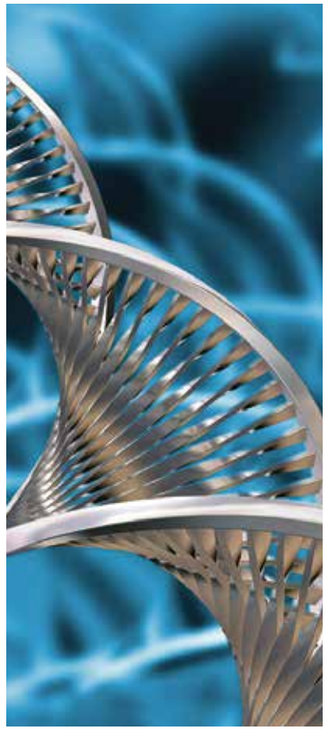
Although researchers have made huge strides over recent decades towards a greater understanding of how cancer develops and propagates, there is much more work to be done. Further unravelling these complex cascades will unveil new targets that we can exploit in future cancer treatments. Studying the biochemical mechanisms that cause healthy brain cells to become cancerous is Dr Maria Stella Carro at University Medical Center Freiburg in Germany. In the first article of this section we introduce her investigations into the complex network of cell proteins that regulate healthy brain cells. In one of her team's projects, they explored the role of a transcriptional repressor protein (a protein that prevents expression of a gene) called ZBTB18. They found that ZBTB18 was very common in healthy brain tissue, but completely absent in a form of brain cancer known as glioblastoma – hinting at its tumour-suppressing activity. Remarkably, when they inserted the gene that codes for this protein into tumour-forming cells, they were much less capable of forming new tumours, meaning that this gene may work as an effective treatment to inhibit tumour growth. Next we showcase the work of Drs Laura Gillespie and Gary Paterno at Memorial University in Canada, who investigate the molecular pathways that lead to the development of breast cancer cells. Their research has led them to focus on MIER1α – a regulatory molecule – and how it behaves in both healthy and cancerous breast tissue. The team discovered that this compound – similar to Dr Carro's ZBTB18 – may function as a tumour suppressor, since it appears to be absent from the nucleus of cells in progressive cancer types.

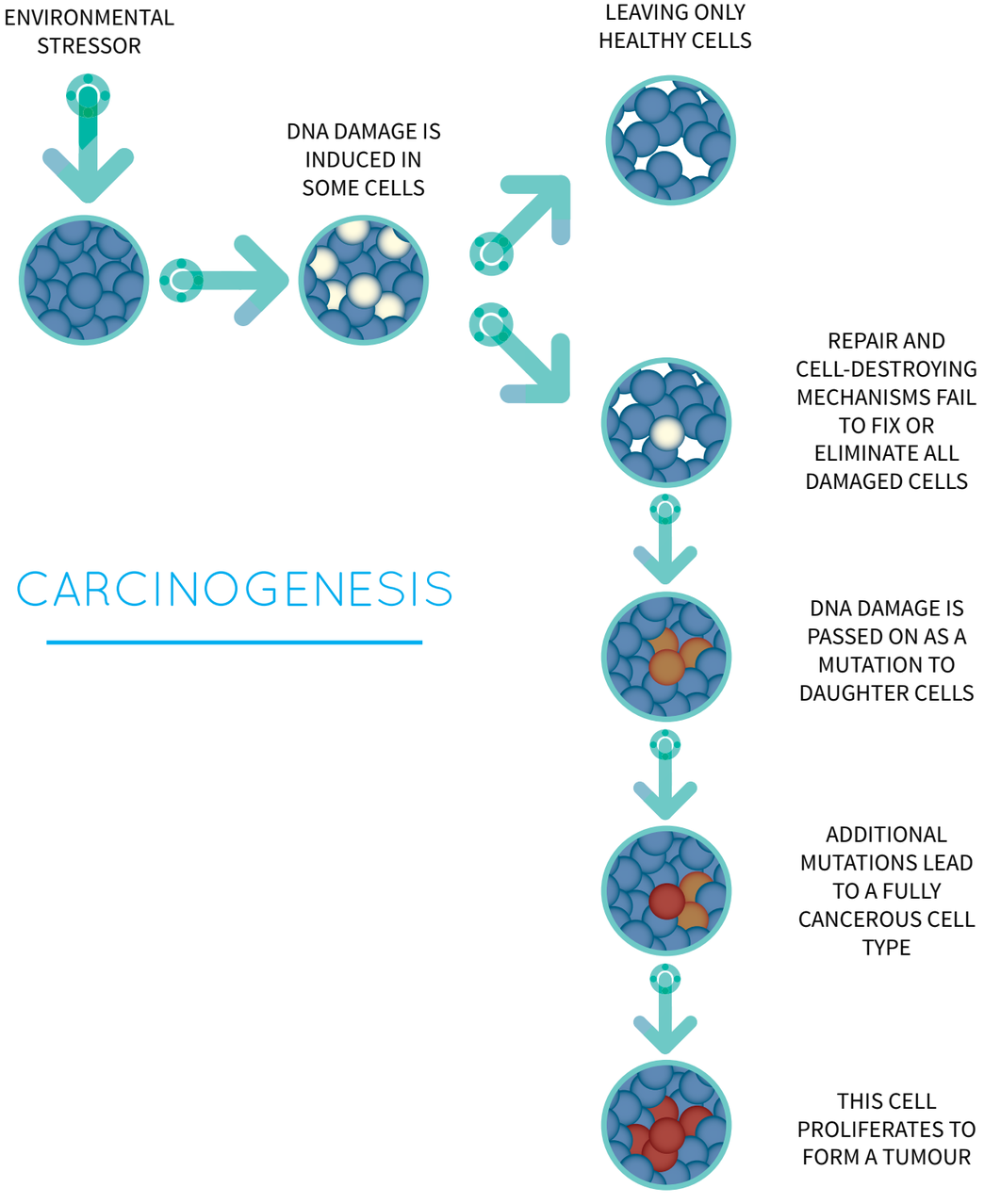




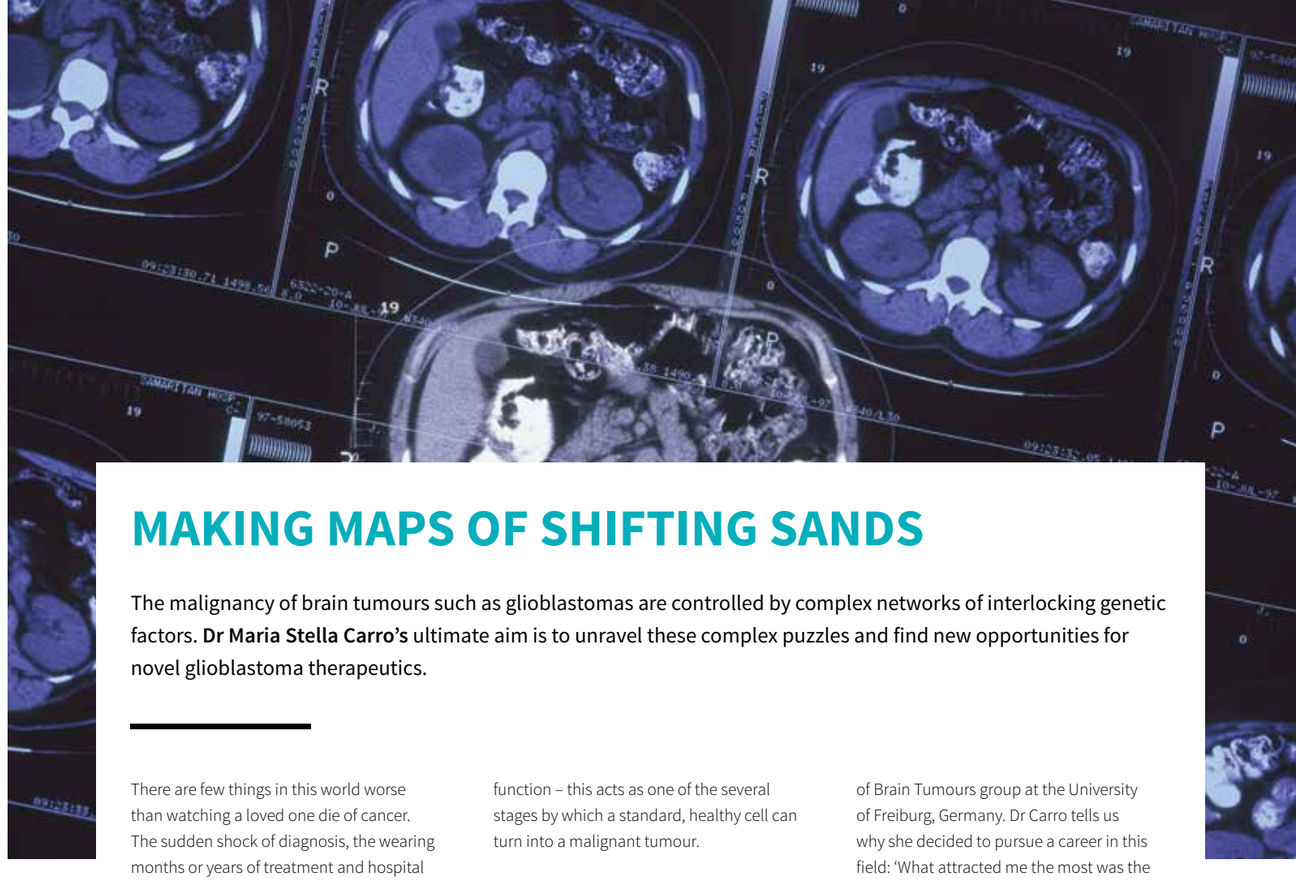
From here we move on to examine how prostate cancer develops. In our third article of this section, we introduce Dr Donald Vander Griend and his research group at the University of Chicago, who explore the gene pathways that cause this all-too-common cancer to arise. In one study, his team investigated one particular pathway that controls gene expression, involving the homeobox (Hox) gene family of transcription factors. This work led to the identification of a new network of genes and cofactors that is unique to prostate epithelial cells. The team found that levels of these cofactors declined as cancer progressed, while low levels also predicted poorer prognosis for patients. Therefore, if levels of these co-factors could be restored, this may be a promising approach to treating prostate cancer.

So once cancer arises, how can we stop it from spreading to other parts of the body? This is where Professor Jonathan Kurie steps in. Along with his colleagues at the University of Texas MD Anderson Cancer Center, Professor Kurie studies how lung cancer metastasises, in order to identify novel therapeutic targets. In one investigation, the team discovered that ZEB1 – a molecule present in lung cancer cells – reduces the expression of a particular microRNA called miR-34a. Because miR-34a decreases tumour cell invasion and metastasis, Professor Kurie's findings support the development of miR-34a as a therapeutic agent in lung cancer patients. Our final article in this section also investigates the molecular mechanisms behind metastasis. Here, we showcase the work of Dr Lei Zheng at the department of oncology at the Johns Hopkins University, who explores how the gene AnxA2 promotes the spread of pancreatic cancer cells. By blocking the action of this gene using antibodies, Dr Zheng and his team were able to prevent pancreatic cancer from metastasising to the liver in a mouse model. Now, the team are planning clinical trials to test a recently developed vaccine to target AnxA2, while at the same time also developing a therapeutic antibody that targets AnxA2. This novel immunotherapy approach leads us nicely on to the next section in the magazine, where we look at ways to harness the immune system to fight cancer.





CARCINOGENESIS



MAKING MAPS OF SHIFTING SANDS

The malignancy of brain tumours such as glioblastomas are controlled by complex networks of interlocking genetic factors. **Dr Maria Stella Carro's** ultimate aim is to unravel these complex puzzles and find new opportunities for novel glioblastoma therapeutics.

There are few things in this world worse than watching a loved one die of cancer. The sudden shock of diagnosis, the wearing months or years of treatment and hospital visits, the moment of realisation when all other options are gone. Brain cancers such as glioblastoma are more terrible still, when patients and their families need to decide between almost certain death and the horrific potential side-effects which come when surgeons slice both tumour and healthy brain tissue away in a last-ditch attempt to save lives. Is it any wonder that we want to understand these diseases, to find out what makes them tick and, in the end, how they can be destroyed?

Corrupted Blueprints

Our story starts in the world of DNA, the blueprint of life and the code for every single one of the proteins which keep our millions of cells alive. Vast amounts of energy are expended by each cell to ensure that DNA is copied exactly, repaired when broken, and kept in a pristine state. Yet despite all of this effort, mistakes slip through. Mutations occur, sections can be deleted and swapped. Normally this is not an issue, as a group of tumour suppressor proteins are able to detect when the damage is too severe and instruct the cell to kill itself, removing the threat to the body as a whole. Yet mutations can occur in these genes themselves, deactivating them or preventing their proper function – this acts as one of the several stages by which a standard, healthy cell can turn into a malignant tumour.

Yet though we say 'cancer' and 'tumour', there are vast differences within these words. The genes which are currently active in any set of cells can be the difference between an easily treatable worry and a highly aggressive disease with no hope of survival. The process by which genes are read and translated into proteins is controlled by transcription factors, protein switches which turn genes on and off according to their individual input signals. Transcriptional networks are made up of many different transcription factors, each of which can affect the overall expression of many targets, including other transcription factors within the network. Networks can be incredibly complex, with different factors altering expression patterns both up- and down-stream, perhaps better thought of less as a series of traffic lights and more like a downtown traffic grid. Identifying which parts of this transcriptional network are active in any one tumour cell can help doctors to target their therapies more precisely, vastly helping patient survival.

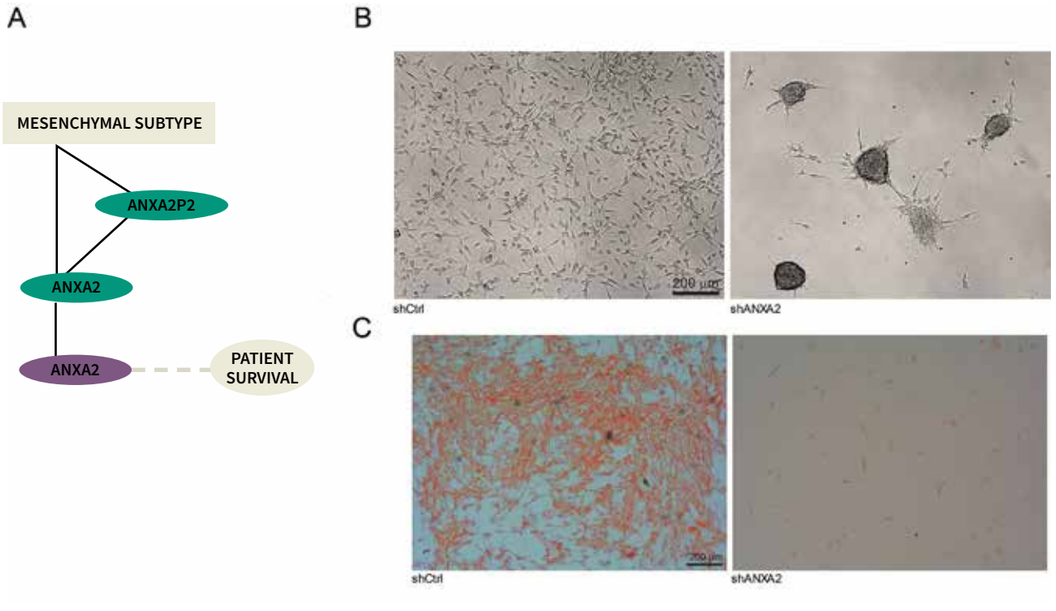
Navigating Gridlock

This complex field has attracted many bright scientists, and one of the well-known researchers active in the area is Dr Maria Stella Carro, Head of the Genetics of Brain Tumours group at the University of Freiburg, Germany. Dr Carro tells us why she decided to pursue a career in this field: 'What attracted me the most was the possibility explore the connection between this pathology and the nervous system,' she explains. 'Of course, I was also motivated by the urgent need to make progress in the understanding and treatment of this terrible disease.' Her work focuses on the transcriptional networks which lie behind the malignancy of glioblastoma, with the goal of understanding both how these networks interact with each other and, eventually, how to treat them.

As we said before, 'tumour' covers a wide range of possibilities, and similarly glioblastomas are not just 'glioblastomas'. Following on from the realisation that transcriptional networks can affect tumour growth and malignancy, many people have tried to identify subclasses of glioblastoma based on their transcriptional fingerprints. One of these subclasses, known as mesenchymal glioblastoma, is often observed as having the most malignant features – this is associated with high numbers of mutations in tumour suppressor genes such as NF1, PTEN and TP53.

'Given the association of the mesenchymal subgroup with the most aggressive features of these tumours,' comments Dr Carro, 'I became interested in identifying and

‘I was motivated by the urgent need to make progress in the understanding and treatment of this terrible disease’



ANXA2 is connected to the mesenchymal subtype of glioblastoma and plays a role in patient survival (A). ANXA2 silencing affects tumour cells morphology (B) and mesenchymal differentiation (C).

studying molecular mechanisms driving this gene expression signature.’ This interest led to her postdoctoral work at Columbia University in the Institute of Cancer Genetics, where she identified and explored a transcriptional network which affected glioblastoma growth and survival. As you can imagine, this was difficult research into highly complex, tangled network.

Difficult, yes, but successfully navigating this complexity led to one of Dr Carro’s first high-profile publications. ‘I identified a group of transcription factors potentially involved in the regulation of a gene signature (mesenchymal signature) associated with glioblastoma,’ she recalls, ‘it revealed the crucial role of the transcription factors Stat3 and C/EBPß and represented the first comprehensive mapping of transcription factors controlling the mesenchymal signature.’ By creating a scientific map, of sorts, the researchers laid the groundwork for further drug discovery and proved to the world that it was possible to find ‘master regulators’ for other types of cancer and disease. These master regulators can be thought of as the central node of the complex network, having a strong effect on all of the following functions – target these central nodes and you can have wide-reaching effects with comparatively little effort.

Ground-breaking work indeed, published in Nature in 2010 with Dr Carro as the first author (the place traditionally reserved for the scientist who performed the lion’s share of the work). Nature, of course, is a goal for almost every scientist in the biology or health fields, accepting only ground-breaking scientific discoveries. This is easily seen in the reception given to Dr Carro’s paper, having been cited in almost 300 other scientific works and having been selected as one of the top 50 cancer biology studies.

Zibbity Zabbity Doo

During this work, the collaborative study also indicated a possible role for a transcriptional repressor protein known by the incredibly catchy name of ZBTB18. Transcriptional repressors act to prevent the expression of genes, effectively acting as an off-switch in the network. ZBTB18 was found to be very common in healthy brain tissue, completely absent in glioblastomas, and so could potentially be acting as a tumour suppressor – blocking the development of pre-tumour cells. ‘Given this function and the connection to the mesenchymal signature,’ commented Dr Carro, ‘I was interested in studying the role of ZBTB18 and its mechanism of regulation in glioblastoma in greater detail.’

Through a lot of further work Dr Carro and her group were able to identify several factors which could support the idea of ZBTB18 as a tumour suppressor. The existence of the protein was shown to block the expression of mesenchymal genes, cells lacking the tumour suppressor quickly switched into the highly invasive mesenchymal phenotype. Similarly, when the gene for the protein was put back into tumour-forming cells they were much less capable of forming new tumours. This work (currently in preparation for publication by Dr Carro’s group) thus points the way for new inhibitors of cancer development.

One protein does not make a cure, however, and Dr Carro is not one to rest on her laurels. Having already identified one potential candidate, her next goal is to, in her words, ‘identify and characterise ZBTB18 interacting proteins to get more insight into its repressive mechanism. ZBTB18 occupies a nodal position in the overall transcription network, which means that there are many potential interacting proteins to be studied. As she commented, ‘Given its putative role of tumour suppressor, I believe that understanding the mechanism and regulation of ZBTB18 could allow for the identification of new molecular targets. These could potentially re-activate the ZBTB18 tumour suppressor function in glioblastoma cells and hinder their malignant properties.’ In other words, ZBTB18 is a promising target for the development of therapeutics which would turn it back ‘on’ in glioblastoma cells, thus slowing down the spread of the tumour.

ANXA2

Dr Carro’s team have also investigated the involvement of other genes (not transcription factors) in the pathogenesis of glioblastoma. A recent finding is that the Annexin 2 (ANXA2, a calcium dependent phospholipid binding protein which is aberrantly expressed in various human cancers) is involved in the invasive mesenchymal subtype of glioblastoma. In collaboration with Dr Nelander’s group at Uppsala University, the team used a network analysis to determine separate transcriptional, epigenetic and genomic regulators of various subtypes of glioblastoma. Epigenetic modulation of ANXA2 was found to drive the mesenchymal subtype of the cancer. Excitingly, this discovery could provide a promising new drug target for the treatment of glioblastoma. Since ANXA2 is mainly located at the cell membrane compartment, it would be a very convenient target because, differently from transcription factors, it would be more easily accessible and provide a new and effective way to reduce the mortality and morbidity of glioblastoma patients. In fact, there are quite a few chemical compounds able to halt ANXA2 function, some of them already under investigation for a potential use in cancer therapy; most of these compounds derive from chlorotoxin, a peptide originally isolated from scorpion venom.

Networks of Success

With so many projects on the go at once, it is difficult to see how Dr Carro manages to find any free time at all. Her secret lies in her willingness to form joint ventures between otherwise disparate fields of research. ‘I believe that a requisite to the success of my research has been the ability to collaborate with system biology researchers, efficiently complementing their expertise in order to provide a deeper insight to the biological questions we raised.’ she explains. The greatest advantage of such cross-cultural collaboration is the ability to learn skills which are outside the normal career path. By working with a number of experts in different fields, Dr Carro was able ‘to expand my expertise and collaborations according to the project needs’ – in effect pushing her to broaden her horizons.

‘Since my first work as a postdoc, the idea has been to make sense of the recent molecular characterisation of glioblastoma and to identify master regulators of specific subclasses that can be targeted to efficiently downregulate a group of genes associated with the disease in order to offer alternative therapies and ultimately, improve patients’ survival.’

Horizons can be a very long way away, so we asked where she will eventually end up, what her ultimate goals would be. Her research, she said, has ‘the ultimate goal of unravelling new opportunities to develop novel therapeutic avenues for glioblastoma.’ Will her work lead to a treatment for glioblastoma? Many patients and their loved ones are hoping that the answer to this question, one day, will be yes.

Meet the researcher

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Born in Italy, fluent in four languages, Dr Maria Stella Carro has worked her way around the world – having been a PhD in Milan, a postdoc in New York, and finally reaching her current position as a group leader at the renowned Universitätsklinikum Freiburg, Germany. With her name on many publications, (including two published in Nature), a group of talented researchers under her investigating the genetics of brain tumours and a gift for collaborations, Dr Carro is as motivated as ever about the future of her research.

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*Co-corresponding authors.

INVESTIGATING BREAST CANCER AT THE MOLECULAR LEVEL

Cancer researchers **Dr Laura Gillespie and Dr Gary Paterno**, along with their colleagues at the Terry Fox Cancer Research Laboratories at Memorial University in St. John's, Newfoundland, dive down obscure molecular rabbit holes in pursuit of a deeper understanding of breast cancer.

Chasing a common killer of women

Cancer, the group of diseases characterised by abnormal cell growth and possible spread to other parts of the body, is one of the most frightening of human diseases. Often perceived as a death sentence, cancer causes almost 15% of all human deaths. And breast cancer is one of the most common and notorious cancers, particularly in women.

In Canada, 26% of cancers diagnosed in women – over 1 in 4 – are breast cancer. In 2015, breast cancer was the most common cancer diagnosis in Canadian women, and deaths from breast cancer were second only to deaths from lung cancer. In the UK, breast cancer is the most common type of cancer, with almost 50,000 cases of invasive breast cancer diagnosed there in 2011 alone. While 80% of those cancers are in women over 50 years of age, more and more are being found in younger women and occasionally even in men. In the United States, breast cancer is the second most common cancer in women after skin cancer. Although very rare in men, each year there are about 2,300 new cases of male breast cancer. However, there are 100-fold more than that – about 230,000 new cases – in women.

No matter where you go, breast cancer is a major women's health issue. It is logical, then, that scientists concentrate efforts to develop newer and more effective ways to diagnose, prevent and treat breast cancer. Today, this often leads to complex investigations deep into the molecular realm of the cell. After all, we think of cancer as a growth of cells that have somehow lost the usual cellular controls that normal cells have, such as the mechanisms that regulate growth, reproduction and specialisation. When cells somehow lose the molecular controls that limit growth or keep cellular functions in order, the cell becomes a cancer cell and may multiply without limit, invading normal tissue or metastasising to distant parts of the body. It is these obscure cellular mechanisms that Dr Laura Gillespie and Dr Gary Paterno are attempting to unravel, to solve the mysteries of why breast cells turn cancerous and how to prevent that transformation from occurring or fight it when it happens.

MIER1α– a new face in the cell regulatory line-up

Initially, the team focussed their research on the molecular mechanisms of cell growth, and the differentiation of these cells into various adult tissues and oncogenesis – cancer formation. 'Our research has evolved over the years; as we make discoveries, we follow the results where they lead us,' Dr Gillespie tells Scientia, 'our initial research program was aimed at identifying genes that play a key role in developmental processes, knowing that often such genes become misregulated in cancer.' They initially investigated the role of various cellular growth factors and the receptors to which those factors attached, since it was known that aberrant functioning of these molecules can contribute to cancer development. One of the approaches they took was to isolate and characterise genes that were the initial targets for growth factor molecules in the nucleus: the 'immediate-early' genes. Immediate-early genes are most likely to function as 'master' regulatory genes that control cellular function. Understanding how such genes work would not only enable the researchers to determine their role in growth and differentiation, but could contribute to the understanding of cancer development. This work led the pair to a relatively new regulatory molecule called Mesoderm Induction Early Response 1 (MIER1).

MIER1 exists in multiple forms with presumably distinct functions. Of interest to the issue of breast cancer is one particular form, MIER1**α**, that specifically interacts with a cellular receptor called oestrogen receptor alpha (ER**α**) and modulates its activities. This is important in breast cancers that are sensitive to oestrogen via of the presence of oestrogen receptors, molecules to which the oestrogen attaches to cause its effect. In fact, regulated overexpression of MIER1**α** in breast carcinoma cells was found to inhibit oestrogen stimulated growth. So it appeared that MIER1**α** was a regulatory molecule that interacted with cancer cells to inhibit the effect of the oestrogen making them grow. It was something exciting to concentrate on, especially in the fight against breast cancer.

MIERα, MIER1α, where is the MIER1α?

The oestrogen receptor ERα plays a key role in tumour development in the breast, so inhibiting its activity remains a prime strategy in the treatment of ERα-positive breast cancers. Current therapies for breast cancer often contain anti-oestrogenic drugs. Thus, unravelling the molecular mechanisms responsible for regulating ERα activity may allow the design of new, more effective breast cancer drugs and therapies.

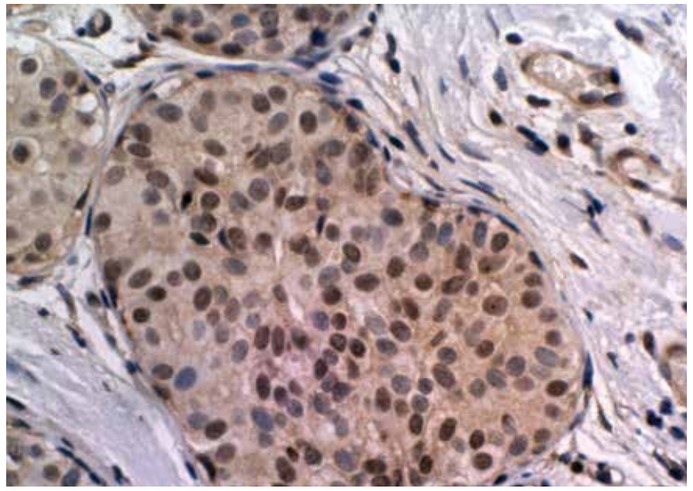
Drs Gillespie and Paterno investigated the ability of MIER1α to bind to ERα in laboratory breast carcinoma cell lines. In these cancer cell lines, they found that MIER1α interacted with ERα in the presence and absence of oestrogen, but the interaction was stronger in the absence of oestrogen. It seemed that increased levels of MIER1α in some breast carcinoma cells resulted in the inhibition of oestrogen-stimulated growth. This implied that MIER1α could play a role in regulating breast carcinoma cell proliferation in living tissue, i.e. cancer patients.

To explore this further, they looked at levels of MIER1α in normal breast tissue versus breast carcinoma, but they found no consistent difference in the MIER1α expression level between the two. However, there was a dramatic shift in the MIER1α localisation. 'This analysis revealed a shift in subcellular localisation during breast cancer progression that was quite striking and very interesting', says Dr Gillespie. Nuclear MIER1α was detectable in 75% of normal breast samples and in 77% of breast tissue hyperplasia (pre-cancer). But in breast carcinoma, only 51% of ductal carcinoma in situ (cancer that has not yet invasive), 25% of invasive lobular carcinoma and 4% of invasive ductal carcinoma contained nuclear MIER1α. This shift of MIER1α from a nuclear to a cytoplasmic location during breast cancer progression looked like the loss of MIER1α from the nucleus might be a contributing factor in the development of invasive breast carcinoma. They published these findings in the British Journal of Cancer in 2008.

That's the What—now for the How

So the team understood that MIER1α was a transcriptional regulator that interacts with ERα to inhibit oestrogen-stimulated growth. They also understood that MIER1α functioned when it was in the nucleus of the cell, but not when it was in the cytoplasm.

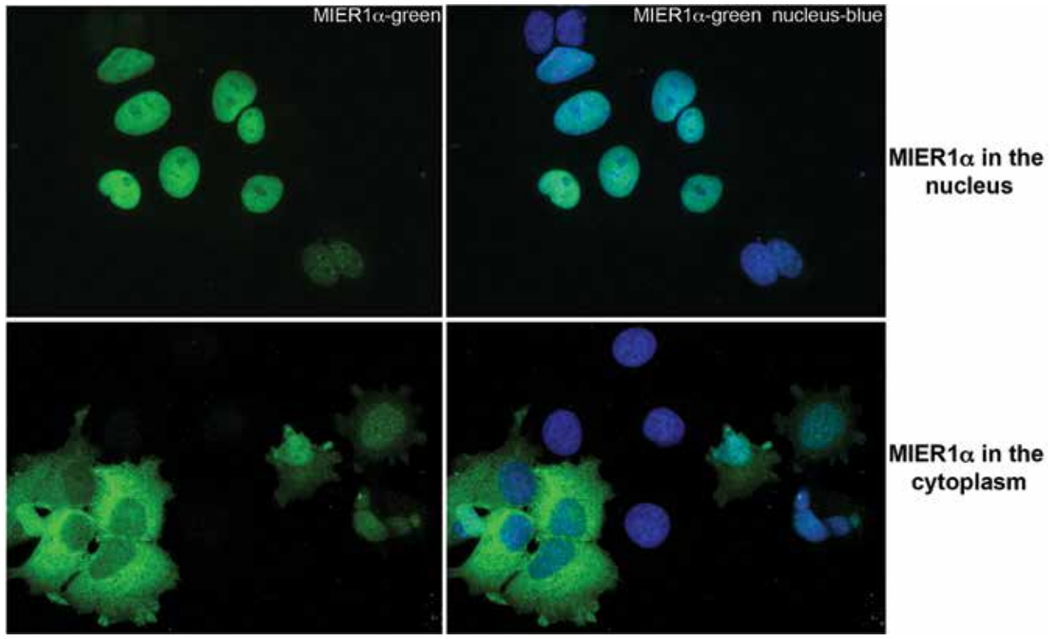
'Our work has the potential to produce a valuable marker for breast cancer screening and to reveal additional targets for the development of more effective breast cancer therapies'



The next question to be answered was how MIER1α's location in the cell is controlled. They first looked at how the molecule got into the nucleus. One obvious possibility was that MIER1α interacted with ERα to make this happen. However, they showed that nuclear targeting of MIER1α does not require ERα. After further study, they determined that MIER1α is actually transported into the nucleus by binding to other nuclear proteins, specifically HDAC1 and 2. The HDAC proteins are enzymes that play an important role in the regulation of gene expression. These interesting discoveries were published in the journal PLoS ONE in 2013.

More important to the development of cancer, however – if MIER1α got into the nucleus via HDAC, how did it not end up in the nucleus? After all, it was looking like the absence of MIER1α from the nucleus was associated with cancer progression. So Dr Gillespie, Dr Paterno and their students worked on this problem and found, in work only published last year, that treatment of breast carcinoma cells with either insulin or insulin-like growth factor 1 (IGF1) affected the subcellular localisation of MIER1α. Both factors reduced the percentage of cells with nuclear MIER1α from 81 and 89 to 41 and 56%, respectively. Conversely, treatment with oestrogen had no effect and MIER1α remained nuclear. Oestrogen did not affect the localisation of MIER1α, but insulin or IGF1 did.

Furthermore, Gillespie and Paterno have evidence that other molecular growth factors – specifically fibroblast growth factor and epidermal growth factor – have the same effect as insulin and IGF1. It appears that all peptide growth factors tested so far, but not steroid hormones like oestrogen, cause MIER1α to be kicked out of the nucleus. So insulin and peptide growth factors might facilitate the production of cancer by causing the nucleus to lose an important regulatory protein, MIER1α.



Where do they go from here?

Breast cancer is a heterogeneous disease of which 95% are carcinomas – the vast majority of these are invasive ductal carcinoma (IDC). The currently accepted hypothesis is that IDC results from progression through multiple stages, including atypical ductal hyperplasia (ADH), then DCIS, and culminating in the potentially lethal IDC. While this strictly linear model may be overly simplistic, there is a general consensus based on cellular and clinical information that DCIS is a non-obligate precursor lesion of invasive disease. This is supported by the results of several retrospective studies of the natural history of untreated cases of DCIS from the pre-mammographic era. And while outcomes for patients with pure DCIS are excellent (>98% survival rate), approximately 30% of patients who develop invasive breast carcinoma will die from their disease. So being able to predict which DCIS lesions will progress to IDC would be vital information for physicians to have. In spite of significant advancements in breast cancer research over the recent decades, the ability to predict progression to IDC for patients with DCIS remains limited. What is needed is a molecular marker or a system of molecular profiling that can be used alone or in combination with histopathologic features to accurately predict those patients who will progress to invasive carcinoma and those who will not. Defining such DCIS patient subsets would facilitate the selection of the most beneficial treatment for each subset. This would not only help prevent the development of IDC in at-risk patients, but it would also identify those who could be managed with less therapeutic intervention without sacrificing positive outcomes.

Several studies evaluating the potential of a number of cellular proteins as markers of progression have been published. While encouraging, all of the current markers lack accuracy and none of the studied proteins on their own serve as predictors of progression to invasive carcinoma. Dr Gillespie's hypothesis is that MIER1α localisation is such a predictor, or at the very least, it may be an important component of such a classification system. After all, their research thus far suggests that MIER1α may function as a tumour suppressor, since its absence from the nucleus correlates with more progressive cancer.

So now, the team are expanding their investigation of the relationship between MIER1α subcellular localisation and progression of DCIS to invasive carcinoma by undertaking a retrospective study to determine the subcellular localisation (i.e. nuclear vs. non-nuclear) of MIER1α in DCIS samples from patients and correlate this with the subsequent development of IDC.

'This work resulted in the discovery of MIER1 as a gene involved FGF-induced mesoderm differentiation'

What's the long-term plan?

If their results establish a positive correlation between non-nuclear MIER1α in DCIS samples and invasive recurrence, the next step would be to determine if the predictive accuracy can be increased by combining MIER1α status with other known biomarkers identified by other research teams. Ultimately, they would undertake a larger, multi-centre study to confirm their results. If validated as a predictive marker, then anti-MIER1α antibodies could ultimately be employed in pathology labs for testing patient DCIS samples to determine if the specimen indicated a higher or lower chance of progression to more invasive disease. This would be extremely helpful, since a significant proportion of patients diagnosed with DCIS will experience a recurrence of their disease, usually 8–10 years later. In about half of these patients, the recurrence will be a deadlier IDC. The identification of predictive markers, such as MIER1α, for DCIS most likely to progress would be of tremendous importance for the health and well-being of these patients, enabling them to opt for a much more aggressive treatment in order to prevent the future development of invasive disease.

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Dr Laura Gillespie received her B.Sc. and PhD in Biology from the University of Ottawa in Canada. Thereafter she did an MRC Post-Doctoral Fellowship in the Department of Biochemistry of McGill University and an MRC Centennial Fellowship with the Imperial Cancer Research Fund, Developmental Biology Unit, of the University of Oxford. In 1990, she joined Memorial University as an assistant professor and a founding member of the Terry Fox Cancer Research Laboratories and became a full professor in 1999. Since 1990 she has received numerous research grants, both as principal investigator and co-investigator, to study a variety of genes and their roles in the genesis of cancer. She is also an external reviewer for a number of international granting agencies and scientific journals.

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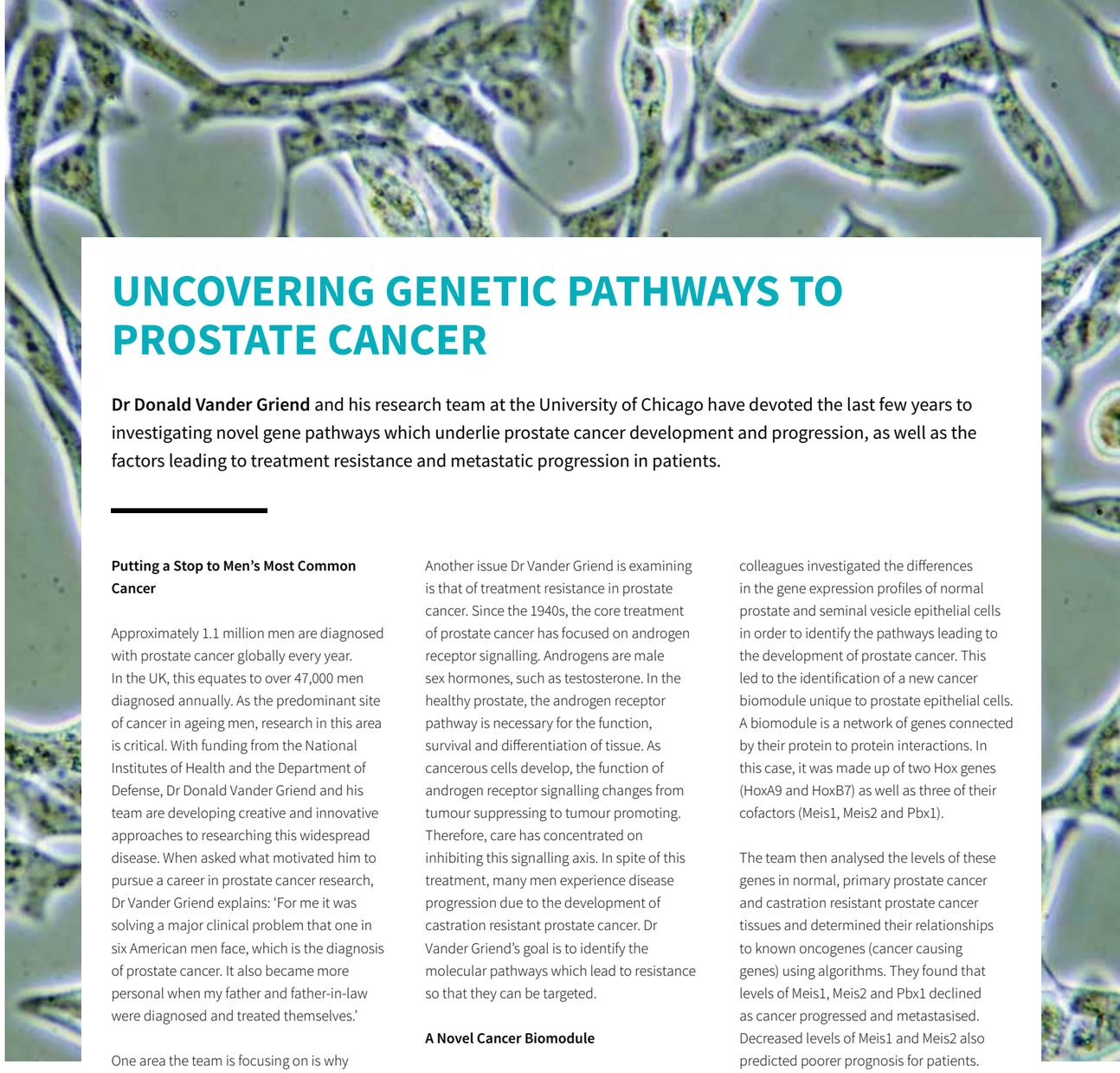


UNCOVERING GENETIC PATHWAYS TO PROSTATE CANCER

Dr Donald Vander Griend and his research team at the University of Chicago have devoted the last few years to investigating novel gene pathways which underlie prostate cancer development and progression, as well as the factors leading to treatment resistance and metastatic progression in patients.

Putting a Stop to Men's Most Common Cancer

Approximately 1.1 million men are diagnosed with prostate cancer globally every year. In the UK, this equates to over 47,000 men diagnosed annually. As the predominant site of cancer in ageing men, research in this area is critical. With funding from the National Institutes of Health and the Department of Defense, Dr Donald Vander Griend and his team are developing creative and innovative approaches to researching this widespread disease. When asked what motivated him to pursue a career in prostate cancer research, Dr Vander Griend explains: 'For me it was solving a major clinical problem that one in six American men face, which is the diagnosis of prostate cancer. It also became more personal when my father and father-in-law were diagnosed and treated themselves.'

One area the team is focusing on is why prostate cancer develops. For example, the prostate gland is developmentally and functionally similar to the seminal vesicles – they are both sex accessory glands, they are both androgen dependent – so why are there 250,000 cases of prostate cancer reported annually in the United States and less than 50 total cases of seminal vesicle cancer have been reported in the English literature? To investigate this question, Dr Vander Griend and his colleagues analysed the genes expressed by the tissues of both the prostate and seminal vesicles in order to highlight the key differences between the organs as well as identifying developmental pathways which could predict tumour initiation and progression.

Another issue Dr Vander Griend is examining is that of treatment resistance in prostate cancer. Since the 1940s, the core treatment of prostate cancer has focused on androgen receptor signalling. Androgens are male sex hormones, such as testosterone. In the healthy prostate, the androgen receptor pathway is necessary for the function, survival and differentiation of tissue. As cancerous cells develop, the function of androgen receptor signalling changes from tumour suppressing to tumour promoting. Therefore, care has concentrated on inhibiting this signalling axis. In spite of this treatment, many men experience disease progression due to the development of castration resistant prostate cancer. Dr Vander Griend's goal is to identify the molecular pathways which lead to resistance so that they can be targeted.

A Novel Cancer Biomodule

So why is it that the prostate gland is so prone to enlargement and tumour formation? As Dr Vander Griend posits: 'My theory is that the prostate becomes diseased because of how it develops and, likewise, the seminal vesicles do not become diseased because of how they develop at the cellular level.' Therefore, his lab has undertaken studies on one pathway involved in the control of gene expression: the homeobox (Hox) gene family of transcription factors. Members of these regulatory pathways have been found to be mutated in several cancers but there is still doubt as to how they function in both normal and cancerous cells.

In a 2012 study, Dr Vander Griend and his colleagues investigated the differences in the gene expression profiles of normal prostate and seminal vesicle epithelial cells in order to identify the pathways leading to the development of prostate cancer. This led to the identification of a new cancer biomodule unique to prostate epithelial cells. A biomodule is a network of genes connected by their protein to protein interactions. In this case, it was made up of two Hox genes (HoxA9 and HoxB7) as well as three of their cofactors (Meis1, Meis2 and Pbx1).

The team then analysed the levels of these genes in normal, primary prostate cancer and castration resistant prostate cancer tissues and determined their relationships to known oncogenes (cancer causing genes) using algorithms. They found that levels of Meis1, Meis2 and Pbx1 declined as cancer progressed and metastasised. Decreased levels of Meis1 and Meis2 also predicted poorer prognosis for patients. The expression of the biomodule was also able to detect overall survival differences in watchful waiting patient groups, distinguish between normal tissue, primary tumours and metastatic disease and identify patients in which disease was more likely to recur post-treatment.

The clinical relevance of this biomodule cannot be ignored. Meis genes have already been shown to play a role in other forms of cancer; if they are implicated in the formation of poor prognosis tumours then they may serve as useful biomarkers or targets for treatment. Such biomarkers could be critical in discerning which patients with low grade tumours would benefit from early treatment. Therapies which restore Meis1, Meis2 or

‘What attracted me to prostate cancer research? For me it was solving a major clinical problem that one in six American men face, which is the diagnosis of prostate cancer.’

Pbx1 to normal levels may prove effective in preventing or treating prostate tumours.

Unlocking the Secrets of Sox2

Another transcription factor to consider is Sox2. This embryonic stem cell regulator is essential for maintaining the survival of undifferentiated stem cells and regulates the expression of over 1200 genes. In other words, Sox2 has a role in dictating what stem cells will become in the body once mature and is particularly important in how the prostate forms and develops. Sox2 expression has also been linked to a number of cancers, including prostate cancer. This leads to a number of questions for Dr Vander Griend: ‘Sox2 regulates other genes; what is it regulating in the prostate? Does Sox2 have unique and novel functions in the prostate that we can exploit for therapeutic benefit? For example, if we can target Sox2 in prostate cancer cells, we could block the ability of cells to acquire resistance to hormone therapy and thus increase the efficacy of hormone therapy.’ These questions have led to a grant of 1.5 million US dollars from the National Institutes of Health to further study and understand how Sox2 works.

By investigating the role of Sox2 in normal and malignant prostate epithelial cells, Dr Vander Griend and his colleagues found that Sox2 is expressed in a portion of basal epithelial cells in normal prostate tissue but tumours were either uniformly Sox2 positive or negative with the percentage of Sox2 positive tumours increasing with more advanced and metastatic tumours. They also observed that Sox2 expression was repressed by increased androgen receptor signalling. Conversely, resistance to anti-androgens was associated with a marked increase in Sox2 and consequent castration resistant tumour growth. Knockdown of Sox2 protein expression led to a significant inhibition of castration resistant cell growth, supporting the data demonstrating the role of Sox2 in acquisition of resistance. Finally, the team found that castration resistant tumour growth was not promoted via reactivation of established processes but rather through new pathways in which Sox2 alone was sufficient to promote castration resistance.

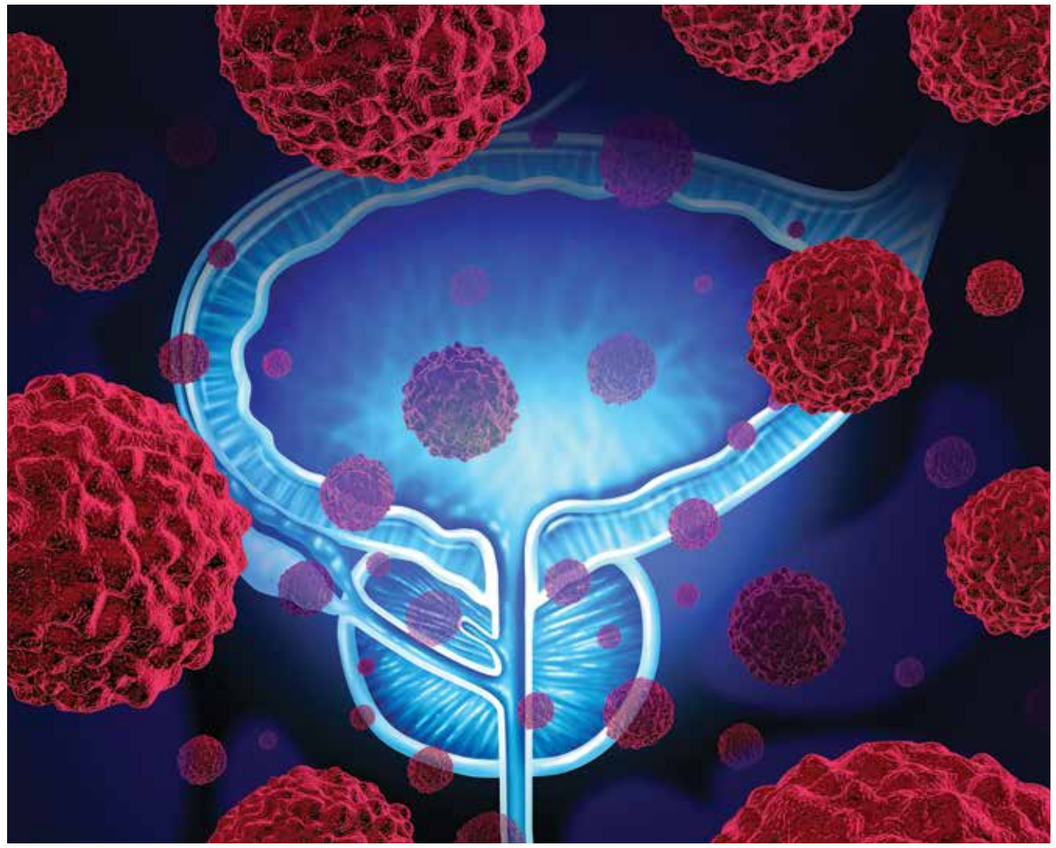
This study offers many clinically significant applications. The finding that tumours of different cell origins may display unique progression characteristics has the potential to become a powerful prognostic tool. Further study could also lead to the identification of clinically targetable pathways to prevent or treat castration resistant prostate tumour formation.

Delving Deeper into Treatment Resistance

Just this year, Dr Vander Griend and his team published a study in which they aimed to develop and characterise a series of enzalutamide resistant prostate cancer cell lines in order to identify pathways which mediate this process. Enzalutamide is an androgen receptor agonist which has proved effective in the treatment of castration resistant prostate cancer. Unfortunately, many patients develop resistance to the drug and the disease continues to progress. Therefore, four distinct cancer cell lines were developed to mirror patient heterogeneity. The team then compared differences in several outcomes, including proliferation, viability and changes in gene expression.

The results showed large diversity in growth and death rate between cell lines upon acquisition of resistance which suggests that there may be heterogeneous mechanisms underlying enzalutamide resistance. For example, two of the cell lines demonstrated increased castration resistant and metastatic growth whereas the other two did not. Although they observed that androgen receptor signalling was altered upon acquisition of resistance, they found no uniform pattern of androgen receptor expression and localisation amongst cells.

‘The next steps in our research, now that we’ve established clinical relevance for our transcription factors in prostate cancer initiation and progression, is we need to understand how they work so that we can start targeting them or their gene targets.’



Global gene expression analyses also revealed that genes which initially changed with short term enzalutamide treatment were restored with the development of resistance. These changes were associated with both androgen receptor pathways and non-androgen receptor pathways. Pathway enrichment techniques allowed the team to prioritise the most promising pathways – Androgen Receptor, ß-Catenin, microRNA 16 and Oncostatin M. Contrary to previous thought, androgen receptor mediated pathways only made up a part of the story, with non-androgen receptor mediated mechanisms contributing a greater influence to the development of enzalutamide resistance.

These enzalutamide resistant cell lines are a valuable tool in the research of castration resistant prostate cancer. Firstly, two of the cell lines developed by the team display metastasis to the adrenal glands and bones (some of the most clinically relevant sites for researchers). Secondly, the heterogeneity of the cells can be used to model the diversity of disease seen in clinical settings which can then set the stage for the development of innovative biomarkers and targets and more personalised diagnosis and treatment for patients.

Translating research into clinical outcomes

These findings are more than just meaningful in the lab. Translational research such as this can be applied to medical contexts in order to produce meaningful clinical outcomes. Dr Vander Griend emphasises the importance of his clinician collaborator, Dr Russell Szmulewitz MD: ‘He is a critical part of our translational research program. Not only is he a great scientist and fun to work with, he brings a perspective to my research program, lab meetings, and training environment which enhances the clinical relevance of my work and better prepares young scientists for meaningful translational science careers.’

The team have clearly established the clinical relevance of their transcription factors in the initiation and progression of prostate cancer. The next step will be to continue exploring how they work so that they can be targeted for treatment, used as diagnostic and prognostic tools and utilised in further research.

Meet the researcher

Dr Donald J. Vander Griend
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Dr Donald J. Vander Griend is Assistant Professor, Director of Resident Research and Director of Urological Research in the Department of Surgery at the University of Chicago. After receiving a BS in Biology from Calvin College, Dr Vander Griend went on to complete his PhD in Cancer Biology in the University of Chicago and a post-doctoral fellowship in John Hopkins University. He has received several honours and awards over the last two decades, including the Elaine Ehrman Award, the Early Career Investigator Award from the Society for Basic Urologic Research and the Young Investigator Award from the International Congress of the Metastasis Research Society. Dr Vander Griend has secured funding from the American Cancer Society, Cancer Research Foundation, Department of Defense and National Institutes of Health. His research focuses on various aspects of prostate cancer including the role of stem cells and development in cancer initiation and progression. He also teaches cancer biology to undergraduate and graduate students in the University of Chicago.

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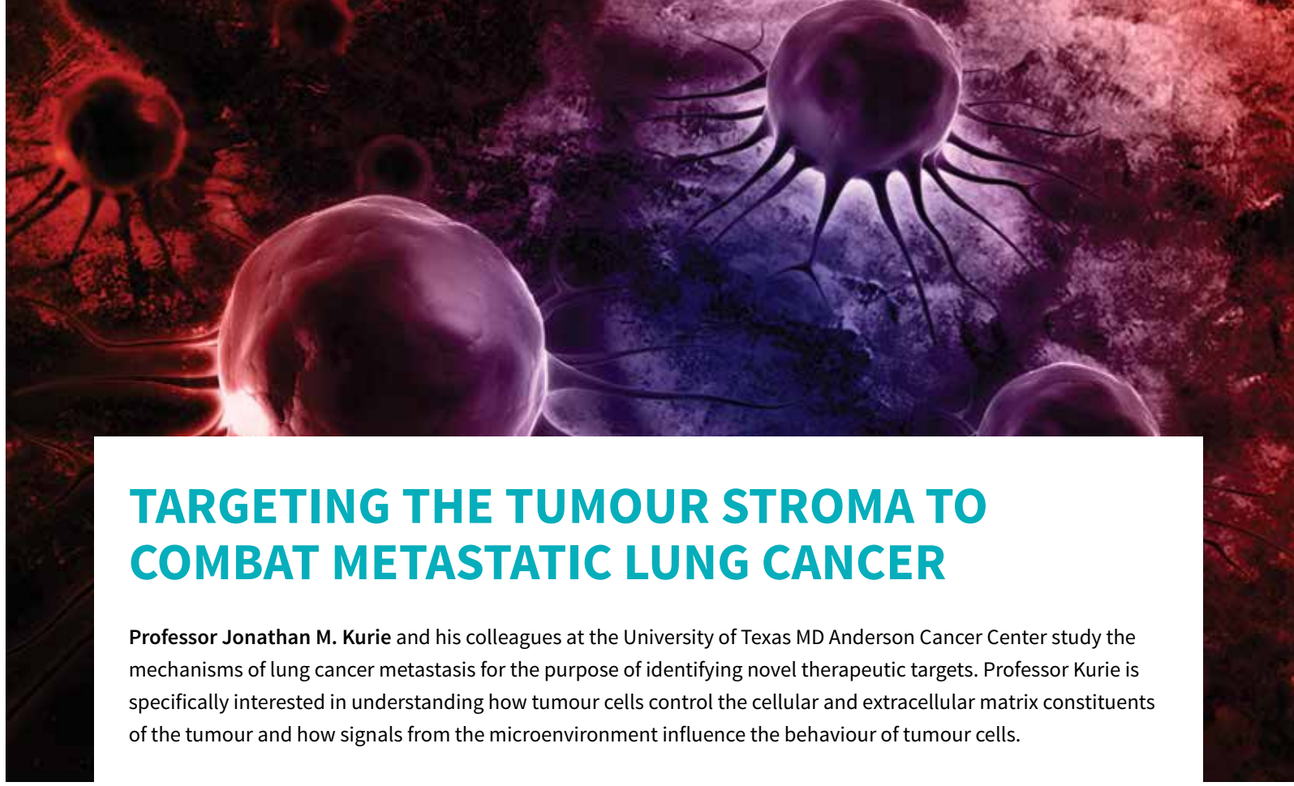


TARGETING THE TUMOUR STROMA TO COMBAT METASTATIC LUNG CANCER

Professor Jonathan M. Kurie and his colleagues at the University of Texas MD Anderson Cancer Center study the mechanisms of lung cancer metastasis for the purpose of identifying novel therapeutic targets. Professor Kurie is specifically interested in understanding how tumour cells control the cellular and extracellular matrix constituents of the tumour and how signals from the microenvironment influence the behaviour of tumour cells.

Lung cancer and metastasis – a deadly combination

When cells break away from a cancerous primary tumour and travel through the bloodstream or through the lymph vessels to other areas of the body this is called metastasis. The metastatic cells that escape from the primary tumour can spread to other organs or tissues in various parts of the body. Lung cancer is the principal cause of cancer-related deaths worldwide. Lung cancer patients primarily die from the metastasis of lung cancer, and not actually from the lung cancer itself. Thus, it is important to understand the biological basis of metastasis in order to develop therapeutic strategies to reduce lung cancer mortality rates.

A tumour cell becomes metastatic in part because of signals from outside of the cell in an area referred to as the tumour stroma. This area consists of cells called cancer-associated fibroblasts (CAFs), inflammatory cells and endothelial cells, all of which secrete molecules known as growth factors, chemokines and cytokines that trigger a complex network of cell-cell interactions within the tumour microenvironment. Professor Kurie's group believes that targets within the tumour microenvironment are a virtually untapped resource from the standpoint of clinical trial development, and this untapped source of information is a therapeutic research opportunity that might have real clinical benefits for lung cancer patients.

The migrating stem cell hypothesis

In an effort to understand how cancer cells from a lung tumour become metastatic, Professor Kurie and his colleagues focused on the mechanism of the 'migrating stem cell hypothesis', a concept proposed and developed by Thomas Brabletz and Robert Weinberg, which is based on the notion that metastases arise from a small population of tumour cells that have the ability to undergo epithelial-mesenchymal transition (EMT). EMT is a reversible process that occurs when cells lose their polarising features, detach from neighbouring cells, become increasingly motile and invasive, and are resistant to standard cytotoxic chemotherapies. There is a factor called ZEB1 that is expressed in lung cancer and is involved in the induction of EMT. ZEB1 represses the genes that could make a cancer cell 'normal' and also represses a microRNA family known as miR-200. The miR-200 family members support normal cell development and suppress the stem cell properties of cells. During EMT, ZEB1 creates tumour cells with stem cell properties, which is a driving factor of metastasis – hence, the migrating stem cell hypothesis.

Environmental cues play an important role in regulating EMT though signals that are transmitted via cell-cell contacts. One of these factors is the Notch axis, which is a system of ligands and receptors that are known to promote EMT, but the mechanism is not clear. Professor Kurie and his team aimed to understand how the Notch signalling axis regulates EMT. Using a lung cancer mouse model they demonstrated that metastatic-prone tumour cells use the Notch ligand Jagged2 to promote EMT by reducing the expression of miR-200. These findings enhance the understanding of migrating stem cell hypothesis and place the Notch pathway upstream of the ZEB1/mir200 axis

The team has shown that in mice that develop metastatic lung cancer, the ZEB1/miR-200 axis plays a central role in the regulation of metastasis. Based on these data, they proposed that mediators of ZEB1 represent potential therapeutic targets for metastasis suppression. In an effort to address this hypothesis, Professor Kurie and his colleagues examined phosphatidylinositol 3-kinase (PI3K), because this molecule is implicated in the

expansion of a variety of normal stem cell populations and tumour-initiating cells in lung cancer models. The findings from their study suggest that ZEB1 activates PI3K in lung cancer cells, and thus makes the mesenchymal tumour cells more sensitive to metastasis suppression using PI3K-targeted therapy. Professor Kurie's group believes that treatments to selectively transform the metastatic behaviour of mesenchymal tumour cells are reasonable and may have clinical value.

Professor Kurie's work has further expanded the understanding of the role of ZEB1 in EMT. Transcriptional profiling studies demonstrate that ZEB1 controls the expression of numerous oncogenic and tumour-suppressive microRNAs, including miR-34a. The expression of miR-34a decreases tumour cell invasion and metastasis and inhibits the formation of cytoskeleton structures that promote migration. Professor Kurie and his colleagues found that ZEB1 reduces the expression of miR-34a, which promotes prometastatic actin cytoskeletal remodelling in lung cancer cells. These findings support the development of miR-34a as a therapeutic agent in lung cancer patients and also contribute to the notion that ZEB1 is a key player in the migrating stem cell hypothesis.

The role of stromal stiffness in metastasis

Within a tissue, the most abundant intrinsic matrix scaffolding protein is collagen. Collagen contributes to the tensile strength of the tissue. Numerous characteristics of collagen metabolism, including its expression, deposition, organisation and turnover, are irregular in lung cancer and are implicated in tumour progression. Tumour extracellular matrix (ECM) stiffness increases when collagen accumulates and is stabilised by the formation of covalent intra- and inter-molecular cross-links. Collagen cross-linking occurs in the space outside of the cell by the action of an enzyme called lysyl oxidase (LOX). Tumour cells highly express LOX and LOX-like family members, and this finding led to the development of pharmacological inhibitors of these enzymes. In animal tumour models, the LOX inhibitors reduce the amount of collagen cross-links in the tumours and suppress metastasis, which suggests that the abundance of collagen cross-links in the tumour regulates the disease progression.

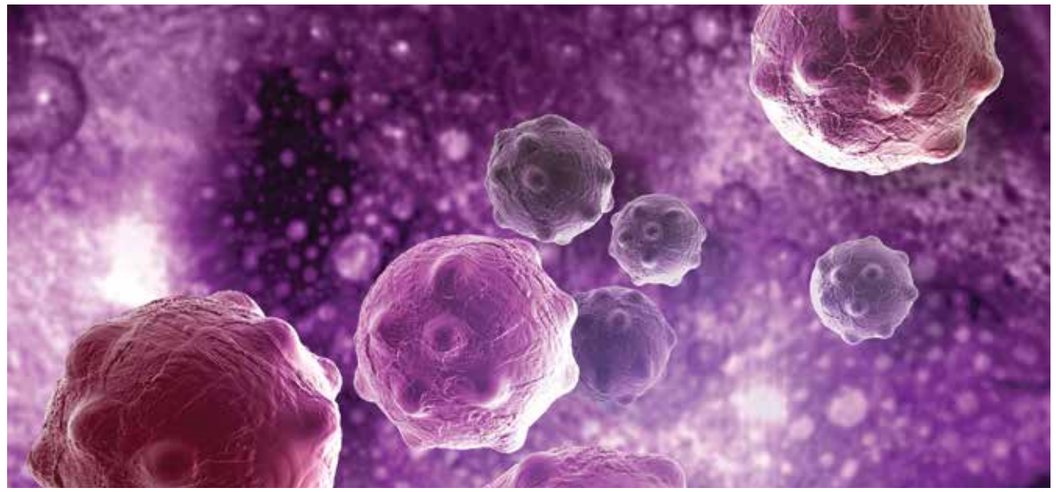
Before tumour cells become metastatic there is an accumulation of collagen cross-links that increases the stromal stiffness and stimulates the invasive properties of the tumour cells, however the biochemical nature of the collagen cross-links in cancer is still unclear. Professor Kurie's team speculated that the progression of lung cancer is accompanied by changes in the biochemical nature of the collagen cross-links. In their study, they utilised human lung cancer tissues and a mouse metastatic lung cancer model to show that the tumour stroma has higher levels of hydroxylysine aldehyde–derived collagen cross-links (HLCCs) and lower levels of lysine aldehyde–derived cross-links (LCCs) compared to normal lung tissues. They performed functional studies in the tumour cells and showed that the enzyme lysyl hydroxylase 2 (LH2) shifts the tumour stroma toward a high-HLCC and low-LCC state and increases the tumour stiffness, which enhances tumour cell invasion and metastasis. The data from Professor Kurie's laboratory indicate that LH2 boosts the metastatic properties of tumour cells and serves as a regulatory switch to influence the relative abundance of biochemically distinct types of the collagen cross-links in the tumour stroma.

'Apart from trying to kill tumours, one might suppress tumour metastatic activity, which holds promise as an adjuvant to more traditional cytotoxic therapies'

Clearly, the stiffness of the tumour microenvironment plays a role in tumour metastasis. This is evident from the development of therapeutic strategies, including inhibitors of LOX and LOX-like family members, which demonstrate anti-metastatic activity in preclinical models. Currently, there are no direct inhibitors of LH2 enzymatic activity, but the catalytic activity of LH2 is dependent on the same co-factors (iron, oxygen, ascorbate, and a-ketoglutarate) used by other LHs, and these antagonists already exist and might be modified to selectively inhibit LH2.

Cancer-associated fibroblasts regulate the stiffness switch

Cancer-associated fibroblasts (CAFs) are mesenchymal cells that are morphologically and functionally heterogeneous with



diverse origins. CAFs regulate tumour fibrosis, immunosuppression, angiogenesis and metastasis. These unique cells are migratory and contractile and they secrete collagen, cytokines and chemokines into the tumour stroma. Eric Sahai has shown in experimental tumour models that CAFs function as 'leader cells' for the invading tumour cells. CAFs lead the group of migrating tumour cells by realigning the impeding collagen fibres to create a path for the invading tumour cells, implying that the realigned collagen fibres within the pathway created by the CAFs have attained stability through the collagen cross-linking. However, it is unclear whether the CAFs actually play a role in regulating the collagen cross-linking.

Professor Kurie and his colleagues wished to address the question of the involvement of the CAFs in collagen crosslinking. Using a lung cancer mouse model, they found that these mice were highly fibrotic and contained CAFs that produced collagen and generated stiffness in collagen gels. In addition, they injected wild-type mice with lung cancer cells alone or in combination with CAFs and found the same total concentration of collagen cross-links in both groups of mice, but the mice that were co-injected with the cancer cells and the CAFs had higher hydroxylysine aldehyde–derived collagen cross-links (HLCC) and lower lysine-aldehyde–derived collagen cross-links (LCCs). Thus, the researchers hypothesised that the LCC-to-HLCC switch induced by the CAFs promotes the migratory and invasive properties of lung cancer cells. To test this hypothesis, Professor Kurie's group generated co-culture models in which the CAFs were positioned interstitially or peripherally in the tumour cell aggregates in order to mimic the distinct spatial orientations of the CAFs in human lung cancer. The CAFs increased the invasive properties of the tumour cells in both of the co-culture models. They also found that LH2 is expressed in the CAFs, and when LH2 is depleted, the ability of the CAFs to promote tumour cell invasion and migration is abrogated.

The Tumour Microenvironment – a bench-to-patient approach

Professor Kurie has established collaborations with a team of scientists at Rice University, the MD Anderson Cancer Center, and the Baylor College of Medicine to integrate cutting-edge technologies from multiple scientific disciplines in order to address the role of the tumour microenvironment in lung cancer progression. This team of scientists plans to follow an approach where the results from the laboratory experiments (at the bench) can be tested in human samples to determine their clinical value (for the patient). The team plans to use these integrated technologies as a platform to identify novel predictive and prognostic factors and therapeutic targets in lung cancer patients. Ultimately, these researchers will incorporate these findings into larger efforts at the MD Anderson Cancer Center and the other institutions to develop personalised targeted therapeutic approaches.

The experimental design is based on three parts. First, Professor Kurie and his team will use a mouse lung cancer model to discover factors secreted by the CAFs that drive lung cancer metastasis. Once they establish candidate factors from the mouse studies, the CAFs and their secreted mediators will be detected immunohistochemically in a large bank of human lung cancer biopsy samples and correlated with clinical outcome.

Secondly, the team will search for the biochemical and physical properties of the tumour ECM that regulate tumour cell polarity and metastasis. After identifying the ECM components using cell culture techniques, they will be detected immunohistochemically in a large bank of human lung cancer biopsy samples and correlated with clinical outcome.

Finally, Professor Kurie's team plans to examine the interplay between tumour blood vessel formation (angiogenesis), the CAFs and immune cell (macrophage) recruitment, and metastatic invasion. Through these studies they hope to discover the cell-matrix and cell-cell interactions that regulate the rate of angiogenesis as well as the structural properties of the resultant vessels. Once they have identified the pro-angiogenic mediators, the candidate targets will be detected immunohistochemically in a large bank of human lung cancer biopsy samples and correlated with clinical outcome.

Clearly, the common goal of all of these projects is to take data generated from laboratory experiments and test it with actual clinical samples to determine if the findings are meaningful. Through these studies Professor Kurie and his colleagues hope to translate their findings into novel clinical trials that target the tumour microenvironment in lung cancer patients.

Meet the researcher

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THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center

IDENTIFICATION OF NERVE-GUIDING PROTEIN AND ITS ROLE IN THE METASTASIS OF PANCREATIC CANCER

Dr Lei Zheng is an associate professor at the department of oncology at the Johns Hopkins University in Baltimore. His research group has developed a pancreatic cancer immunotherapy research program on a neoadjuvant therapy platform. His group also tries to understand the roles of the tumour microenvironment in cancer development and the spread of cancer, so called metastasis. They hope to identify new targets for pancreatic cancer therapies.

Pancreatic cancer

Pancreatic cancer strikes nearly 50,000 people in the United States each year. While it accounts for only 3% of all cancers in the US, it remains the fourth-leading cause of cancer related deaths in the US in 2015. Pancreatic cancer is equally common in men and women. There are different forms of pancreatic cancer, however the most common is pancreatic ductal adenocarcinoma (PDA). This type accounts for about 85% of all pancreatic cancers.

PDA is devastating and highly malignant. When a patient is diagnosed with PDA, his 5-year survival is less than 5%. This means that 5 years after the diagnosis has been made, the patient has only a 5% chance of being alive. The 5-year survival is even less for patients with metastasis at the time of the diagnosis – only 2%.

There are several reasons why pancreatic cancer is so devastating. Unfortunately, pancreatic cancer is extremely difficult to diagnose at the early stages of the disease. So, most patients are only diagnosed after their cancer has already spread. Also, pancreatic cancer has a high degree of metastasis compared to other types of cancer. At the time of diagnosis, fewer than 20% of the patients have a local form of pancreatic cancer and are therefore candidates for surgical resection. However, even after surgical removal of a localized tumour, there is a high risk that the disease will recur with metastasis. Pancreatic cancer is resistant to most conventional chemotherapeutics and to radiation therapy. Thus, improved strategies for treating pancreatic cancer are desperately needed.

Metastatic cancer: what do we know so far?

A recent study identified antibodies against a metastasis-associated protein, namely annexin A2 (AnxA2), in PDA patients. These patients demonstrated prolonged and recurrence-free survival after resection of the primary tumour. In another study, metastases were suppressed in a tumour model because of an antibody-mediated blockade of AnxA2.

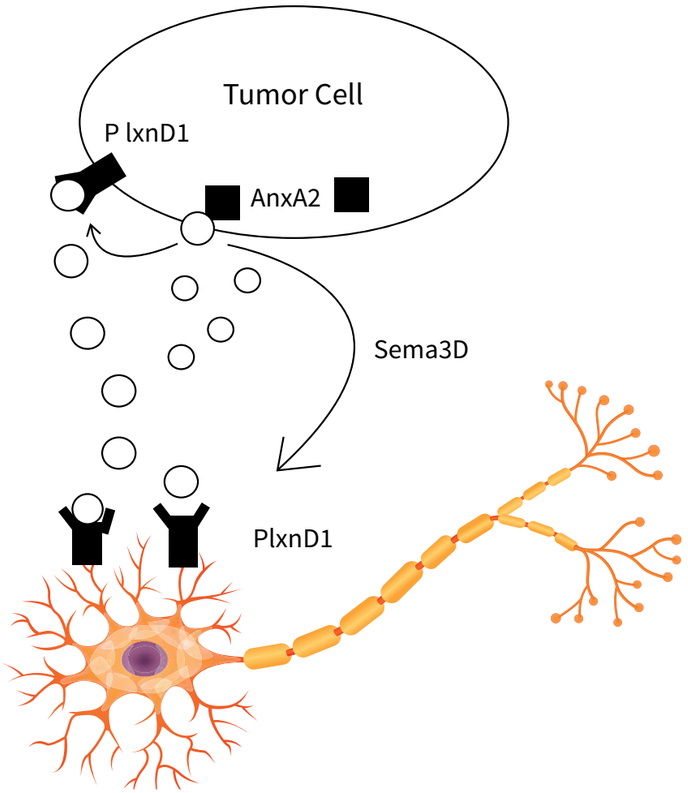
Human PDA genome studies have uncovered genetic alterations of molecular pathways that may regulate the process of metastasis. Genes encoding semaphorins and their receptors were found to be among the pathways that were most frequently altered at genetic level in PDA. Semaphorins are molecules that guide nerve fibres, or axons. Aside from semaphorins, plexins are other axon guidance molecules. It is known that plexins play a role in the development and progression of other cancer types. For instance, PlxnD1 plexin abundance is associated with high-grade primary and metastatic melanomas, a very malignant form of skin cancer. Additionally, Semaphorin 3D (Sema3D) and PlxnD1 have been shown to promote metastasis in various types of cancer.

Dr Zheng's research group investigated the mechanisms through which Sema3D and PlxnD1 signalling functions are responsible for the development of PDA and metastasis formation.

Transgenic mouse model of PDA

To evaluate the mechanisms by which AnxA2 influences the development of PDA and the occurrence of metastases, Dr Zheng's research group set up a mouse model using two types of mice, 'KPC mice' and 'KPCA -/- mice'. The KPC mice developed PDA without the antibody mediated AnxA2 blockade, while the KPCA -/- mice also developed PDA but with the AnxA2 blockade.

In 16 of the 17 KPC mice, metastatic lesions were observed in the liver, lungs, or



abdominal cavity. However, no observable gross metastatic lesions were seen in the 23 KPCA -/- mice. So, despite the presence of PDA tumours that grow relatively close to the liver in both mice, only mice with PDA tumours that expressed AnxA2 were able to invade and metastasise into the liver.

These same mice where then used to investigate the downstream pathways that mediate the function of AnxA2 in PDA metastasis formation. Genes of particular interest were Sema3D and PlxnD1 because they belong to gene families that are frequently amplified and mutated in PDA. They found that in KPCA -/- mice the protein abundance of Sema3D was decreased compared to KPC mice. However, the protein abundance of PlxnD1 was similar in both mice.

They also investigated the role of AnxA2 and they found that in the absence of AnxA2, the secretion of Sema3D was diminished. These data support the role of AnxA2 in regulating the secretion of Sema3D from PDA cells. To understand how AnxA2 mediates the secretion of Sema3D, they examined the protein-protein interaction between Sema3D and AnxA2 in PDA cells. In KPCA -/- mice lacking AnxA2, the secretion of Sema3D was diminished, so no Sema3D would bind to PlxnD1 on the surface of the cell. These results suggest that AnxA2 is required for Sema3D and PlxnD1 to form a complex, probably through controlling the secretion of Sema3D from PDA cells. This then would facilitate the subsequent interaction between Sema3D and PlxnD1 in the surface of the tumour cell.

Because it is known that both Sema3D and PlxnD1 are involved in cell motility, Dr Zheng's group examined whether Sema3D is also involved in PDA invasion and formation of metastasis. They found that Sema3D appears to have a role in controlling PDA invasion and metastasis formation.

These results suggest that Sema3D and PlxnD1 represent an AnxA2-downstream pathway that mediates the role of AnxA2 in PDA invasion and the formation of metastasis.

Results from human PDA tissue specimens

Dr Zheng's research group then wanted to further establish the role of Sema3D in PDA metastasis formation so they performed Sema3D immunohistochemistry on human PDA tissue specimens.

Tissue specimens of resected PDA presenting abundant Sema3D were observed in 15 out of 20 patients (75%). These patients had a disease-free survival of less than 1 year. Only 4 out of 15 patients (26.7%) with abundant Sema3D in their tissue specimens of resected PDA had a disease-free survival of more than 2 years. These data also suggest that Sema3D abundance in PDA is significantly associated with early recurrence after surgical resection. In all PDAs examined, Sema3D abundance was positively correlated with PlxnD1 abundance, suggesting that Sema3D and PlxnD1 may be co-regulated.

Furthermore, 14 out of 22 patients (63.6%) with widely metastatic disease demonstrated abundant Sema3D in their primary PDA tumour. Also, 17 out of 22 patients (77.3%) demonstrated abundant Sema3D in their metastatic tumour. These results suggest that Sema3D is preferentially enriched in metastatic or primary PDA tumours from patients that have a poor prognosis or patients who died with widely metastatic disease.

Which conclusions can be drawn from this study?

Dr Zheng's research group discovered that one mechanism of PDA metastasis formation is linked to the secretion of Sema3D mediated by AnxA2. The secretion of Sema3D subsequently activates PlxnD1. Their immunohistochemistry studies, linking the increase in abundance of Sema3D and PlxnD1 in human PDA metastasis with lower survival, provide evidence suggesting that they are important for human PDA metastasis development.

They also discovered that AnxA2 regulates the function of Sema3D by controlling its secretion. Sema3D present outside the cell will bind PlxnD1 on the surface of the PDA tumour cells.

This study has also revealed the mechanistic role of axon guidance genes in PDA metastasis.

Recommendations for further research

Dr Zheng's research team discovered some very interesting concepts involving the role of AnxA2, Sema3d and PlnxD1 in PDA metastasis. However, they made several recommendations for further research to elucidate this process.

Sema3D and PlnxD1 are not the only downstream mediators of the AnxA2 pathway. It is important to explore other downstream pathways of AnxA2 in the future. Furthermore, future studies are required to delineate the mechanism and the extent to which AnxA2 controls the Sema3D secretion. Possibilities include genetic regulation of the gene and altered transport of the molecule within the tumour cell. It is possible that AnxA2 regulates the packaging of Sema3D into vesicles.

Future studies are also needed to clarify how the interaction of Sema3D with PlxnD1 functionally promotes tumour metastases. It is important to know whether the Sema3D-PlxnD1 interaction activates the signalling cascade during the metastatic process in PDA.

Finally, they are also hoping to carry out further studies to uncover the exact processes by which Sema3D and PlxnD1 induce invasion of PDA cells from the primary tumour site into the surrounding blood vessels, nerves and lymphatic vessels.

The GVAX vaccine

Pancreatic cancer is naturally resistant to radiation and chemotherapy, so alternative treatment options are desperately needed. The use of therapeutic vaccines has shown to be a promising alternative therapy. These vaccines involve administering pancreatic tumour antigens to stimulate the patient's immune system. The immune system will recognize the small differences between tumour cells and normal pancreatic cells, so the patient's own immune system is recruited to fight the cancer. This treatment is organ specific and results in minimal toxicity.

One promising therapeutic vaccines is the GVAX vaccine, which was first developed by Johns Hopkins researchers, namely Dr Jaffee and colleagues. The GVAX vaccine, also known as GM-CSF gene transduced allogeneic pancreatic cancer vaccine, is made up of pancreatic cancer cells that have been modified to produce a protein that stimulates the immune system.

Patients who were treated with this vaccine, demonstrated prolonged survival rates. So, Dr Zheng's laboratory investigated the mechanism behind this increased survival. They discovered that the post-treatment blood sera of these patients contained a PDA-associated antigen, namely ANXA2. They found that the surface of the cell was increasingly covered with ANXA2 with the development and progression of PDA. Furthermore, the results of in vitro experiments suggested that therapeutic anti-ANXA2 antibodies were induced by the vaccine. These antibodies inhibited in vitro invasion of PDA cells. Therefore, they concluded that ANXA2 was a new target for the development of PDA therapeutics.

Recommendations for the development of new therapies for PDA

With the newly discovered data discussed in this article, Dr Zheng's research group is currently pursuing three possible therapeutic targets to stop pancreatic cancer metastasis driven by AnxA2 and Sema3D.

They are planning clinical trials to test a recently developed vaccine to target AnxA2. At the same time, they are developing a therapeutic antibody that targets AnxA2. They are also looking for a small molecule that will inhibit Sema3D.

Currently, they do not know precisely how Sema3D encourages the spread of pancreatic cancer. Dr Zheng believes that Sema3D may help cancer cells surround and track nerves to travel away from the main tumour, and thinks that this neural pathway might be especially important in pancreatic cancer. Pancreatic cancer causes fewer blood vessels to grow that can carry cancer cells to the rest of the body compared to other forms of cancer. Also, pancreatic cancers tend to invade nerves.

The exact role of AnxA2 in the spread of cancer cells in PDA is still unclear. Dr Zheng suspects that AnxA2 may act like a bodyguard to Sema3D, sheltering and guiding Sema3D as it makes its way toward an exit at the cell surface. Another theory is that AnxA2 acts more like a professional packer, helping to enclose Sema3D in tiny molecular bubbles before it is secreted by the cell. Dr Zheng and Dr Jaffee hold a patent on AnxA2 as a target for cancer therapy. Dr Zheng also has a pending patent involving AnxA2 as an immunologic target.

Meet the researcher

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Dr Lei Zheng is an associate Professor at the department of oncology and surgery at the Johns Hopkins University School of Medicine in Baltimore. His research interest focuses on developing a pancreatic cancer immunotherapy research program on a neoadjuvant therapy platform and understanding the mechanistic roles of tumor microenvironment in cancer development and metastasis and identifying new targets for pancreatic cancer therapies.

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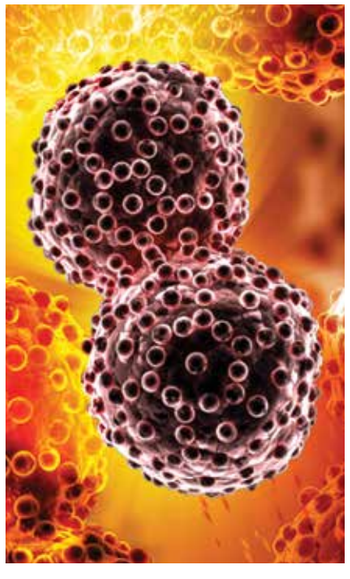
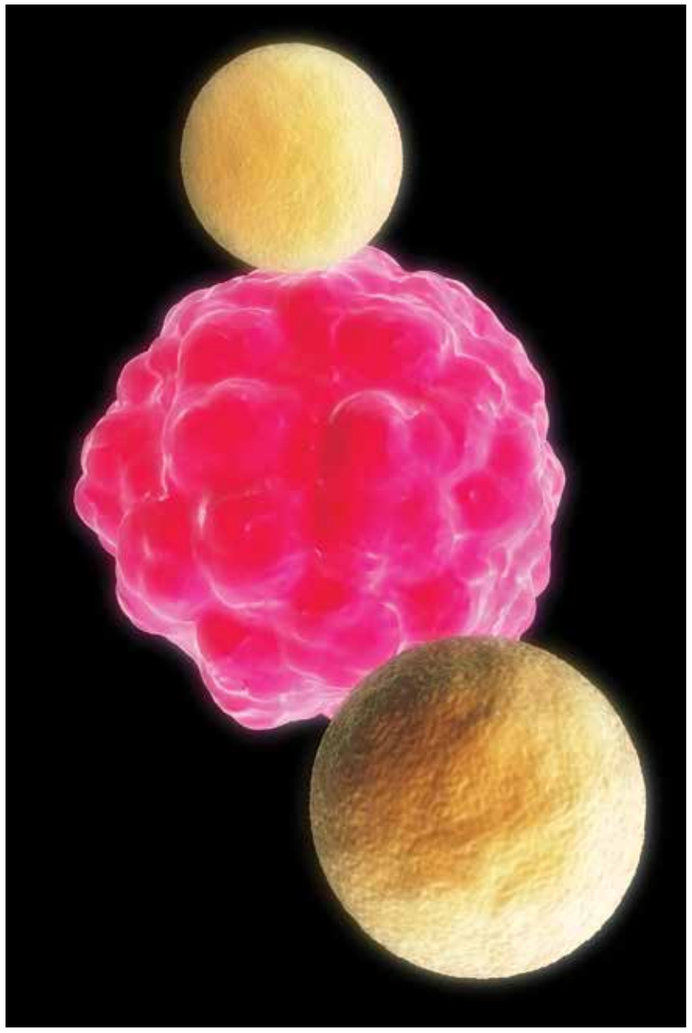
HARNESSING THE POWER OF THE IMMUNE SYSTEM IN THE FIGHT AGAINST CANCER

Every day, our immune systems work hard to detect and destroy damaged cells that have the potential to transform into cancer. However, mutant cells may not always be recognised by the immune system, enabling them to escape destruction. If one of these cells then undergoes carcinogenesis, transforming into a cancer cell, it becomes even harder to detect – as cancer cells can create an environment that suppresses our natural immune response, thus repressing the normal function of our white blood cells. Therefore, our immune systems need help to seek and destroy cancer, and that's where the relatively new world of cancer immunotherapy steps in.

Cancer immunotherapy is described as stimulating our own immune systems to kill cancer cells, and is believed by many to be the most promising new way to eradicate cancer. So far, many different immunotherapies have emerged as cancer treatments, including monoclonal antibodies, adoptive cell transfer, cytokines and treatment vaccines.

Monoclonal antibodies are drugs designed to bind to specific targets, thus triggering an immune response that destroys cancer cells. Other types of these antibodies work by marking cancer cells, so that our white blood cells can detect and destroy them more easily. Adoptive cell transfer, on the other hand, is a process whereby T cells – a type of white blood cell – are first taken from the tumour tissue of a cancer patient. The most active of these are then genetically modified to enhance their ability to kill cancer, and are then grown up and introduced back into the patient to work their magic. The third type we mentioned are cytokines, or more specifically, interferons and interleukins – signalling proteins that are produced by immune cells during inflammatory responses. Interferons help to alert our immune systems to the presence of cancer, by activating white blood cells, such as natural killer cells and dendritic cells, while one of the ways that interleukins work is by stimulating the growth of white blood cells. Finally, certain vaccines have shown some promise in the treatment of cancer, one

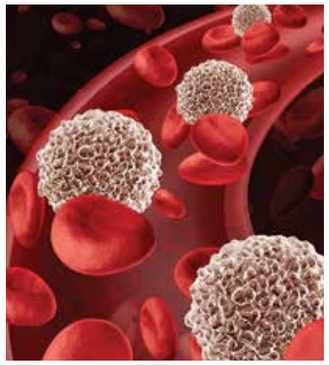




of which is a bacterium known as Bacillus Calmette-Guérin (BCG). Although this is the primary vaccine used for tuberculosis, it also happens to be a highly effective immunotherapy for bladder cancer.

In this section of the edition, we highlight the latest in cancer immunotherapy research. To introduce this promising field, we speak to Dr Jill O'Donnell-Tormey, the chief executive officer of the Cancer Research Institute (CRI). The CRI is the world's first non-profit organisation dedicated exclusively to advancing the development of cancer immunotherapies. The institute achieves this in many different ways, through funding academic projects, accelerating clinical studies by creating partnerships across industry and academia, and encouraging patients to enrol in clinical trials for new immunotherapies. Here, Dr O'Donnell-Tormey tells us all about the CRI's activities, and what the future holds for this relatively new approach to cancer therapy.

From here, we feature the work of Dr Stephanie Watkins at Loyola University in Chicago, who has been working hard to increase our fundamental understanding of how our immune system responds to cancer. In particular, Dr Watkins has made some exciting discoveries about the activity of a transcription factor known as FOXO3. This important factor plays a role in regulating cell death, and when it malfunctions, our immune cells can fail to recognise cancer cells as being harmful, allowing them to take over. However, Dr Watkins found that the function of FOXO3 in immune cells is influenced by exposure to oestrogens or



androgens, which may lead to differences in cancer incidence, aggressiveness, and outcomes between genders. This fascinating research may lead to the development of new targeted cancer immunotherapies.

Next, we showcase the multidisciplinary research of Professor Seamas Donnelly and Dr Ciaran O'Reilly and their diverse team of scientists at Trinity College Dublin in Ireland. Through computational screening and cell culture experiments, the team investigate the ability of small molecule drugs to perturb the function of Macrophage Migration Inhibitory Factor – a protein that plays a key role in the body's natural inflammatory response. By doing this, the researchers have been able to pin down the role that this protein plays in inflammation and how this inflammatory response can trigger the development of cancer. This promising research will hopefully lead to the development of new small-molecule based immunotherapies.

Our final article in this section describes the work of Professor Iraldo Bello-Rivero and his team at the Centre for Genetic Engineering and Biotechnology in Cuba. The team have developed a new immunotherapy to treat skin cancer, using interferons – cytokine molecules that we mentioned previously. His 25 years of experience working with these molecules has enabled him to discover a winning combination of two interferons that exhibits synergistic activity in attacking cancer cells. Due to the outstanding clinical trial results obtained, this new formulation has been approved in Cuba for the treatment of basal cell carcinoma. However, the team now hope to find international collaborators to help them further test the efficacy and safety of the treatment, with the hope of spreading their new treatment worldwide.

CANCER RESEARCH INSTITUTE

THE CANCER RESEARCH INSTITUTE

Founded in 1953, the Cancer Research Institute (CRI) is the world's first non-profit organisation dedicated exclusively to harnessing the power of the immune system to defeat cancer. This important branch of research has led to a new class of cancer treatments known as cancer immunotherapy, believed to be the most promising new approach in the fight against cancer. These treatments strengthen and sustain our immune system's natural ability to track down and kill cancer cells, wherever they are in the body. The Cancer Research Institute supports cancer immunotherapy research all over the world, through a variety of different means, from funding academic research projects to accelerating clinical studies by creating partnerships across industry and academia, and encouraging patients to enrol in clinical trials of new immunotherapies. Here, we speak to Dr Jill O'Donnell-Tormey, chief executive officer of CRI, who tells us all about the organisation and what the future holds for this new approach to cancer therapy.

To start, please give us a brief introduction to the Cancer Research Institute (CRI), and tell us a little about its history.

The Cancer Research Institute (CRI) is the world's first non-profit organisation dedicated exclusively to harnessing the immune system's power to conquer all forms of cancer. Established in 1953, CRI has supported the laboratory and clinical research of more than 3,000 scientists at top academic institutions around the world, whose work has enabled the development a new class of immune system-based cancer treatments called immunotherapy. Considered the most important breakthrough in cancer treatment today, immunotherapy has already transformed the treatment of some cancers, and promises ultimately to revolutionise the treatment of all cancers.

Why is harnessing the immune system's power the best strategy to adopt in the fight against cancer?

Conventional treatments like chemotherapy and radiation have reached their maturity in terms of effectiveness. New treatments for cancer are urgently needed. Scientists funded by the Cancer Research Institute have demonstrated that the immune system is capable of seeing and eliminating cancer, and that it is possible to intervene therapeutically in patients whose immune systems are no longer able to keep cancer under control. Immunotherapy offers significant advantages over conventional treatments. Rather than directly targeting tumours, immunotherapy treats a patient's immune system, 'educating' it to identify cancer targets, 'empowering' it to act against cancer unimpeded, or 'reinforcing' it with boosted numbers of tumour-targeting immune cells. As a result, the dynamic and complex immune system is better equipped to keep pace with cancer, not only during treatment, but also long after. Early clinical studies have also indicated that immunotherapy can work well in combination with conventional treatments, enhancing the overall benefit to patients.

Describe some of the many ways that CRI promotes and supports cancer immunotherapy research?

The Cancer Research Institute supports scientific research along the entire spectrum of discovery. CRI funds young postdoctoral fellows working under the tutelage of world-leading immunologists, facilitates translational research that brings insights from the clinic back to the laboratory with the goal of improving immunotherapy's effectiveness, and accelerates clinical research through partnerships across industry and academia that power innovative drug combination studies. CRI convenes scientists from throughout the field at annual meetings and workshops. And CRI encourages patients to enrol in clinical trials of promising immunotherapies – an essential step in the development of new treatments.

What are the main types of cancer immunotherapy that are currently available to patients, and what are the most promising treatments currently under development?

Currently the type of immunotherapy that has generated the most interest and has seen the most success in treating patients is called checkpoint blockade. Antibodies like those that naturally play a role in the immune system are engineered in the lab to interfere with specific pathways known to inhibit the immune system. The most prevalent targeted pathways include CTLA-4 and PD-1/PD-L1. By blocking these pathways, checkpoint blockade therapy 'takes the brakes off the immune system', neutralising the signals that stop immune cells from attacking cancer. These treatments have proven effective in melanoma, lung cancer, kidney cancer, head and neck cancer and others. Another promising immunotherapy is CAR T cell therapy. These T cells are taken from a cancer patient, engineered to express a chimeric antigen receptor (hence 'CAR') targeting a specific protein found on cancer cells, grown to exponential numbers, and then given back to the patient. This approach has been highly successful in treating leukaemia, and is now being tested in other types of cancer. Personalised therapeutic cancer vaccines that target patient-specific mutations are an exciting area of research today that could offer yet another immunotherapy approach in the near future. Finally, older generations of antibody therapies have been available to patients since the late 1990s, including Herceptin for breast cancer and Rituxan for lymphoma, for example.

Where does CRI receive its funding from?

The Cancer Research Institute relies on philanthropic support from individuals, corporations, and foundations. CRI also participates in combined campaigns through the Community Health Charities. CRI does not receive funding from the government, and raises its operating budget each year.

What are the main routes by which cancer researchers can avail of CRI grants and fellowships and how does CRI ensure that its funding is best allocated?

For our open application programs, such as the CRI Irvington Postdoctoral Fellowship Program or CRI's Clinic and Laboratory Integration Program, scientists may apply directly to CRI for funding for projects within the field of tumour immunology. Application instructions and forms are available at www.cancerresearch.org. All funding decisions are guided by a Scientific Advisory Council composed of world-leading immunologists and tumour immunologists, and including three Nobel Prize winners and 26 members of the National Academy of Sciences. Scientists seeking funding for clinical research can apply to become members of CRI's global clinical trials network.

Who are your main collaborators, and what countries are involved in CRI funded research?

CRI's longest standing partner is the Ludwig Institute for Cancer Research, with whom CRI co-manages its global CVC Clinical Trials Network. This international network of more than 60 leading clinicians with expertise in immunotherapy clinical trials powers CRI's innovative clinical research program, the Clinical Accelerator. Both the members of this network as well as the larger community of scientists funded by the Cancer Research Institute span the globe, ranging from Japan and Australia to countries in Europe and North America. CRI also partners with disease-specific cancer research and patient advocacy organisations to provide CRI's expertise in immunotherapy for the benefit of different cancer patient communities. Such partnerships may result in clinical trials that are co-funded by partnering organisations, or educational programming such as webinars, patient summits, and literature designed to provide patients with a basic understanding of immunotherapy as a springboard for further discussion with their doctors.

What are the biggest challenges facing cancer immunotherapy today, and how is CRI working to overcome these challenges?

A key hurdle in the field is the difficulty scientists have accessing tissue samples from patient biopsies pre-, during, and post-treatment with immunotherapy. These precious tissue samples are important because they allow scientists to see what is happening (or not happening) inside the tumour immunologically. Such observations would permit scientists to better identify the causes of immunotherapy success or failure in individual patients and could lead to improved treatment strategies overall. CRI's Clinic and Laboratory Integration Program and Clinical Accelerator facilitate this by providing funding for tissue acquisition and immunological analysis. A second challenge is the lack of awareness of cancer immunotherapy options for patients. To address this, CRI has developed theanswertocancer.org, a website for patients and caregivers who are seeking information about cancer immunotherapy and who wish to connect with other people who have received immunotherapy treatment. The site has also proven useful to healthcare professionals such as community oncologists and oncology nurses whose patients have expressed interest in immunotherapy treatment.

Finally, can you please share your thoughts on the future of cancer immunotherapy, and the role of CRI in that future?

There has been significant progress recently in the successful application of immunotherapy to treat some cancers. This is only the beginning, however, and more research is needed if we are to realise immunotherapy's full potential. Nevertheless, immunotherapy is well on the way to becoming a backbone of treatment for many types of cancer, and we at the Cancer Research Institute believe it one day will become the standard of care for most if not all cancers. In this not-too-distant future, cancer will be understood to be an immunological problem. And while chemotherapy, radiation, and surgery will continue to have a place in cancer treatment, it is likely these will be used in combination with immunotherapy treatment. The Cancer Research Institute will continue to lead the way in cutting-edge laboratory and clinical research, breaking down research silos, encouraging collaboration, and solving for the complex challenges that are certain to arise. Throughout, CRI will continue to seed the field with the brightest scientific minds, ensuring a steady stream of talent and inquiry to drive future innovation in the field of cancer immunotherapy.

CANCER RESEARCH INSTITUTE

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BUILDING IMMUNITY AGAINST CANCER

A growing body of evidence supports how harnessing the power of the immune system may be the ultimate way to fight cancer. Therefore, researchers such as **Dr Stephanie Watkins** at Loyola University Chicago have been striving to increase our understanding of cancer immunology, by exploring the role of gender on immune cell activation. This fascinating research may lead to the development of new targeted cancer immunotherapies.

Cancer Complexities

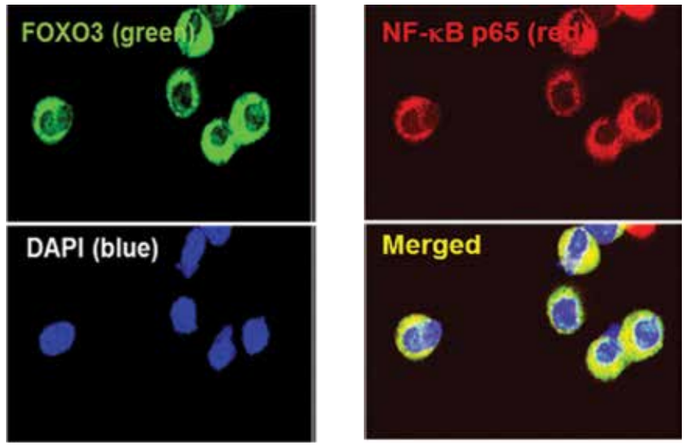
For every 100,000 people, there are around 455 new cases of cancer reported every year, while about 171 people die due to this terrifying disease. For this reason, oncology is an extremely important field of research, receiving much deserved attention from both researchers and governmental authorities. Cancer can arise in a number of different ways, each involving the hijacking of multiple pathways. Thus, cancer therapies must enrol and target a wide range of systems in order to be as effective as possible.

Every single day, a large number of cells in our bodies are damaged by internal and environmental factors. Some of these cells cannot repair themselves effectively and die out, while others survive despite their damaged DNA and produce new generations of cells with incorrect information. When enough of these so-called mutations appear in a single cell, the mechanism that ensures our survival transforms into one of the deadliest known diseases, where cells become virtually immortal and start multiplying out of control. Every day, it is up to our immune systems to clear up damaged cells with oncogenic potential to preserve our health.

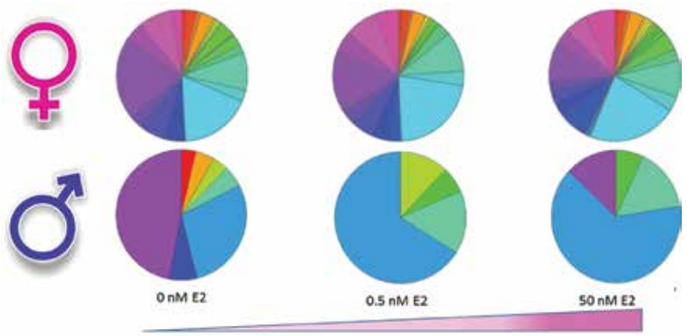
Yet tumour cells are not always recognised by the immune system, which enables them to escape destruction. Tumours have been found to create microenvironments that suppress immune response, where anti-tumour activity and the function of T cells are repressed. Dendritic cells play a critical role in our immunity against tumours because of their ability to trigger a strong immune response to the abnormal substances – or antigens – produced by cancer cells. Although these cells produce chemical signals to enable the recognition of tumours, they can be tricked into tolerating the tumour by the tumour microenvironments and therefore fail to send warning messages. Dr Stephanie Watkins at Loyola University Chicago investigated this phenomenon, specifically looking at a transcription factor known as FOXO3. FOXO3 is a protein class specific to humans, belonging to a family of transcription factors that play roles in regulating cell death – malfunctions of the FOXO3 system lead to dendritic cells becoming more tolerant of tumours. However, this relationship was found to be rendered more complex by the gender of the patient. Dr Watkins and her collaborators showed that there are significant differences between males and females in the frequencies and function of dendritic cell sets that infiltrate tumours. More precisely, the function of FOXO3 in dendritic cells was shown to be affected by exposure to oestrogens or androgens, which may lead to differences in cancer incidence, aggressiveness, and outcomes between genders.

In addition to inter-gender differences, cancer evolution is also known to be also influenced by alcohol, which reduces the ability of dendritic cells to react to antigens and promotes inflammation in response to trauma and infections. Despite its known role, the influence of alcohol on anti-tumour response had not previously been explored. Furthermore, vaccines for triggering a dendritic response are often produced from blood taken from the same individual, which means that previous exposure to alcohol can play a role in the efficacy of the vaccine. In a study examining the gender-dependent impact of alcohol on anti-tumour immune response, Dr Watkins and her team found differences between the signalling dendritic pathways of males and females, while also discovering that these differences are amplified by exposure to alcohol. In the team's experiments, female mice that were given alcohol showed reduced levels of granzyme B and interferon gamma (both having roles in immunity) and decreased function of CD44 and CD69 (two proteins found on the membranes of healthy cells). This showed that the female mice were unable to activate antigen-specific cytotoxic T cells and obtain immunity against the tumours. Furthermore, the alcohol countered even the extra FOXO3 produced by dendritic cells in response to the threat.

'I am interested in identifying the role of gender and sex hormones on immune cell activation and the outcome of targeting these pathways to enhance the activation of the immune response against cancer'



Tumour infiltrating dendritic cells (DC) express FOXO3 – mediates DC induced T cell tolerance/suppression.



Human tumour antigen specific cytotoxic T cell polyfunctional profiles in response to estrogen. Each color of the pie chart represents a population of T cells that produce a different combination of cytokines (TNFa, IL-2, IL-4, IL-17, IL-22, and IFNg or have lytic function as determined by CD107a).

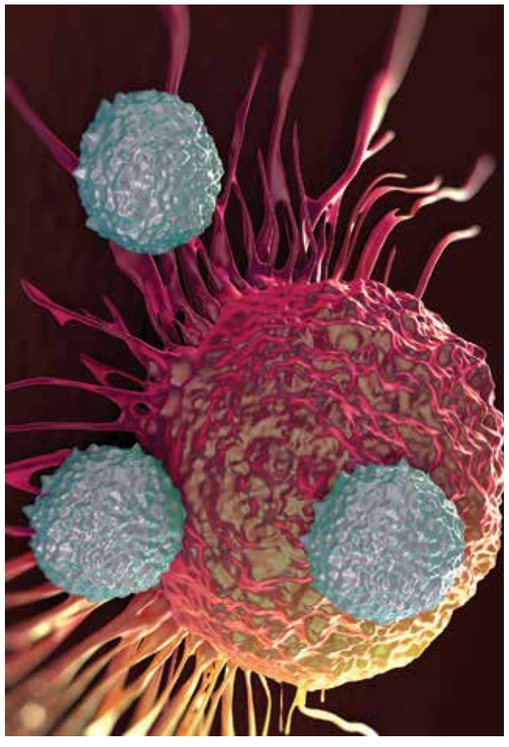
The Work of a Cancer Researcher

In her early career under the mentorship of the late Dr Robert D. Stout, Dr Watkins investigated the permanently changing behaviour of macrophages, a type of large white blood cells that migrate through the body engulfing and digesting foreign compounds, cellular remains, cancer cells, and microbes. This process is called phagocytosis, and healthy cells avoid this fate due to their surface proteins, which macrophages can recognise. Dr Stout was one of the first immunologists to recognise and thoroughly demonstrate that macrophages have the unique impressive ability to continually change their functions in response to their changing environment. Based on this, Dr Watkins found that macrophages which infiltrate lung tumours secrete anti-inflammatory cytokines and growth factors and are extremely immune suppressive. After injecting IL-12, or interleukin 12, into the tumour, Dr Watkins found that the macrophages quickly changed their behaviour – they produced a signalling substance to alert the immune system to the presence of cancer. She also discovered that the cells continued to change their function according to all the new stimuli they encountered – a process which persisted even after they were extracted from the cancerous microenvironment of the tumour.

Later on, Dr Watkins found that when macrophages infiltrating tumour sites release cytoplasmic interleukin 15, a process is initiated that leads to tumour regression. They knew that although anti-tumour vaccines can increase immunity in healthy mice, benefits in ill mice have been found to be minimal, due to the strong immunosuppressive environment created by the tumours in the host bodies. Although previous studies had tried to destroy the cells with immune-suppressing roles, success had been extremely limited. However, some scientists had reported that injecting interleukin 12 in mice bearing tumours initiates tumour destruction even if the mice have not been vaccinated to increase their immunity. Therefore, Dr Watkins injected interleukin 12 into mice to encourage leukocytes to invade cancerous tissues. Within two hours, they noticed that serum interleukin 15 increased, while the cytoplasmic interleukin 15 of the macrophages in the tumour decreased. The reverse was also true: injecting anti-interleukin 15 within an hour before interleukin 12 counters the beneficial effects – leukocytes are no longer interested in infiltrating the tumours and the reduction of the primary mass and clearance of metastases under the influence of interleukin 12 no longer occurs. However, injecting anti-interleukin 12 18 hours after interleukin 12 had no detectable impact of the activity of leukocytes. Together with the team's previous studies, this research proved the beneficial impact of interleukin 12 and its potential to

reduce the cancer-supporting behaviour of macrophages in tumours. The results suggest that resetting the function of macrophages is important for triggering the correct immune response to fight tumours, and deprives cancer of the growth support it normally receives. If integrated into current cytokine therapies, this finding has the ability to increase success rates and improve patient prognosis. For this work, she received her PhD from the University of Louisville in 2007.

With the knowledge that bone marrow, macrophages, and dendritic cells associated with tumours become hijacked and change their protective behaviours into immune-suppressive ones, Dr Watkins and her collaborators further looked into the pathways controlling myeloid cell function. In their experiments, they identified a direct interaction between two proteins, FOXO3 and NF-kB RelA. While FOXO3 regulates dendritic signalling, the NF-kB protein family (nuclear factor kappa-light-chain-enhancer of activated B cells) is present in most cells and controls DNA transcription, cell death, and the production of cytokines. Its role is to control the response of cells to external stress, for example UV radiation, free radicals, microbe antigens, or heavy metals. RelA, in particular, belongs to an NF-kB group responsible of transactivation, or an increase in the rate of gene expression. Dr Watkins found that FOXO3 binds NF-kB RelA in the cytosol – the liquid part of the cell cytoplasm. Consequently, neither protein can migrate to complete its activity, and instead, they remain locked near the location where proteins become attached to the transcription factor. However, when deleting the FOXO3 sequence, the NF-kB RelA resumes its activity. Although researchers are momentarily unable to suggest the consequences and applications of this finding, the study is important for being the first attempt in exploring the activity and roles of two proteins known to have a direct role in cancer propagation.



Future research directions

Dr Watkins' main research goal is to enhance immune based therapies for cancer by targeting pathways that regulate immune tolerance. At this point, she has three projects on the table that focus on advancing her previous cancer research.

The first project seeks to gain insight into the role of hormone receptor signalling in dendritic cells infiltrating tumours. Having identified the regulating role of FOXO3, which interacts with sex hormone receptors, she completed a preliminary study on mice and found that interventions targeting these transcription factors increased immunity towards tumours. The other finding was that decreasing the FOXO3 levels in female mice with tumours increased the growth rate of the tumours by approximately 4 times, a phenomenon accompanied by a lowered immune response. Although current therapies involve hormone receptors, the effect of targeting these pathways is not well understood at this point. Once Dr Watkins uncovers the impact of tuning the hormone receptor response, these findings will enable researchers to leverage the phenomenon and obtain better patient outcomes.

The second project is an inter-university collaboration between Loyola and Notre Dame, intended to produce molecule inhibitors that will break the interaction between FOXO3 and NF-kB – a transcription factor with a role in stress response and triggering immune reactions to infection. When incorrectly produced, NF-kB is linked to inflammation, cancer, and autoimmune disease. The research collaboration works with the premise that interrupting the FOXO3–NF-kB interaction

'We found that dendritic cells that entered tumours had an upregulated expression of the transcription factor FOXO3'

promotes the immune potential of dendritic cells, and has applications in prostate cancer therapies.

Dr Watkins' third project focuses on understanding the multiple functions of T cells in melanoma and vitiligo, the latter being a condition predominantly affecting women, in which skin patches lose their normal colour. By means of polyfunctional cytokine analysis, her team found that certain patterns of cytokine production by T cells are linked with the appearance of vitiligo. Currently, the team is looking to understand whether the same patterns are responsible for ensuring immunity against melanoma. Dr Watkins' current graduate student and PhD candidate, Ms Flor Navarro, recently discovered significant differences in human male and female T cell polyfunctional profiles, especially upon stimulation in the presence of oestrogen. Further analysis will be required to determine the impact of therapeutically targeting these distinct patterns of function to control tumour growth and simultaneously prevent autoimmune disease.

Meet the researcher

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Dr Stephanie Watkins obtained her PhD from the University of Louisville in 2007 for a project investigating the plasticity of tumour-associated macrophages. Here, she showed that anti-inflammatory tumour-associated macrophages become pro-inflammatory under IL-12 cytokine therapy and induce cytotoxic T cells to infiltrate lung tumours. She continued her work as a postdoc studying the relationship between cancer and inflammation and earned her track to tenure at Loyola University Chicago through a Pathway to Independence NIH grant. She is a member of the American Association of Immunologists (AAI), American Association for Cancer Research and several other oncology and immunology dedicated associations. Over the course of her career has received no less than 14 awards for her work, including a prestigious Cynthia Chamber-memorial Award from the AAI and a Research Scholar Grant from the American Cancer Society. In addition to her dedication to cancer research she is involved raising awareness through Public policy and community outreach programs to enhance science education in public schools.

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TRANSLATIONAL MEDICINE: FUNDAMENTAL RESEARCH, DRUG DISCOVERY AND MORE!

Professor Seamas Donnelly and Dr Ciaran O'Reilly are currently working together in a cross-disciplinary team, uncovering the role of macrophage migration inhibitory factor in inflammation and cancer, and investigating promising small molecule therapeutic approaches.

Your team seems to represent the archetype of Translational Medicine. How is it like for two researchers from such distinct backgrounds to collaborate?

When doing translational research, I think it's important to surround oneself with scientists from many different backgrounds. Each person brings with them a unique set of skills and tackles problems from a slightly different perspective. Having this wealth of experience together also allows us to develop novel ideas and exploit any potential opportunities that arise over the course of our projects. We are fortunate in our group to have clinicians, immunologists, microbiologists, cell biologists and medicinal chemists working together resulting in a productive synergy within the team. Translational Medicine is a very exciting space to be in. The opportunity to make a positive impact on the lives of patients is the key driving force behind everyone in the group.

CADD generates enormous amounts of candidate compounds with good predicted therapeutic characteristics. How do you select which ones to develop further? What are the main reasons for CADD hit compounds to be excluded? Does your CADD software also include predictions pertaining to drug metabolism/safety?

We tend to conduct virtual screening campaigns using commercially available libraries to look for hits against our target of choice, in this case, the pro-inflammatory protein, MIF. Within each program there are scoring functions that will rank compounds based on the likelihood it will bind to your target. We validate the accuracy of these scoring functions using training sets and docking of the virtual library into the binding site on the protein of interest follows this. Once the compounds have been docked, we cluster the compounds based on their similarity and then choose based on their rank within the clusters in order to get as many different chemotypes as we can for screening. We do filter out a lot of potentially reactive compounds and also any compounds which have moieties which may interfere with an assay (these compounds are called PAINS-pan assay interference compounds). We also explore the IP space around the structure to make sure it's not too crowded. Some of the programs do have a toxicity prediction facility integrated within them but our group rarely uses them, we tend to test the compounds in house once they've arrived from the vendors.

Is there typically a great difference between the *in-vitro* and *in-silico* properties of the compounds you chose to pursue further?

In the past, we have found a strong correlation between our *in-silico* leads and in vitro validation experiments which suggests our models are quite accurate at predicting which molecules will bind to our target of interest.

Given that MIF has both intracellular and extracellular activity, have there been efforts towards modulating the inhibitor's diffusion/transport across the cellular membrane?

Drug discovery is a big part of our work. It complements one of out strategic objectives, namely, enhancing our understanding of the mechanisms behind the development of chronic inflammation and our drug discovery programs have evolved from that space. Our group seeks to examine antibody drug conjugates and nanoparticle delivery systems to target organs and specific cells.

Are the small molecule MIF inhibitors expected to be delivered as IV formulations or is there hope for oral formulations? Are their safety profiles compatible with out-patient regimens?

We are a respiratory science group and have demonstrated a role for MIF in many respiratory diseases including ARDS, cystic fibrosis, asthma and lung cancer so a particular interest to us is exploring aerosolised systems for delivery of anti-inflammatory/anti-cancer compounds directly to the lungs.

How does the synthesis of novel small molecules compare with the development of monoclonal antibodies? Are new compounds that eventually make it to oncologist's arsenals expected to break the trend of escalating prices?

From our perspective, low molecular weight small molecules can usually be synthesised through well-defined routes and purified to give rise to a single product that is always identical. Small molecules are also more stable than their monoclonal antibody counterparts. In terms of cost, development of antibody based therapies is more expensive compared to their small molecule counterparts – so that has certain advantages in cost-containment strategies.

TARGETING MACROPHAGE MIGRATION INHIBITORY FACTOR, IN CANCER

New therapeutic approaches must focus on increasingly less conspicuous targets, seeking weak links in the chain of oncogenesis. The research conducted by Professor Seamas Donnelly and Dr Ciaran O'Reilly at Trinity College Dublin is expanding our understanding of cancer, while developing new, potentially lifesaving drugs.

Targeting MIF in Cancer Therapeutics

The Macrophage Migration Inhibitory Factor (MIF) is a protein that plays a key role in the body's natural inflammatory response. This strange molecule has been known to be an integral part of the inflammatory process since the 1950s, but its behaviour and properties are so unlike any other cellular messenger molecules that it has become a class of its own.

Contrary to other immunomodulatory molecules, MIF is present in cells even before any inflammatory processes have begun. It lies dormant, waiting to play its role in the inflammatory cascade, whenever the need may arise. Furthermore, this molecule acts not only as a messenger, but also as an enzyme in its own right.

Chronic inflammation and cancer, two sides of the same coin

From an evolutionary perspective, defending the body from invading microorganisms is the immune system's top priority, with protection from cancer cells playing a somewhat secondary role. The inflammatory cascade seeks to deploy a massive counter-response to external stresses, recruiting immune cells to infection sites, promoting the development of additional blood vessels, and preventing cells from dying before their defensive or support capabilities have been exhausted. The fact that MIF drives a number of pro-inflammatory pathways, explains why it has such a key role within inflammation. It is well recognised that many chronic inflammatory diseases predispose one to the development of cancer. Exaggerated MIF activity has been shown to be is a key driver in the development of chronic inflammation, causing persistent cell damage and stress long after the initial triggering stimulus has been removed. In addition, many of the unique biological activities of MIF are harnessed by cancer for its survival advantage. Consequently, MIF has become a prime pharmacological target in our fight against cancer.

The small molecule paradigm

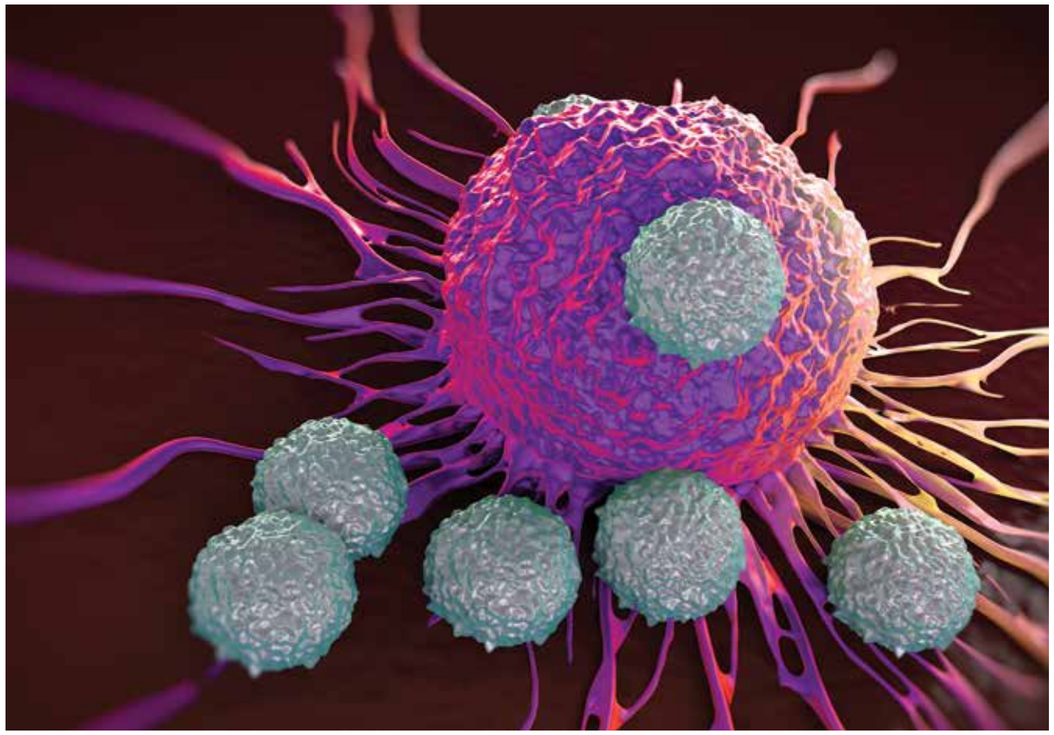
While monoclonal antibodies are renowned for their selectivity and effectiveness, there is also something to be said about the new generation of small molecule drugs. These relatively simple compounds of low molecular weight, are able to interact with biologically active sites. These compounds are small and often stable enough to be delivered orally. Furthermore, small molecules build upon a foundation of decades of chemical synthesis, making them relatively inexpensive to produce and purify, and of high value from a pharmacoeconomic perspective.

When compared to monoclonal antibodies, small molecules also provide a more rational approach to drug design. In the case of antibodies, candidate molecules are the product of random variation, that may eventually produce affinity to biologically relevant sites. For small molecules, the process of drug design is carefully directed, and aimed towards producing a specific biological effect, better pharmacokinetics or greater shelf life.

Lastly, it is also possible to benefit from the best of both worlds. By binding small molecule drugs to monoclonal antibodies or encapsulating them within nanoparticles, it is also possible to achieve systemic distribution, with very precise delivery to target cells.

Targeting MIF with small molecules

One of the classical methods for studying biological function is to structurally disrupt your protein of interest, and trace the consequences in cellular systems. Professor Seamas Donnelly and Dr Ciaran O'Reilly and their team at Trinity College Dublin are using tailored molecules to temporarily perturb the function of MIF. In this way, the researchers have been able to better ascertain its role in inflammation and in the development and progression of cancer. By disrupting the



catalytic function of MIF and/or its binding to both extracellular and intracellular receptors, these candidate drugs in-vivo have been able to significantly reduce tumour lethality.
Alternative strategies include utilising covalent inhibitors that bind irreversibly with MIF's active site, destroying its functionality permanently. 4-Iodo-6-phenylpyrimidine is an example of one of these chemicals which, although normally shunned by researchers for fear of unwanted reactions, actually has the same mechanism of action as naturally occurring dietary isothiocyanates, present in vegetables such as Brussels sprouts and watercress. Having an irreversible mechanism of action allows for much lower effective dosages, placing significantly less metabolic burden on the patient's system.

Finally, it is also possible to target other proteins responsible for stabilising the MIF trimer, like Heat Shock Protein 90, as is the case of 17-(alkylamino)-17-(demethoxygeldanamycin). This approach also benefits from the fact that other pro-inflammatory factors are stabilised by this protein, magnifying a drug's potential effect.

The virtual lab

The simplicity of small molecules makes them prime candidates for Computer Aided Drug design (CADD), highlighting once again the power of working in a cross-disciplinary research environment. Using this technique, the cancer fighting properties of never before seen compounds, can be assessed long before they are even synthesised, allowing researchers to bypass potentially expensive and time consuming projects.

In a fashion that is both faster and cheaper than the drug discovery of old, thousands of compounds are screened for their potential usefulness and are sieved by algorithm design to find patterns that equate to clinical effectiveness. Once the virtual world has yielded a great diversity of potentially effective new drugs, they can then be synthesised and assessed in the real world.

> **'The opportunity to make a positive impact on the lives of patients is the key driving force behind our research group'**

The physical lab

In a world of perfect computational models, the entire process of drug discovery could be conducted in the black and white realm of computer programs. Unfortunately, although both commercial and open source CADD software are improving their ability to mimic and predict the real world, there is still no substitute for lab work.

Cell culture studies and animal models are an invaluable part or the team's efforts. These experiments provide a picture of the sometimes unforeseeable behaviour that compounds exhibit in actual living systems. Cell culture work allows researchers to probe the intricacies of a drug's interaction with the multitude of intracellular metabolic and signalling pathways, confirming software predictions more often than not. Animal models yield insights into how drugs behave in complex biological systems, as well as into how normal bodily function arises. Using genetically engineered animals has allowed researchers to shed light on the actual mechanism of MIF's action – a non-trivial feat, given the protein's inherently complex nature.

Meet the researchers

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Professor Seamas Donnelly is the Head of Medicine at Trinity College Dublin and leads the Translational Medicine Group. He holds an Honorary Professorship from the University of Edinburgh and has obtained significant external funding from the Wellcome Trust, Science Foundation Ireland (SFI), the European Union and other national and international funding bodies. He was one of the first clinicians to be awarded a Science Foundation Ireland's (SFI) Principal Investigator Programme award. A leader in medical research, he specialises in small molecule anti-inflammatory agents, with a recent focus on their role as possible cancer treatments.

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A SYNERGISTIC IMMUNOTHERAPY FOR SKIN CANCER

Professor Iraldo Bello-Rivero, of the Centre for Genetic Engineering and Biotechnology, Havana, Cuba, has conducted several clinical trials to investigate the potential of a synergistic interferon combination therapy for the treatment of skin cancers.

Skin cancer – current therapies and challenges

Cancers of the skin are one of the most common types of cancer globally and their incidence has increased in recent decades. The vast majority of skin cancers arise as a result of exposure to ultra-violet radiation, most commonly in the form of sunlight, or through the use of tanning beds. However, the increased incidence is also attributable to an aging global population, depletion of the ozone layer and improved rates of detection. Light skinned, Caucasian populations in countries that receive a lot of sunlight, such as Australia, are at higher risk, but skin cancers are prevalent across all ethnicities. There are several forms of skin cancer, including basal cell carcinoma (this is the most common form, at 80–90% of all skin malignancies), squamous cell skin carcinoma and melanoma. Professor Bello-Rivero focuses on the treatment of non-melanoma skin cancers. Basal cell carcinoma can present as a raised bump or crusty lesion, whereas squamous cell skin carcinoma can present as thickened skin with redness or scaling. These most commonly present on sun-exposed skin, such as that of the neck, shoulders and face. Basal cell carcinoma and squamous cell skin carcinoma can present in difficult to treat areas, such as around the eye or on the eyelid, where surgical removal would require specialised reconstructive techniques to preserve eyelid function. Cancer in this location is known as periocular carcinoma. Periocular basal cell carcinoma rarely undergoes metastasis (where it spreads and forms tumours elsewhere in the body), but can cause significant morbidity when it arises on the eyelids. Mycosis fungoides is another type of skin cancer, which arises as a result of lymphoma in white blood cells associated with the skin, called CD4 T cells. This causes rashes and lesions of the skin.

The treatment of skin cancer is patient specific and depends on the type of cancer, its location, whether it is a new growth or is a recurrent cancer, whether the cancer is treatment-resistant (to chemotherapy, for instance) and the age of the patient. Many types of skin cancer can be treated successfully with surgery, chemotherapy, radiotherapy or cryotherapy. The current gold standard treatment for many skin cancers is surgical removal. However, this may not always be possible or desirable, because of cosmetic concerns. Surgery can cause permanent scarring and disfigurement and for certain facial cancers other treatments are often preferred. Professor Bello-Rivero is particularly interested in the treatment of 'difficult' skin cancers, for which other treatment modalities are not suitable, either as a result of treatment resistance or cosmetic concerns which prohibit surgery.

Interferon therapy in cancer treatment

Another treatment option for skin cancer patients is interferon therapy. Interferons are pleiotropic cytokines. That is, they are substances that are naturally secreted by immune cells and exert effects on multiple cell types in the body, as part of immune responses. A range of interferons have been identified and several have been trialled as potential anti-cancer therapeutics. Interferons are thought to exert anti-cancer action through a variety of mechanisms. These include the direct effects of interferons on cancer cells to reduce their proliferation and survival, their action on immune cells which then go on to attack the cancer in an anti-tumour immune response, and their involvement in processes that are vital to tumour growth and survival such as blood vessel growth, which is termed angiogenesis. Professor Bello-Rivero explained to Scientia why he thinks interferons are promising as anti-cancer therapeutics: 'Interferons are naturally designed proteins with fabulous properties that impact all the organs and tissues of the body. They are very powerful pleiotropic homeostatic proteins that are yet not well understood.' Professor Bello-Rivero has extensive experience with interferons. He is credited with discovering the serum soluble interferon gamma receptor, which led to a new paradigm that this receptor could potentially function as an antagonist of interferon gamma.

To date, interferons have been used with varied success in the treatment of a variety of cancers. However, in cases of aggressive basal cell carcinoma the use of single interferon treatments has not yet been shown to be effective. Patients with aggressive basal cell carcinoma or squamous cell skin carcinoma tend to suffer high levels of tumour recurrence and secondary primary tumours and consequently there is still an unmet clinical need in this patient population. While the anti-tumour potential of interferons is well known, there has not been a breakthrough in interferon cancer treatments in the last decade.

HEBERFERON – a synergistic interferon formulation

Efforts to change or enhance the effects of interferon treatments have previously involved combining more than one type of interferon. However, to date, these combinations have not always been rationally conceived to maximise efficacy, by capitalising on known synergistic effects. Professor Bello-Rivero has identified a combination of interferons with proven in vitro synergistic activity, which he decided to trial in the clinic. He discussed his experience with interferons and motivation for this combination therapy: 'I have been involved in the study of interferons from more than 25 years. I decided to improve the clinical impact of the use of interferons in the treatment of cancer exploiting the synergism between these two very potent molecules, in a rational way not previously tried.' This strategy involves combining two different interferons, interferon alpha and interferon gamma, in one treatment. Professor Bello-Rivero has conducted extensive research on the interaction of interferon alpha and interferon gamma receptors and discovered that they interact with a likely common protein in the interferon alpha and/or interferon gamma receptor complex. This was a surprising discovery at the time, and it was contrary to mainstream scientific thought, which made it challenging to get these results published. However, in 2001 the paper detailing this interaction was finally published in Biochemical and Biophysical Research Communications.

'Interferons are naturally designed proteins with fabulous properties that impact all the organs and tissues of the body'

A combination of interferon alpha and interferon gamma has previously been shown to produce synergistic effects in reducing the proliferation of several cancer cell lines. Interferon alpha and interferon gamma share similar biological activities and it is thought that this may underlie the reinforcing effect that they have on each other. In vitro results suggest that these interferons may affect similar genes, albeit through different mechanisms, such as the activation of different protein receptors, thereby preserving the specificity of each interferon but providing a means for synergy. One research group found that when mouse cells were treated with interferon gamma that interferon alpha receptors were cross-recruited and became activated and other research groups have found that interferon alpha and interferon gamma receptors move closer together and become associated in the presence of interferon alpha and interferon gamma. Therefore, considering the results of Professor Bello-Rivero and other research groups, there is tangible evidence of interferon alpha/interferon gamma crosstalk, mutual activation and synergistic anti-cancer efficacy. Consequently, this interferon combination represents a promising anti-cancer treatment modality.

Results to date with HEBERFERON

Professor Bello-Rivero and his research team have formulated a combination interferon alpha/interferon gamma therapeutic for the clinical treatment of skin cancers, called HEBERFERON. The team has applied the therapeutic in a number of clinical trials to assess its potential. Here, we discuss some of their key results, to date.

In 2009, the team published the results of an early trial for the HEBERFERON treatment of patients with basal cell carcinoma and squamous cell skin carcinoma, which was advanced, recurrent and was resistant

to previous treatments. This specific trial had the primary goal of assessing treatment safety but the rationale was to find an effective non-surgical therapy for this patient subtype, who had exhausted other therapeutic options. Patients received injections of HEBERFERON into or beside cancerous lesions, three times a week, for three weeks. Although the patient cohort was small (16 patients), 46.7% of the HEBERFERON-treated patients experienced a complete response to the therapy and 40% experienced a partial response. Given that this is a treatment-resistant patient sample, these results are very encouraging. Side effects were largely well tolerated and included chills and fever, which is typical for interferon therapy.

In a slightly larger trial (InCarbacel-II) the team compared HEBERFERON treatment of basal cell carcinoma with individual interferon treatments, to assess if synergistic efficacy was possible. They found that more patients treated with HEBERFERON responded to therapy, compared with those treated with individual interferons, including a complete response rate of 42% in the HEBERFERON treatment group. They also found that combination therapy produced a more rapid and prolonged effect, compared with treatment with individual interferons. Patients who responded to the therapy were left with excellent cosmetic results, compared with surgery and associated reconstructive procedures.

In a third small trial, the team focused on assessing the potential of the formulation in treating periocular cancer (basal cell carcinoma or squamous cell skin carcinoma), which is difficult to treat, as surgery is often precluded or difficult without causing damage to the eyelids. After 12 weeks of treatment 47.6% of patients experienced a complete response and 23.8% experienced a partial response. Another clinical trial to assess the potential of HEBERFERON treatment in patients with mycosis fungoides has also been carried out by the team. All patients received a single high dose of HEBERFERON, intramuscularly, to assess the pharmacodynamic and pharmacokinetic parameters of the drug. Interferon inducible markers were measured in the blood over the next 96 hours. The drug was largely well-tolerated and produced an increase in interferon inducible markers that was greater than conventional individual interferons, showing that it has the potential to be efficacious in this patient cohort. Further efficacy studies are required to determine the potential of the drug to alleviate or resolve mycosis fungoides in affected patients.

‘We are now receiving interest and international recognition for our work, and several groups are interested in using HEBERFERON to perform molecular biology studies, or to combine it with other products to see the impact on the treatment of patients with cancer’

Future work

The HEBERFERON formulation has been approved in Cuba for the treatment of basal cell carcinoma, based on the extremely encouraging results achieved to date. However, the team would like to undertake larger trials to further assess its potential. Professor Bello-Rivero tells Scientia about his plans for future research and collaboration. Financial support and new collaborators are key for undertaking further trials: ‘We are now receiving interest and international recognition for our work, and several groups are interested in using HEBERFERON to perform molecular biology studies, or to combine it with other products to see the impact on the treatment of patients with cancer. We would like to perform additional clinical studies to further determine the efficacy and safety of this formulation in the treatment of skin cancer. To achieve this we would like to find partners, and collaborate with other groups or companies, because this is a costly route for the treatment of cancer patients.’

Meet the researcher

Professor Iraldo Bello Rivero
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Professor Iraldo Bello-Rivero obtained his Ph.D. from Havana University (2005). He carried out fellowships with Dr Michel Aguet (1988) at the Institute of Immunology and Virology, Zurich, Switzerland, with Dr Erik Lundgren (1994) at the Department of Molecular Biology, Umea University, Sweden and with Dr Marco Soria (1995) at Department of Biological and Technological Research, San-Raffaele Scientific Institute, Italy. He was also the head of the Clinical Trial Laboratory at CIGB between 1994 and 2005. He has published more than 25 papers in reputed journals and received Cuban Academy of Sciences Awards in both 1990 and 2006 and a Public Health National Award (Cuba) in 2010. He is author of three patents and is the product manager at CIGB.

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KILLING CANCER CELLS WITH NOVEL DRUG THERAPIES

Given the complexity of cancer as a disease, its increasing incidence and consequent effect on patient mortality and morbidity, the development of new anti-cancer drugs is of the utmost importance. This is particularly true given the terrible side effects and often poor effectiveness of existing therapies.

Classical chemotherapeutic drugs are specific only in that they target actively dividing cells. This would be fine if the only actively dividing cells in the body were found in the tumour. However, high levels of cell division occur in a number of tissues including our hair follicles (hair loss is a frequent side effect of chemotherapy) and our bone marrow (immune suppression is also a common side effect of these drugs). In addition to causing serious side effects, classical chemotherapy drugs are not always particularly effective – cancers can become resistant to their action and they are often so toxic that effective doses cannot be used. Consequently, developing newer anti-cancer agents is a highly active area of research. In this section of the magazine we showcase the work of four scientists – each striving to improve our drug treatment options for cancer. In the first article of this section, we discuss the research of Dr Catharine Smith, an Associate Professor at the University of Arizona. Professor Smith studies the fundamental role of histone deacetylases in cancer, and looks at the downstream effects of inhibiting such enzymes using drugs. Two types of drug in particular, called HDACis and KDACis, show huge promise as treatments for combatting cancer. Next, we introduce Dr Peter Houghton of the Greehey Children's Cancer Research Institute at the University of Texas Health Science Center in San Antonio. Dr Houghton's research involves implementing preclinical trials to identify new drugs that may be effective and less toxic treatments for childhood cancers. To do this, his research team use a variety of preclinical models, including immune deficient mice and canine models, and by doing so they have identified over 80 new drugs or drug combinations for the treatment of these diseases.

The third researcher in this series is Professor Paul Dent – a leader in the field of applied translational cancer research who works at Virginia Commonwealth University. Here, we detail a clinical trial undertaken by Professor Dent and his colleagues, to test the safety of a new combination of anti-cancer drugs, which has shown highly promising results so far. Finally, we examine the work of Professor Jatinder Lamba, of the University of Florida, who studies the genetic basis for inter-individual variability in the response to anti-cancer drugs, and in particular in the response of patients with acute myeloid leukaemia to antibody therapies. Understanding the basis for this variability could help to tune treatments for personalised medicine.

TARGETING AND ENHANCING THE EFFECTS OF DEACETYLASE INHIBITORS IN CANCER TREATMENT

Dr Catharine Smith is an Associate Professor at the University of Arizona. Her interest in the actions of lysine deacetylases (KDACs, also known as histone deacetylases or HDACs) led her to join the University of Arizona Cancer Centre and translate her research to aid the clinical treatment of diffuse large B-cell lymphoma (DLBCL)

I'd like to start by getting an idea of your background and how you came to be interested in KDACs

My background is in epigenetic mechanisms of transcription using steroid receptors as a model system, so epigenetic regulatory proteins like KDACs are of great interest to me.

Due to the availability of small molecule inhibitors of KDACs, we were able to quickly ascertain that KDAC inhibition impaired the ability of the steroid receptor GR to activate the mouse mammary tumour virus promoter. This was contrary to expectations from the established model that KDACs oppose transcriptional activity and suggested that the role of KDACs in gene expression was more complicated than initially thought.

How is your research continuing to explore the (perhaps unexpected) roles of KDACs in transcription and other cell processes?

Models of the role of lysine acetyltransferases (KATs, also known as histone acetyltransferases or HATs) and KDACs in transcriptional regulation tend to focus on histones as their targets, however more researchers are beginning to acknowledge a role for acetylation of non-histone proteins. Many transcriptional regulatory proteins are now known to be acetylated, however in most cases the function of acetylation is unknown. In addition, several thousand proteins in multiple cellular compartments have been identified to be acetylated through proteomic studies. The localisation of these proteins and deacetylases suggests that they are likely to be involved in regulation of cellular processes other than transcription, such as metabolism. Although there are many unknowns, it is an exciting time to be in the field.

With this challenging topic, what was your motivation for translating your research to a clinical setting?

About 10 years ago I got to a point in my career at which I wanted to see my research benefit people suffering from disease within my lifetime. With several KDAC inhibitors being approved for use in humans, mostly to treat lymphoma, I saw an opportunity to contribute. I joined an interdisciplinary group of researchers at the University of Arizona Cancer Centre, who focus on finding new treatment strategies for lymphomas that have proven to be difficult to treat, including particularly aggressive forms of diffuse large B cell lymphoma (DLBCL).

How is understanding KDACs critical to the clinical use of KDACis?

Understanding the actions and cellular functions of KDACs is crucial for developing the most effective uses of their inhibitors (KDACi) in humans to treat various diseases. For example, as cancer therapeutics KDACi are not very effective unless given in combination with other therapeutics. Because we lack a comprehensive understanding of the functions of KDACs in various cell types, it is difficult to predict which therapeutics can be combined effectively with KDACi.

Our lack of knowledge also means that many side effects of KDACis are not well understood. For example, the drug Depakote is a KDACi used clinically for epilepsy for close to 40 years, however it causes unexplained metabolic and reproductive problems in about 50% of users. More research could contribute to better dosing strategies or more selective deacetylase inhibitors to alleviate or minimize some of these side effects.

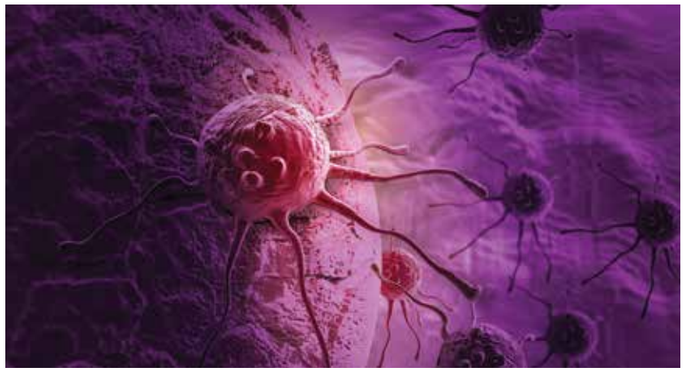
How will your research in KDACs translate to clinical treatment?

We developed a cell-based model of sensitivity and resistance to KDAC inhibitors using DLBCL cell lines that share characteristics with aggressive forms of DLBCL. We are using this system to explain the variable efficacy of these drugs in the DLBCL context. The knowledge gained from our model system will allow rational selection of companion therapeutics that would sensitize aggressive DLBCL to the cytotoxic effects of deacetylase inhibitors. We have already identified one effective combination and are working to secure grant funding to evaluate it in the clinic.

How has your collaboration with the Southwest Oncology Group furthered your research into effective DLBCL therapies?

Dr Persky from the Southwest Oncology Group has conducted several clinical trials that evaluated KDAC inhibitors in DLBCL treatment, so we have a common interest in the effective use of these drugs. This collaboration has really taught me about patient issues and helped me translate my research to a clinical setting, and our teamwork led to a recently approved grant application to the Hope Foundation.

This grant will take advantage of next generation sequencing (NGS) technologies to examine the mutations and gene expression profiles of DLBCL patients treated with R-CHOP, the standard DLBCL treatment, and vorinostat, a KDACi. We will compare the markers and patterns that correlate with patient clinical outcome to those from a trial in which patients were not exposed to vorinostat. The verification of biomarkers in further studies would help clinicians to determine which DLBCL patients might respond to the inclusion of KDACis in their treatment.



UNDERSTANDING THE ROLE OF DEACETYLASES ON THE PATH TO TREATING CANCER

Enzymes that deacetylate proteins are important regulators of cell processes, and their inhibitors are used in medical treatments to sensitise and destroy specific blood cancers. Dr Catharine Smith from the University of Arizona Cancer Centre has been extending her fundamental research on deacetylases to clinical studies. This research aims to identify mechanisms of deacetylase inhibitor action and biomarkers of clinical response that have potential to increase the efficacy of these drugs and identify cancer patients that would most benefit from their inclusion in treatment.

What are HDAC/KDACs (and what do they do)?

Many of the proteins in living cells are in a constant state of flux – not only their concentrations, but also in terms of how they act and appear to other proteins. Most cellular proteins are constantly tagged and untagged with a variety of molecules, in a process called post-translational modification. These chemical tags act as signals that determine what the protein should be doing, what it should be targeting, or where it should be going. Acetylation is one such tag, about twice the size of a water molecule, which is attached and removed from lysines within proteins by acetylation and deacetylation enzymes, respectively.

Traditionally, the enzyme groups: histone acetyltransferases (HATs) and histone deacetylases (HDACs), have been established to add/remove acetyl tags from histone proteins. As histones are responsible for the packaging of DNA into chromatin, the modification of these proteins plays an important role in regulating the expression of genes. However, recently the targets of HATs and HDACs have been found to include a large number of non-histone proteins, and are now increasingly referred to as lysine acetyltransferases (KATs) and lysine deacetylases (KDACs) to reflect the broader range of their protein targets. The expansion of possible KAT and KDAC targets has led researchers to believe that these enzymes are involved in a far greater range of cellular processes than once thought, for example, having roles in metabolism and signalling.

How can they cause cancer?

Many types of cancer involve the misregulated expression of typically helpful genes, and naturally KAT and KDAC enzymes have been implicated in the formation of several cancer types. For example, several KATs have been found to be mutated and inactivated in a variety of cancers. In addition, KDACs have been shown to be present at abnormally high levels in cancer cells. Together these findings indicate the level of cellular protein acetylation becomes unbalanced in cancer. It is very likely that misregulated acetylation of select proteins, rather than all proteins, contributes to the development or progression of cancer and identifying them is a key goal of research in this field.

How HDAC/KDAC inhibitors (HDACis and KDACis) can be used to treat cancer

The association of increased KDAC levels with cancer led researchers to find direct solutions to this problem by screening for small molecule inhibitors of KDACs. Three (vorinostat, romidepsin, and belinostat) have been FDA approved for the treatment of advanced cutaneous or peripheral T Cell lymphomas (CTCL or PTCL). These KDAC/HDAC inhibitors (or HDACis) appear to be effective against this cancer type by reducing the cancer's growth and survival by a mechanism that is not fully explained.

Despite the effectiveness of HDACis in treating CTCL or PTCL, they are relatively rare forms of cancer, and researchers and clinicians have attempted to expand the use of HDACis to the treatment of other blood cancer types, with the hypothesis that the effects of HDACis would be useful for sensitising these cancers to combinatorial treatments.

One of the most common forms of blood cancer is diffuse large B-cell lymphoma (DLBCL). Although initial treatment of this cancer is effective, 40% of patients relapse within 5 years of diagnosis. Multiple clinical trials have been designed to determine whether combinatorial treatments that include HDACi will prevent or reverse relapse. Although some of these trials are ongoing, the results suggest that specific patient characteristics impact the efficacy of HDACis. This highlights the need for biological markers (or biomarkers) that are predictive of patient response to HDACis.

How the gaps in KDAC knowledge affect fundamental and clinical research

As described above, there are numerous gaps in the knowledge of KDACs that stem from the unknown range and involvement in regulation of many cellular processes. These holes compound the difficulty of clinical research with KDACi, where data indicates that the activity of KDACs is being blocked in

patients but this does not predict whether these patients will respond with tumour regression. It is important for researchers to understand the fundamental functions of KDACs in both normal and cancer cells, as this will refine the pool of drugs and other molecules to mix with KDACis in cancer treatment, and enable the focused selection of patients sensitive to these drugs

Dr Catharine Smith's research

Dr Catharine Smith is an expert in fundamental KDAC research, who became interested in KDACs due to their interactions with glucocorticoid receptor (GR) and their function in regulating gene expression. An early discovery saw her turning the established KDAC models on their head, by showing that KDACs increased gene expression induced by GR, rather than the more commonly held belief that KDACs act universally to reduce gene expression. She continues her fundamental research by using biochemical approaches combined with single cell microscopy to examine the effects of KDAC inhibition on downstream gene expression in extensive detail.

Translating fundamental KDAC research to the clinical side

About 10 years ago Dr Smith saw an opportunity to help people by contributing her expertise to the clinical assessment and treatment of HDACi-sensitive cancers. She joined an interdisciplinary group at the University of Arizona Cancer Centre, and integrated her research with the team's to improve treatments for cancer types that have remained resistant to traditional approaches, such as DLBCL. Within the team, Dr Daniel Persky from the Southwest Oncology Group was also interested in the use of HDACis, and had run several HDACi trials on DLBCL patients. The alignment of Dr Daniel Persky and Dr Catharine Smith's interests enabled them to re-focus their research by taking an integrated approach, from the fundamental cellular functions of KDACs all the way to the specific use of KDACis in clinical treatments.

The tools of Dr Smith's lab

The collaboration with Dr Persky enabled Dr Smith to re-align her research with clinical goals in mind. To this end her lab developed cell culture models for DLBCL, with multiple cell lines exhibiting either resistance or sensitivity to HDACis. These models enable her team to define the mechanisms that make cells resistant or sensitive to HDACis and then use that knowledge to predict which other drugs might be combined with HDACi to overcome resistance. The advantage of using a lab model is that research progresses much faster than in clinical trials, and treatment solutions can be assessed quickly. For example, Dr Smith's group found that, in resistant cell lines, HDACi affected the activity of cyclin-containing complexes, which are involved in the division and growth of cells. This finding suggests that drugs which impact the action of these complexes might be combined with HDACi to make them more efficient in killing cancer cells.

This collaboration has formed an integrated research flow, from fundamental lab-work to the treatment of patients, and cycling back again to inform the next fundamental questions

The use of patient data for genetic research

The partnership took another step forward with a successful grant from the Hope Foundation. This grant enables Dr Persky and Dr Smith to utilise next generation sequencing techniques to analyse patterns of gene expression and mutations in DLBCL patient tumours. By comparing tumours from patients treated with a traditional therapy (called R-CHOP), to those from patients treated with both R-CHOP and HDACi, the team will look for patterns of gene expression or mutations that tend to associate positively or negatively with a patient's response to the treatments. The eventual goal is to then validate these patterns (called biomarkers) for their ability to predict the efficacy of KDACi treatment in patient groups. These biomarkers may then feed back into the fundamental research, giving clues as to the specific actions of KDACs in the complex environment of human DLBCL tumours.

The future for HDAC research and clinical therapy

The initiative of Dr Catharine Smith in joining with members of the Southwest Oncology Group and The Arizona Cancer Centre has formed an integrated research flow, from clinical trial data to fundamental lab work, and cycling back again to inform the next fundamental questions. This could translate to more effective cancer treatments in the future, including treatments tailored specifically to the patient, dependent on the specific genomic characteristics of their tumour. As the varied functions of KDACs are revealed, future research could use this as a basis for understanding acetylation in more diverse diseases.

Meet the researcher

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Dr Catharine Smith received her PhD in Biochemistry from the University of Vermont, did her postdoctoral work at the National Institutes of Health, and is now an Associate Professor in the University of Arizona College of Pharmacy and a member of the University of Arizona Cancer Center and Bio5 Institute, USA. Her research focuses on epigenetic mechanisms of gene expression, and in particular their regulation through signalling pathways and modulation by anti-cancer drugs.

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NOVEL THERAPEUTICS FOR CHILDHOOD SOLID TUMOURS

Dr Peter Houghton's group implements preclinical trials to identify new drugs that may be effective and less toxic treatments against childhood cancers. From patient-derived xenografts in immunodeficient mice to utilising canine cancer patients as models, they have identified over 80 new drugs or drug combinations for the treatment of these diseases.

Solid tumours in children

Approximately 12,400 cases of cancer are diagnosed in the U.S. annually in individuals under 20 years of age. Solid tumours comprise approximately 30% of all childhood cancers. There are critical and unique aspects surrounding the study of paediatric tumours as compared to adult tumours, one of which is that many of the most common paediatric cancers are rarely observed in adults. Some of these include osteosarcoma, rhabdomyosarcoma, Ewing's sarcoma, retinoblastoma, Wilms' tumour, and neuroblastomas. Complicating the discovery of novel therapeutic options for these tumours are their low incidence rate, exponentially increasing the time for successful clinical trials to take place. For instance, only approximately 250 cases of Ewing's sarcoma are diagnosed in the US annually. Currently available chemotherapeutic or surgical protocols, while curative in the majority of patients, are often toxic and invasive, calling for further advancement in the field of therapies to treat childhood cancers.

Dr Peter Houghton, a researcher at the University of Texas Health Science Center, focuses on improving treatments for these paediatric solid tumours. Since the 1940s, when the first chemotherapeutic and radiation protocols that aimed to improve the prognosis of cancer patients were implemented, researchers have been striving to develop new approaches to battle neoplasia. Frequently used chemotherapeutic agents in paediatric cancers currently include vincristine, cyclophosphamide, cisplatin, irinotecan and others. While many protocols using these agents in combination with radiation therapy have dramatically improved cure rates, many of them have deleterious side effects, including marked immunosuppression leading to infections, clotting disorders, malnutrition, and long-term sequelea such as cardiac dysfunction and for survivors of brain tumours, cognitive impairment. These side effects may be especially significant in young children where brain development is ongoing. Fewer than 15% of anti-cancer therapies indicated for use in adults are also approved for use in children, further limiting drug options for practicing oncologists. Further, chemotherapeutic or radiation therapy is often coupled with invasive surgical resection, providing increased opportunities for secondary post-operative bacterial infection and slow healing times. While overall national cure rates for childhood cancers is 70%, and 5-year disease-free survival is approaching 80%, the success rate is not nearly as high for patients with advanced disease or where the cancer has metastasised. It is for these patients where exploration of alternative therapeutic protocols is required. However, as a consequence of the rarity of these cancers, the high cure rate, and the prolonged disease-free survival of current clinical treatment, relatively few patients are eligible for new experimental therapies. Thus, approaches to develop preclinical models of paediatric cancer that can identify effective new agents and combinations have been a focus of Dr Houghton's research.

Patient-derived xenografts

One recent area of research has been the development and characterisation of patient-derived xenografts (PDX). Xenografts are the implantation of tissues or organs from the donor of a different species from the recipient. Many xenotransplantation efforts have focused on the transplant of tissues and organs from pigs and non-human primates into human patients. However, Dr Houghton's work focuses on the use of patient derived tumour xenografts. These are created by transplanting cancerous tissues directly from the patient into immunodeficient mice to more accurately mimic the physiologic environment and histopathology appreciated in patient tumours. For these studies, it is crucial the mice do not have functioning immune systems so that they do not reject the transplant. Several strains of immunodeficient mice exist for these studies, including athymic nude mice, severely compromised immune deficient (SCID) mice, and non-obese diabetic SCID (NOD-SCID) mice. After the tumour specimens have

Dr Houghton's research has led to the development of approximately 100 PDX models representing a multitude of childhood cancer types and the subsequent development of several preclinical drugs used in clinical trials.

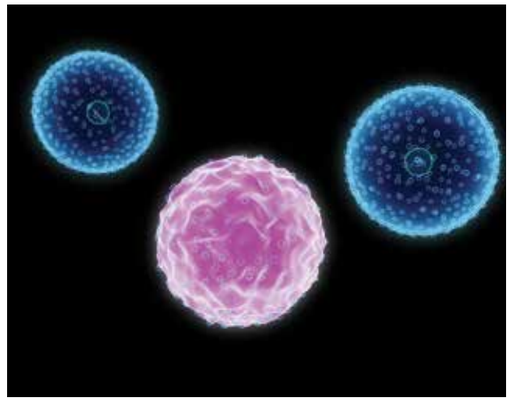
successfully grown, they are harvested and implanted into further generations of mice to study the molecular fidelity of the graft, as well as its biologic characteristics. These models have also been used extensively to identify novel therapeutic agents for treating childhood cancers.

Dr Houghton's research has led to the development of approximately 100 PDX models representing a multitude of childhood cancer types and the subsequent development of several preclinical drugs used in clinical trials. Using these PDX models, they have identified a class of drug (camptothecins) as successful in the regression of sarcomas and neuroblastomas, as well as other cancer types. One of the camptothecin drugs, irinotecan, is now incorporated into many protocols for the treatment of childhood solid tumours worldwide, exemplifying the translation of preclinical results to clinical trials and effective clinical outcomes. To date, Dr Houghton and his group have published over 66 papers on the activity, metabolism, and clinical relevance of camptothecins in paediatric solid tumours.

The importance of the insulin growth factor axis in neoplastic inhibition

Early studies using PDX models identified the insulin-like growth factor axis as important for tumour progression in several types of childhood sarcoma. In one study, Dr Houghton's lab successfully inhibited type 1 insulin-like growth factor receptor (IGF-1R), a common receptor often actively involved in signalling in childhood sarcomas, in conjunction with rapamycin, an immunosuppressive drug that blocks mammalian Target of Rapamycin complex 1 (mTORC1), downstream of IGF-1R. Capitalising on the antitumour properties of CP-751, 871 (Figitumumab), a human antibody blocking IGF-1R ligand binding, in concert with effects of rapamycin (also known as sirolimus), Dr Houghton and colleagues demonstrated a synergistic effect on tumour cell death in 5 of 13 xenograft models examined and elicited tumour remission in rhabdomyosarcoma and osteosarcoma models.

Currently, Dr Houghton's lab is focusing on identifying resistance mechanisms to therapeutics targeting the insulin growth factor axis and further identifying the signalling pathways in the maintenance of malignant phenotypes. It is unclear whether or not these drugs lose efficacy due to the selection of a pre-existing clone within the patient or through a therapy-induced mutation. Further, Dr Houghton and colleagues aim to identify biomarkers that may allow for future patient stratification based on potential for the presence of drug resistance. Expression profiling of these xenografts may allow for this in the future.



Dr Houghton's lab successfully inhibited type 1 insulin-like growth factor receptor (IGF-1R), a common receptor often actively involved in signalling in childhood sarcomas, in conjunction with the immunosuppressant rapamycin.

The Houghton lab collaborates with individuals from The Ohio State University, Tufts University and University of Maryland on this project. Resources provided by the Program Project Grant to Greehey Children's Cancer Research Institute are comprised of three cores. One core, based at OSU provides communication and biostatistical support and another, at GCCRI provides the xenograft and cell line for *in vitro* and *in vivo* mouse models. The third, at Tufts University, the Comparative Animal Core, employs a translational approach using canine osteosarcoma patients for drug evaluation. This core provides canine tumour specimens and expertise in histopathologic examination of the tumours. They also evaluate the novel therapeutics of interest in these animals.

The Paediatric Preclinical Consortium

Building upon the in vitro and in vivo mouse model studies Dr Houghton's group has focused on, the National Cancer Institute implemented the Pediatric Preclinical Testing Program (PPTP). The PPTP comprised a consortium of investigators in the US and Australia that used PDX models for testing new therapeutic agents against not only childhood solid tumours, but also brain tumours and acute lymphoblastic leukaemia models. To date, over 80 therapeutic agents have been tested against 83 models of childhood cancers and these studies have successfully identified drugs and drug combinations currently in clinical trial. New models are characterised via exome sequencing, gene expression profiling and single nucleotide polymorphisms. Agents and combinations that have moved into clinical trials, based in part on PPTP studies, include IGF-1R-targeted antibodies, the combination of a rapamycin derivative with standard cytotoxic chemotherapy, as well as other biologic and small molecule agents.

By continuing to collaborate with other cancer research groups worldwide through open web portals, genomic data spanning numerous cell lines and stemming from both in vivo and in vitro studies may be centralised in one location. Several open web portals exist providing cancer genomic data to the public, much of it from the Tumor Cancer Genome Atlas (TCGA). Using these resources, researchers may quickly access information on a gene of interest, neoplastic pathways, and cancer therapy sensitivity or resistance patterns. One use of relating tumour sensitivity to genomics databases is to identify the underlying mechanism for 'exceptional responders', those rare tumours that demonstrate a dramatic response to a drug. Such genomic signatures may then be used as biomarkers to identify patients who may benefit from that treatment. Breast cancer with biallelic mutations in BRCA genes, for example respond better to PARP inhibitors.

Brain tumours

An example of an 'exceptional responder' was identified in the PPTP screen. Specifically, an astrocytoma PDX model completely regressed upon treatment with the MEK inhibitor, selumetinib (AZD6244). Low-grade astrocytomas are associated with BRAF activation and/or mutation and are the most commonly diagnosed neoplastic disease of the central nervous system in children. Utilising genotyping and expression profiling through deep sequencing, Dr Houghton and his group are using astrocytoma xenografts as a model to investigate the possibility of BRAF mutation as a potential drug target. More recently selumetinib, and a BRAF inhibitor dabrafenib have shown promising clinical activity against low-grade glioma in children. This has indications for clinical trials examining novel therapeutics against paediatric astrocytomas in the future. While most of these astrocytomas are considered low-grade tumours, many are inoperable and require chemotherapy and radiation as surgical excision is not a viable option due to the fragile structures frequently involved within or adjacent to the tumour. For this reason, adjuvant or combination therapy is frequently indicated, namely chemotherapy and/or radiation therapy. Preliminary results in the astrocytoma model indicate that selumetinib may enhance radiation sensitivity selectively in tumours with BRAF aberrations. Potentially, this may allow lower doses of radiation therapy to be used in curative treatment with decreased long-term toxicity which is largely a consequence of radiation treatment.

Future work

Dr Houghton and his group of graduate students and post-doctoral students will continue making strides towards the identification of novel drugs or drug combinations in the treatment of paediatric solid tumours and other neoplastic diseases, such as acute lymphoblastic leukaemia. With new advances in whole genome and exon sequencing, the identification of biomarkers using patient derived xenografts to monitor potential efficacy or resistance will help accelerate these drugs into clinical trials.

Meet the researcher

Dr Peter Houghton
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Dr Peter Houghton completed his Bachelor's degree at the University of Bradford in the UK, where he studied pharmacology. He then assumed a PhD position in biochemistry and pharmacology at the Institute of Cancer Research at the University of London. He was Chair, Molecular Pharmacology at St. Jude Children's Research Hospital for 17 years and currently serves as the Director of the Greehey Children's Cancer Research Institute (GCCRI), and holder of the Greehey Distinguished Chair for the Children's Cancer Research Institute. Primarily, Dr Houghton's group aims to elucidate the mechanisms of tumor initiation and progression in children and uses this knowledge to identify less toxic drugs with higher cure rates. Houghton has largely been funded through the NIH for over 30 years and has directed NIH program grants for over 25 years.

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DEVELOPING CANCER KILLING COMBINATIONS

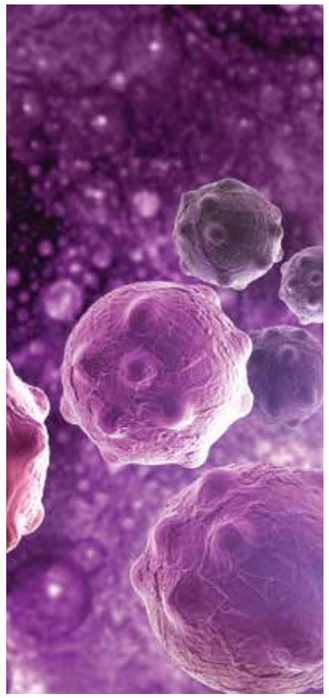
Professor Paul Dent is committed to taking an idea from the bench to the bedside. He is doing just that by designing clinical trials that investigate how drugs can combine and synergise to kill tumour cells.

Maximising the potential of cancer treatments

Professor Paul Dent and his colleagues at the Massey Cancer Centre are leaders in the field of applied translational cancer research. Over the last decade, Professor Dent and his team have researched and contributed novel treatment strategies for a number of different forms of cancer, including hepatoma, soft tissue sarcoma, glioblastoma, pancreatic carcinoma, renal cell carcinoma and breast cancer. Other trials have been open to include all solid tumor patients. This work continues today and focuses on the development of therapeutic interventions which combine two or more drugs to kill tumour cells. The two primary drugs being investigated in clinical trials are pemetrexed and sorafenib. So what are these drugs and how do they work?

Pemetrexed is an anti-folate drug which is used in the treatment of advanced and metastatic non-small cell lung cancer. Anti-folate drugs block the action of folic acid which then inhibits cell division and the production of proteins, DNA and RNA. Specifically, pemetrexed inhibits an enzyme called aminoimidazole-carboxamide ribonucleotide formyl-transferase (AICART). This leads to a series of biochemical events which ultimately stimulates autophagy (the destruction of damaged cells).

Sorafenib is a multi-kinase inhibitor which is used to treat liver and kidney cancers. Multi-kinase inhibitors block the action of certain enzymes (or kinases) which add phosphate groups to particular proteins in a process known as phosphorylation. This is important because the overexpression of these kinases can cause a number of diseases, including cancer. Sorafenib works by inhibiting a proto-



Ongoing preclinical experiments show that it could be possible to pinpoint exactly how the cancer cells are developing resistance to therapies, which might eventually allow oncologists to develop in real time a personalised therapy designed to overcome drug resistance in an individual patient's tumour

oncogene called RAF-1. Proto-oncogenes are genes which could potentially cause cancer if activated by mutations or changes in protein expression. RAF-1 is associated with the ERK1/2 pathway which plays a crucial role in the regulation of the cell cycle. The inhibition of RAF-1 reduces levels of a protein called MCL-1 which is associated with increased levels of apoptosis (programmed cell death).

Because pemetrexed and sorafenib exert their effects through different pathways, it was hypothesised that together they could lead to a synergistic increase in tumour killing. Through clinical trials, Professor Dent and his team are investigating how best to combine these two drugs in order to achieve clinically significant outcomes in the fight against cancer.

Beginning Phase I Clinical Trials

You may have heard the term 'clinical trial' before, but what exactly do the different phases of a clinical trial involve? Phase I involves testing the new treatment on a small group of participants in order to determine the optimal dose, identify adverse effects and evaluate its overall safety. If this is successful, the treatment is then given to a larger group to further assess its safety and effectiveness in Phase II. Phase III compares the treatment to other common interventions and confirms its efficacy in large groups of participants. Phase IV looks at outcomes and side effects associated with long term use and occurs once the drug is on the market.

Between 2011 and 2014, Professor Dent and his colleagues carried out a Phase I study (ref: NCT01450384) to determine the safety and tolerability of a combination of pemetrexed and sorafenib in patients with advanced solid tumours. The study, run by Dr Andrew Poklepovic, used a '3 + 3' design to explore the effect of various doses of pemetrexed with continuous sorafenib. '3 + 3' designed studies involve incremental dose escalation in order to determine the maximum tolerated dose of a drug. Three patients will be enrolled in the first cohort with the lowest dosage. If no patient experiences dose limiting toxicity (side effects which are serious enough to prevent further treatment), three more patients can be enrolled in the next cohort at a higher dose. If one or more patients develops dose limiting toxic effects, three more participants will be added to that dose cohort. The development of adverse effects in more than one patient in a group suggests that the maximum tolerated dose has been surpassed and that the treatment should be stopped or de-escalated. The researchers in this study slightly adapted this design in a novel way which allowed for the escalation or de-escalation of one or both drugs depending on the dose limiting toxicities observed. 36 patients with various forms of cancer were treated over the course of Professor Dent's study with breast cancer being the most common diagnosis amongst participants. Participants were divided into two groups: 24 in cohort A and 12 in cohort B.

Individuals in cohort A were given escalating doses of pemetrexed every 14 days with continuous doses of sorafenib twice daily. Although no dose limiting toxicities were observed at lower doses, higher doses induced a number of adverse side effects amongst participants. High blood pressure, inflammation of the mucous membranes, cytopenia (reduction in number of blood cells) and gastrointestinal symptoms were included amongst the dose limiting toxic effects observed. Therefore, it was determined that pemetrexed at any dose combined with continuous sorafenib was not tolerable and the drug schedule was adjusted for cohort B. The new treatment protocol involved administering sorafenib only on the first five days in each two week pemetrexed cycle. Intermittent dosing was found to be far more tolerable with no dose limiting toxicity and fewer dose delays or modifications required amongst participants. As a result, phase II (ref: NCT02624700) will involve a schedule of pemetrexed 750 mg/m2 every 14 days with sorafenib 400 mg given twice daily on days one through five.

So now that the tolerable dose has been determined, how effective is the actual protocol against advanced solid tumours? Of the 33 participants who were assessed for anti-tumour activity, 20 experienced stable disease or tumour regression. Let's break that down: disease progression was stabilised in 15 patients with responses lasting up to one year; four patients had a partial response (meaning their tumour shrank by at least 30 per cent); and one patient had a complete response in which all traces of the tumour had gone. The treatment was found to be particularly effective in cases of breast cancer. All partial and complete responses occurred in breast cancer patients and 58 per cent of breast cancer patients (and all patients with triple negative breast cancer) experienced stable disease or response from the treatment.

The third piece in the puzzle

Professor Dent and his team have undertaken a number of pre-clinical studies in order to determine if a third drug could be added to enhance the killing effect of pemetrexed and sorafenib. Previous studies by Professor Dent indicated that the addition of the drug vorinostat to sorafenib prolonged stable disease in patients with liver cancer. Animal studies showed that the addition of AR-42 (a drug in the same family as vorinostat) to pemetrexed and sorafenib significantly reduced tumour growth and increased survival when compared to pemetrexed and sorafenib alone.

The team explored the possibility of adding an ERBB1/2/4 inhibitor to the combination of pemetrexed and sorafenib after a surprising discovery. The ERBB proteins are known as receptor tyrosine kinases which are enzymes with the ability to phosphorylate a protein and influence cellular function. The overexpression of ERBB signalling, particularly ERBB1/2, is associated with solid tumours and has previously been thought to protect cancerous cells from treatment. However, using multiplex assays (which allowed the team to analyse the activities of enzymes in tumour cells), Professor Dent and his colleagues were able to the determine that, contrary to expectation, ERBB1 was activated in response to pemetrexed and sorafenib. When tested, lapatinib and afatinib (both ERBB1/2/4 signalling inhibitors) enhanced the lethality of pemetrexed and sorafenib in a variety of cell lines.

Another finding of the study was that in order for the combination of pemetrexed, sorafenib and afatinib to be effectively lethal, there needed to be elevated endoplasmic reticulum stress. Endoplasmic reticulum stress signalling leads to the unfolding of proteins and autophagy. Therefore, the team investigated the upstream signalling pathways that control autophagy and found that the knockdown of certain regulatory proteins protected cancer cells against pemetrexed and sorafenib. The triple drug combo facilitated autophagy by increasing the expression of these key regulatory proteins through endoplasmic stress signalling.

Endoplasmic reticulum chaperones (proteins which assist in the folding of molecular structures) also played a significant role in the tumour killing ability of pemetrexed and sorafenib. The overexpression of the chaperone GRP78 protects cancer cells from pemetrexed and sorafenib because it diminishes the ability of the drug combination to reduce protein expression. By combining pemetrexed and sorafenib with an ERBB1/2/4 inhibitor such as afatinib, researchers were able to rapidly reduce the expression of GRP78 and other chaperone proteins.

Finally, reduced GRP78 expression correlated with increased phosphorylation of the endoplasmic reticulum stress mediator eIF2ɑ (an essential factor in protein synthesis). These processes collectively promoted autophagy and the release of toxic lysosomes in in vitro models of disease.

Where do we go from here?

So what effect does this drug combination have in a living organism? In vivo models of breast cancer were exposed to lapatinib or vandetanib (another type of signalling inhibitor) for five days which boosted the lethality of pemetrexed and sorafenib without any evidence of toxicity to normal cells. Similar findings were observed in non-small cell lung tumours when afatinib was added to pemetrexed and sorafenib. These findings are particularly pertinent in the face of treatment resilient disease states. Other drugs, such as flavopiridol and copanlisib, also show promise in subverting secondary drug resistance mechanisms. Cells treated with the combination of pemetrexed, sorafenib and afatinib showed greater sensitivity to both the above drugs, suggesting beneficial synergistic effects. These researchers firmly believe that resistance mechanisms to the drug combinations can be overcome. In light of this, they are currently submitting a new grant to fund a phase I cancer trial in all solid tumour patients using the combination of pemetrexed, sorafenib and afatinib.

It is clear that Professor Dent and his team are not done yet. A phase II study testing the combination of pemetrexed and sorafenib in patients with recurrent or metastatic triple negative breast cancer is now open at Massey Cancer Centre and Professor Dent encourages people to participate, particularly if they live in the United Kingdom.

Meet the researcher

Professor Paul Dent
Professor and Universal Chair in Signal Transduction
Department of Biochemistry & Molecular Biology
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Professor Paul Dent is Professor and Universal Chair in Signal Transduction in the Department of Biochemistry and Molecular Biology in Virginia Commonwealth University. After receiving a 1st for his BSc degree in Biochemistry from Newcastle University, he went on to do a PhD at the University of Dundee in Scotland. After graduation, Professor Dent became a Postdoctoral Fellow in the University of Virginia, US, and later set up a laboratory dedicated to developmental cancer therapeutics. Through securing funding from the National Institutes of Health and the Department of Defence, Professor Dent and his colleagues have explored the ways in which drugs can be combined to kill tumour cells. Clinical trials have focused on breast, brain, liver and pancreatic cancers respectively and the team are preparing further studies into colon cancer and other solid tumours. Professor Dent has also served as Assistant Editor-in-Chief for the academic journal Cancer Biology & Therapy.

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THE GENETIC PUZZLE: WHY DO WE RESPOND DIFFERENTLY TO CANCER THERAPY?

Professor Jatinder Lamba, of the University of Florida, studies the genetic basis for inter-individual variability in the response to drugs, and in particular in the response of patients with acute myeloid leukaemia to antibody therapies. Understanding the basis for this variability could help to tune treatments for personalised medicine.

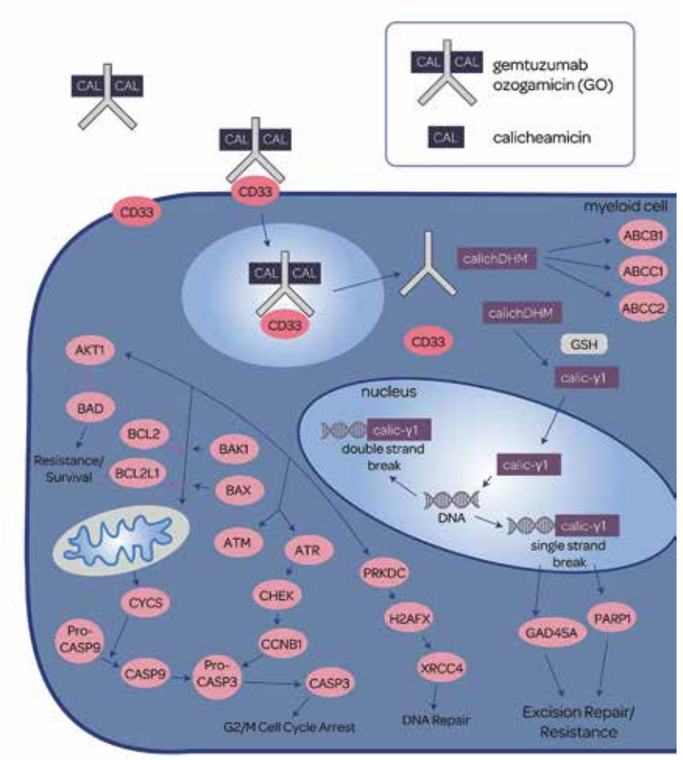
Variable responses to drug therapy

Why do some patients respond well to certain drugs, and get better, while others show no response, demonstrate an adverse response or signs of drug overdose, all on the same dose of the same drug? How a given patient responds to a drug is due to a variety of factors, but in large part the basis for inter-patient variability lies in our genetic makeup. Studying the influence of our genes on drug responses is called Pharmacogenetics. This fascinating discipline seeks to understand what genetic polymorphisms result in a change in drug responses, and how these polymorphisms cause those changes. The effectiveness of a drug or the manifestation of side effects could be influenced by changes in a variety of systems that interact with the drug on its path from ingestion, to the site of action, and eventual excretion. These include such entities as the cell membranes and drug transporters therein that permit the drug to pass through on its way to the target site, the enzymes which break the drug down or convert it into an active form, or the drug target itself. In many cases, the genetic variants that produce changes in these systems are tiny, but can have life or death consequences in terms of a patient responding to life-saving cancer therapies, or having a life-threatening adverse reaction to a drug. Our genome comprises billions of nucleotide bases, which form the genetic code that encodes for our unique biological makeup. In conjunction with the environmental factors we are exposed to throughout our lives, our genome determines so much about us, including the colour of our eyes, our height, and whether we will respond to a cancer therapy. A genetic variant causing a change in just one of those nucleotide bases, termed a single nucleotide polymorphism, can potentially result in a significant change in our drug responses, provided it occurs at the right location in the genome. Discovering where these locations are, and what effect a given single nucleotide polymorphism will have on the response to a certain drug forms the basis of the research of Professor Jatinder Lamba. In this article, we look at the results her team have produced to date, discuss her motivations for this type of research and her future plans.

Professor Lamba's motivations for pharmacogenetic research

Professor Lamba studies single nucleotide polymorphisms, which affect drug responses to acute myeloid leukaemia. She tells Scientia how she became interested in such research: 'My graduate research was focused on understanding how genetic differences impact patients' responses to drugs. At that time, the field of pharmacogenetics was in its infancy and I was really intrigued by how the genetic makeup of a person can influence therapeutic response or occurrence of adverse events in a patient.' She explains how personal motivations initially influenced her decision to get more involved with research into variability in drug responses: 'My interest grew stronger when I observed that my dad was not responding well to one of the hypertension medications he was taking and had to switch it due to certain side effects. This is a very common observation, not every patient responds to prescription drugs in the same way, yet for a particular disease, all patients are treated uniformly.' Eventually, her focus moved to acute myeloid leukaemia:

‘We are looking forward to using the results from our research to develop novel therapeutic agents of relevance to acute myeloid leukaemia’



‘I moved to St. Jude Children’s Research Hospital in 2000 for my postdoctoral training, where state of the art research in the area of precision medicine to improve treatment outcomes in children with cancer was being carried out. Research in last decade or so for childhood acute lymphoblastic leukaemia has resulted in improving 5-year overall survival, so that it now lies at over 90%. However, such an improvement has not yet been observed in acute myeloid leukaemia and being at St. Jude and seeing kids in the hospital inspired me to focus my research on this disease. I wanted to scientifically contribute to enhance our understanding of acute myeloid leukaemia as well as interpatient differences in drug responses and the development of drug resistance. My current research is focused on all these areas in acute myeloid leukaemia and I am hopeful that our results will be utilised in clinics to design personalised therapeutic strategies to improve treatment outcomes.’

Acute myeloid leukaemia and antibody therapies

Leukaemia is a cancer that results in a rapid growth of abnormal white blood cells and normally begins in the bone marrow. Acute myeloid leukaemia is a leukaemia subtype. The intense proliferation of the abnormal white blood cells is usually coupled with a drop in other types of blood cells. Common symptoms include fatigue and paleness, as a result of a drop in red blood cells. Patients may also have a greater susceptibility to infection due to a drop in normal white blood cells and an increase in abnormal white blood cells, which have no ability to fight infection. Leukaemia patients may also have a greater propensity for bleeding and bruising. Currently, the 5-year survival rate for acute myeloid leukaemia in adult patients is approximately 27%, which highlights the difficulties clinicians and scientists face in establishing effective treatments for this disease. Professor Lamba discussed the poor prognosis of acute myeloid leukaemia patients with Scientia: ‘Acute myeloid leukaemia has a dismal outcome. In spite of advances made in the past decade, the overall survival is not great for these patients. There is an urgent need to develop therapeutic strategies which incorporate new drugs as well as design chemotherapeutic regimens using a patient’s genome in conjunction with disease characteristics to achieve maximum treatment benefit.’ A particularly promising therapeutic approach involves the use of cytotoxic agents that can kill leukaemia cells, coupled with monoclonal antibodies, which are protein structures that can bind with great specificity to biological targets. The concept involves attaching a cytotoxic agent to a monoclonal antibody that is specific to a protein target present on leukaemia cells. This can enable the drug conjugate to preferentially bind to the cancer cells, enhancing the effectiveness of the cytotoxic drug and preventing it from producing off-target side effects elsewhere in the body. One such example is gemtuzumab ozogamicin, which combines the cytotoxin calicheamicin with a monoclonal antibody specific for CD33, a target on the surface of the leukaemia cells of 85–90% of all patients with acute myeloid leukaemia. The drug conjugate causes breaks in DNA in treated cells, which lead to cell death. The antibody-drug conjugate must bind to CD33, whereupon it is taken into the cell, in order to exert its effects. Given that the vast majority of acute myeloid leukaemia patients express the CD33 target, one might imagine that this drug combination would be highly effective in almost all patients. However, inter-individual variability means that this is not the case.

Inter-individual variability in acute myeloid leukaemia – results to date

Gemtuzumab ozogamicin has been shown to improve survival of a subset of newly diagnosed patients with acute myeloid leukaemia. However, there is significant

‘I wanted to scientifically contribute to enhance our understanding of acute myeloid leukaemia as well as inter-patient differences in drug responses and the development of drug resistance’

variability in patient responses to this drug. For example, it is effective in only one quarter of patients who have previously relapsed, when used as a single agent. The reasons underlying this variability remain poorly understood. In order to begin to understand the genetic basis for this variability the team initially undertook a small pilot study involving the genetic analysis CD33 gene in genomic DNA from 22 paediatric patients with acute myeloid leukaemia, who were all treated with gemtuzumab ozogamicin. Interestingly, they found that a single nucleotide polymorphism in the gene encoding the drug target itself, CD33, was a predictor of drug response. The team concluded that the polymorphism could potentially affect how CD33 functions, which could interfere with gemtuzumab ozogamicin binding or the proteindrug complex internalisation into the leukaemia cells following drug binding.

Encouraged by these results the team planned and undertook a much larger study. This involved a larger clinical trial of 242 paediatric patients with acute myeloid leukaemia, who were treated with gemtuzumab ozogamicin, as part of a combination chemotherapeutic treatment. These patients were compared with 172 others, who were treated in a similar manner, but did not receive gemtuzumab ozogamicin. The team found several CD33 single nucleotide polymorphisms that correlated with improved outcomes in patients treated with gemtuzumab ozogamicin. For patients treated with gemtuzumab ozogamicin, who had two copies of one particular polymorphism, the 3-year overall survival rate from remission was 84% +/- 8% compared with 68% +/- 15% for other genotypes. In addition, these patients had a superior disease-free survival and a lower risk of relapse. Most recent results from Dr Lamba’s recent study in another cohort of ~1000 pediatric AML patients, 500 of whom received standard therapy and 500 received gemtuzumab ozogamicin along with standard therapy again confirmed that CD33 polymorphisms can be used as biomarkers to identify patients who will or will not benefit from addition of gentuzumab to chemotherapy. Using this information can help in designing the most effective chemotherapeutic regimen based on a patient’s genetics and identifying patients who should or should not be given gemtuzumab.

Future work

The value of being able to predict patient responses to gemtuzumab ozogamicin, through a simple genetic test would be immense. The results of such a test could help patients and their physicians to better plan their treatment. It would also help to manage patient expectations about treatment outcomes. One of the most valuable aspects of accurate treatment outcome predictions, using simple genetic testing, is the avoidance of exposing patients who are unlikely to benefit from a given treatment to unnecessary toxicity and wasted time that could be spent on treatment opportunities elsewhere. Conversely, identifying which patients will most benefit from a given treatment could greatly enhance treatment outcomes. In their future research, the team wish to identify single nucleotide polymorphisms which are indicative of treatment response in acute myeloid leukaemia. They are particularly interested in genes involved in cell death, DNA damage, DNA repair and detoxification enzymes. Professor Lamba talked to Scientia about her hopes for future research, in terms of clinical translation of these valuable results: ‘The next steps involve moving the genetic testing to clinics and developing personalised therapeutic approaches. We are also looking forward to using the results from our research to develop novel therapeutic agents of relevance to acute myeloid leukaemia.’

Meet the researcher

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Professor Jatinder Lamba obtained her PhD from the Postgraduate Institute of Medical Education and Research, Chandigarh, India on the 'Pharmacogenetics of CYP2C19 in North Indians', following which she pursued postdoctoral research in the USA at St. Jude Children's Research Hospital, Memphis, Tennessee. She is currently an Associate Professor (with Tenure), Preeminent Scholar and the Graduate Program Coordinator in the Department of Pharmacotherapy and Translational Research in the College of Pharmacy at the University of Florida. She is the Chair of the Pharmacogenomics Focus Group of the American Association of Pharmaceutical Scientists and is an author on over 70 journal articles and two book chapters.

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UF UNIVERSITY of FLORIDA

FIGHTING CANCER WITH PHYSICS

When people think of physics they might think of Einstein, Stephen Hawking, the click of chalk on a blackboard and unintelligible mathematical equations wrought by people with elbow patches. However, physics has informed a variety of biomedical techniques, many of which are invaluable in the fight against cancer. From imaging modalities such as x-rays to ablative lasers and radiotherapy, many sophisticated biomedical techniques owe their inception, development and optimisation to physics and physicists.

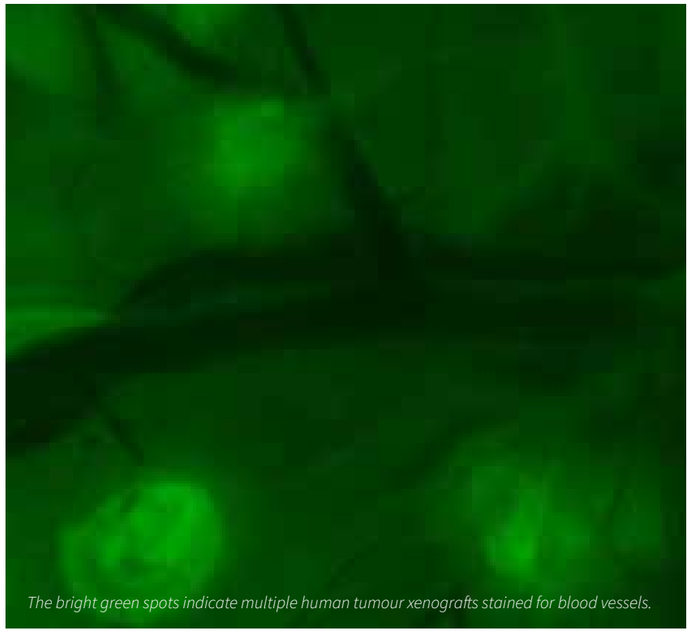
In this section we showcase the research of several scientists who have benefitted from physics in the techniques that they use to target malignant disease. In the first article in this series, we look at the work of Professor Andrew Webb and his undergraduate research students at Wellesley College, who have developed a highly selective nano-therapeutic that could be used to treat solid tumours. The method involves gold nanoparticles loaded with boron-10, which can selectively accumulate in tumours. Irradiation with a beam of neutrons causes these boron atoms to become unstable and decompose, releasing high-energy alpha particles and lithium atoms that destroy the tumour. Next we showcase the work of Dr Kazuhiro Yasufuku and his colleagues at the University of Toronto in Canada, who also employ the latest nanoparticle technology to treat cancer, without the need for major surgery. Their technique involves using minimally invasive surgery to deliver photo-ablative therapies for lung cancer. The third researcher in this series is Professor Eli Glatstein and his colleagues at the University of Pennsylvania, who apply photodynamic therapy for the treatment of a variety of types of cancer. Here, we illustrate the rationale behind this technique and discuss some of the key results achieved by this research group in their ground-breaking work. Finally, to close this edition of Scientia, we have had the pleasure of speaking to Professor Richard Marais, President of the European Association for Cancer Research (EACR) – an organisation set up to advance cancer research for the public benefit. Here, Professor Marais tells us all about the EACR's role in the advancement of cancer research in Europe and beyond.

TUMOURS IN THE CROSSHAIRS: NEW TOOLS TO RESURRECT AN OLD STRATEGY FOR TARGETED CANCER THERAPY

Professor Andrew Webb and his undergraduate research students at Wellesley College have developed a highly selective nano-therapeutic that could be used to treat solid tumours. The method involves gold nanoparticles loaded with boron-10, which can selectively accumulate in tumours. Irradiation with a beam of neutrons causes these boron atoms to become unstable and decompose, releasing high-energy alpha particles and lithium atoms that destroy the tumour.

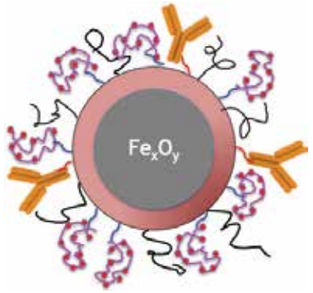
Treatment of solid tumours. At the cutting edge of nanotechnology

The classical and most widely used approaches for treating cancer are surgery, radiotherapy and chemotherapy, and more recent approaches include immunotherapy and hormonal therapies. Surgery is not always possible, is highly invasive, and can sometimes lead to complications, such as post-operative infections. Conventional radiotherapy involves irradiating a tumour with beams of radiation to shrink it in size or cause its complete destruction. It is often used as a supplementary therapy, in conjunction with surgery and/or chemotherapy. However, current limitations include localised side-effects, caused by the destruction of healthy tissues in the path of the radiation beams. In addition, the only specificity for the tumour is conferred by the use of radiation beams which intersect at the tumour core, meaning that the tumour receives more radiation than the surrounding tissues. However, significant destruction of healthy tissues is unavoidable, limiting the dose of radiation that can be delivered and the effectiveness of the treatment. Chemotherapy remains the most commonly used treatment and typically involves giving patients large doses of cytotoxic drugs that circulate throughout the entire body, and which demonstrate a poor specificity for cancerous tissue. Consequently, they cause significant toxic effects elsewhere in the body. Common side effects include nausea, hair loss and suppression of the immune system. Professor Webb sums up this issue, which motivates his research, by saying: 'Conventional chemotherapy suffers from the complications of adverse, off-target side effects. It is important to investigate and develop systemic treatment options that are less harmful to patient health in general'. Therefore, significant efforts have been devoted to developing new systemic therapies that are more targeted, reducing or eliminating side effects and increasing toxic effects at the tumour site.



The bright green spots indicate multiple human tumour xenografts stained for blood vessels.

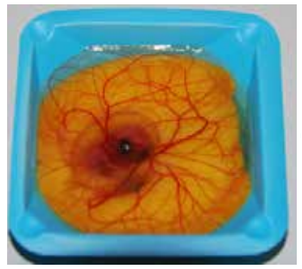
Nanoparticle construct. Orange - targeting antibody; purple - synthetic peptide with boron 10 molecules attached (red balls); m-PEG (black squiggly lines) stealth coating agent.



At the forefront of this research is an advanced suite of nanotechnologies, to permit for highly specific tumour targeting and controlled release of therapeutic drug payloads. Typically, this constitutes nanoparticles, introduced to the bloodstream, which are loaded with a drug and have the ability to passively accumulate in tumour tissues, due to the so called leaky vasculature inherent in most tumours, or are actively targeted to tumour cells. Of those types of nanoparticles that employ active targeting, many are decorated with tumour-specific antibodies to aid binding and uptake into tumour cells. Antibodies are specialised protein structures that can bind to a specific target antigen molecule with high specificity and are produced by the body to neutralise pathogens. Modern biotechnology provides a means to produce antibodies in the lab tailored to bind to antigens that are only expressed, or are more highly expressed, by cancer cells. Antibodies have been used as anti-cancer therapeutics in their own right. For example, Herceptin is an antibody therapeutic used against HER2, an antigen over-expressed on the surface of certain breast cancers. Nanoparticles decorated with tumour-specific antibodies selectively bind to and are absorbed by tumour cells, significantly enhancing the ability of their payload of drugs to produce toxic effects in the tumour itself and reducing the likelihood of drug exposure in healthy tissues.

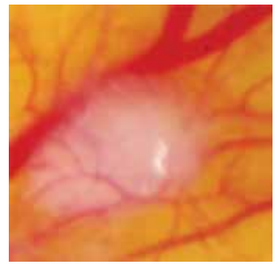
Nanoparticles have been designed using a range of biocompatible materials, including gold. Gold is a particularly useful material for nanoparticle design, as gold nanoparticle surfaces are highly amenable to chemical conjugation of proteins such as antibodies. In addition, gold nanoparticles demonstrate low toxicity, a large surface-to-volume ratio and are extremely small (10-20,000 would fit across the width of a human hair) thus enabling them to readily penetrate leaky tumour vessels to accumulate preferentially in cancerous tissues, all of which render them ideal as systemic anti-cancer carrier vehicles.

‘It struck me that applying nanotechnology was a great way to overcome the shortcomings of an older but promising technology like boron neutron capture’



10 day ex ovo chick embryo

Bolus of human cancer cells injected into CAM



Tumour xenograft 6 days later

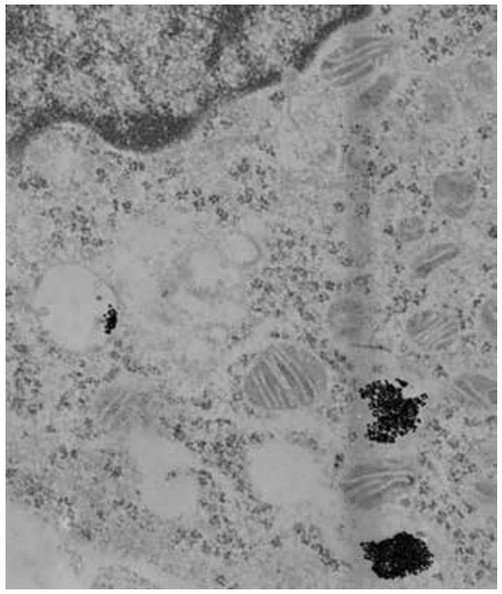
Boron neutron capture

One clever approach to targeting cancerous tissues while avoiding damage to healthy tissues is boron neutron capture. The method is sophisticated, as it uses two treatments that are much less harmful to healthy tissues than many conventional cancer therapies, and which only become toxic when they combine inside the tumour. The binary technique involves the intravenous delivery of a non-radioactive isotope, boron-10, into the bloodstream of a cancer patient. As the boron-10 is not radioactive, it causes little or no damage to the tissues of the body. The boron-10 permeates the cells of the tumour. Tumours tend to have leaky blood vessels, resulting in more boron-10 accumulating there, than in healthy tissues. The tumour is then irradiated, using a modified nuclear reactor, to create a specialised type of radiation, which consists of a beam of low-energy thermal neutrons. The boron-10 in the tumour captures these neutrons, and undergoes nuclear fission to yield high energy alpha particles and lithium atoms. which by virtue of their large mass and short path length destroy only the tumour cells they occur in, resulting in the destruction of the tumour with little collateral damage. Healthy tissues surrounding the tumour and elsewhere in the body contain less boron-10, and are largely unaffected by the neutron bombardment at clinically relevant doses.

While this is an elegant solution to achieve tumour specificity, unfortunately, in practice the technique has been hampered by poor or unpredictable uptake of boron-10 into cancer tissues, resulting in insufficient tumour toxicity under neutron irradiation. In fact, to date, the technique has only been applied with a measure of success in brain tumours, which allow for the uptake of sufficient quantities of boron-10 to be successfully treated with neutron bombardment. The technique also faces another difficulty, whereby it has been impossible to adequately monitor boron-10 levels and distribution in the body, so that optimal dosage and timing of neutron bombardment can be achieved to maximize therapeutic effects. Consequently, while the method underwent extensive trials in patients it has been largely abandoned since the 1990s, due to poor therapeutic outcomes and difficulties in optimising the process.

Putting it all together – Professor Webb’s approach.

Professor Webb’s team of undergraduates have considered the challenges facing boron neutron capture as a promising method of cancer therapy and have applied modern nano- and biotechnological methods to



Electron microscope image of nanoparticles (black dots) engulfed by cancer cell

resurrect this once neglected technique. They have developed tumour-specific antibody-decorated gold nanoparticles, loaded with boron-10 enriched compounds. These should allow more boron-10 to be absorbed by the tumour cells, compared with previously used boron-10 compounds that have been tried as delivery agents in prior attempts at boron neutron capture cancer therapy. Not only this, but the team have also designed these nanoparticles with a superparamagnetic core, to allow them to be visualised using magnetic resonance imaging (MRI). This means that the distribution and loading of the particles in a tumour can be visualised and assessed, permitting for optimal timing of the neutron beam to achieve maximal effects.

Given that these nanoparticles can preferentially bind to tumour cells and can be visualised with non-invasive imaging such as MRI, they could also potentially be used for diagnostic applications such as mapping and identifying cancerous tissue. This combination of diagnostic and therapeutic applications in one deliverable therapeutic means that the particles can be designated as 'theranostic'. Professor Webb tells Scientia: 'It struck me that applying nanotechnology was a great way to overcome the shortcomings of an older but promising technology like boron neutron capture. Nanoparticles have proven to be attractive platforms as theranostic agents in oncology by coupling high concentration targeting of therapeutics with imaging. For binary treatment modalities such as boron neutron capture, this is an ideal combination'.

The chick embryo chorioallantoic membrane assay

The team have applied a novel in vivo assay to test the potential of their nanoparticle system to specifically seek out and bind to cancer cells. Most research groups use immune deficient mice when examining the ability of a developed therapeutic to produce a cytotoxic effect in a human tumour, or even at an early proof-of-concept stage, where the ability of the therapeutic to bind to a tumour is assessed. The tumour is formed by simple injection of human cancer cells beneath the skin of the mouse, and different cancers can be studied by delivering cells derived from different types of cancer. Once the tumour is established and has acquired a blood supply, the therapeutic can then be delivered, and effects such as changes in the size of the tumour, animal mortality and determining whether the therapeutic has localised to the tumour, can all be assessed. However, such mammalian models are highly expensive. The mice must be immune deficient so that they do not reject the introduced human cells (growing cells or tissue of a different species in an organism is known as a xenograft) and consequently need to be housed under stringent pathogen-free conditions. In addition, such studies also raise ethical concerns, especially for early stage proof-of-concept projects, where preliminary data are collected and evaluated, concerning completely untested treatments.
Professor Webb recognised that another technique, the chick embryo chorioallantoic membrane (CAM) assay, could be used for early-stage assessments of nanoparticle behaviour in tumour xenograft, as a viable alternative to an immune deficient mouse tumour model. His research team have employed this technique as a platform to assess nanoparticle uptake and targeting specificity in xenografts of pancreatic tumours. This approach is ethically preferable for early-stage work as it where mammalian animals like mice, are substituted with 4-day-old chick embryos which possess only limited sentience. Additionally the assay is simple to perform. Fertilised chicken eggs are cracked and the contents are deposited in a dish, which is cultured in a humid incubator. The highly vascularised CAM is exposed, and samples of human cancer cells can be injected, grown and visualised under the membrane, without the risk of immunological attack. The injected cancer cells become highly vascularised and form discreet tumours in the membrane tissue within 2–3 days. Nanoparticles can be injected into a vessel near the tumour xenograft and nanoparticle uptake and binding can easily be measured.

'Nanoparticles have proven to be attractive platforms as theranostic agents in oncology by coupling high concentration targeting of therapeutics with imaging'

To date, the team have used the platform to investigate the binding of their nanoparticles to pancreatic adenocarcinoma (PDAC) cells. They cultured two different PDAC cell lines in the fertilised egg membranes, both of which express different biomarkers, to assess the specificity of their antibody-decorated nanoparticles.

Future work for the team.

The results to date have been extremely encouraging. The team have demonstrated that their antibody-decorated gold nanoparticles can undergo highly specific targeting to tumour-cell biomarkers and subsequent cellular uptake in vivo. The next steps are to begin to test the system as a whole, in its potential to successfully and specifically deliver sufficient amounts of boron-10 to tumours in vivo and determine if the delivered boron-10 can produce cell-destroying nuclear reactions when bombarded with neutrons. This will involve using MRI to visualise the nanoparticles in the CAM xenografts, and irradiating them at the nearby Massachusetts Institute of Technology nuclear reactor, where boron neutron capture was first pioneered for clinical use in the 1990s. Professor Webb is keen to emphasise that this research is a highly collaborative effort, and the hard work and dedication of both his students and faculty collaborators has made these innovative approaches possible.

Meet the researcher

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Professor Andrew Webb is a Professor in the Department of Biological Sciences at Wellesley College. After graduating from Southampton University in the UK with a bachelor's degree in Zoology and PhD in Developmental Biology, Professor Webb took a post-doctoral fellowship at Purdue University, Indiana USA. He was appointed to the faculty of Wellesley College just outside Boston in 1975 where he has taught courses in Organismal & Developmental Biology, Cell & Molecular Biology, Genetics, Comparative Anatomy, Bioinformatics & Cancer Genomics. His undergraduate research group was instrumental in cloning several human genes of medical interest in the 1980s (e.g. interleukin-1, parathyroid hormone-related peptide, plasminogen activator inhibitor-2), before moving to the development of nanoparticles as theranostic agents in oncology. He has authored over 50 peer-reviewed articles, is the inventor on over a dozen biotechnology patents and has served as a consultant and expert witness for numerous pharmaceutical and biotechnology companies.

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GETTING REALLY, REALLY SMALL TO TREAT LUNG CANCER

Thoracic surgeon and research scientist **Dr Kazuhiro Yasufuku** and his colleagues at the University of Toronto in Canada aim to use the latest in nanoparticle and high technology to treat lung cancer without major surgery.

Lung Cancer is not Just Any Cancer

Cancer is scary enough, but lung cancer – cancer arising in the lungs and airways – is one of the most distressing diseases imaginable, and with good reason. While lung cancer is not always the most common malignancy, depending on where you live, it is certainly one of the deadliest. In Great Britain, for example, lung cancer ranks below breast cancer and prostate cancer in incidence, but according to Cancer Research UK, it is the most common cause of cancer death. More than 35,000 people died from lung cancer in the UK in 2012. Cancer Australia reports that lung cancer is the fifth most common cancer in that country, yet it is the leading cause of cancer death, with almost 9,000 deaths due to lung cancer yearly. Things are dire in Canada, where Lung Cancer Canada claims that lung cancer is both the most common cancer and more people die of lung cancer than breast cancer, colorectal cancer and prostate cancer combined – with almost 21,000 people dead in 2015. Similarly, the United States Centres for Disease Control documents that more Americans die from lung cancer than any other type of cancer – 157,423 in 2012. No matter where you live, lung cancer is deadly, so it makes sense that medical researchers are keenly interested in new and better ways to treat it.

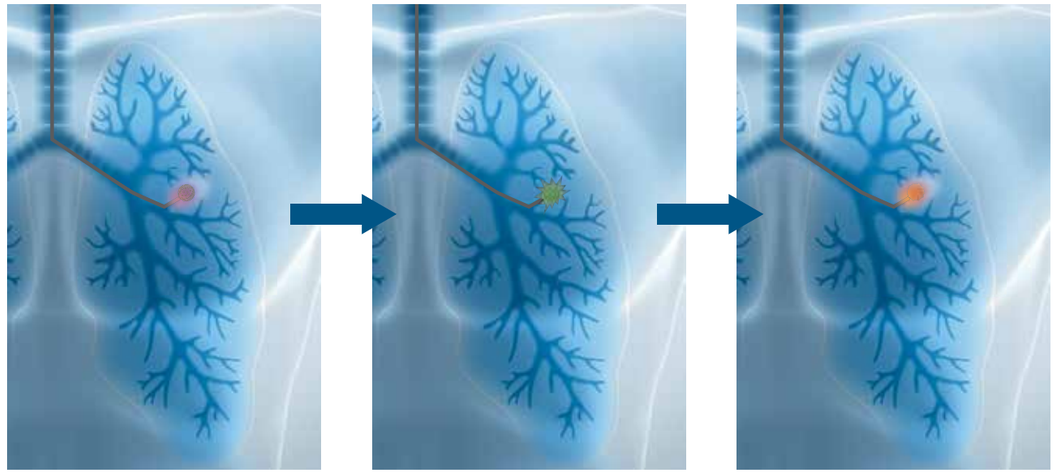
As a surgeon trained in operations on the chest, Dr Kazuhiro Yasufuku knows how difficult traditional lung cancer treatments can be. For one thing, the chest is well protected by a bony cage of bone and cartilage, making traditional chest surgery challenging for the patient. Incisions through the chest muscles, spreading or even breaking ribs and cutting or stretching cartilage – all this is physically demanding and can lead to long convalescences and pain for the patient. It is also technically challenging to remove tumours that may be close to vital structures, like the heart and large arteries and veins. Of course, you may also be taking out pieces of the lung, or even an entire lung. And non-surgical treatments for lung cancer are not all peaches and cream. Radiation beams aimed at a tumour in the lung can also do significant collateral damage to adjacent normal tissue. Chemotherapy can affect the whole patient. After all, chemotherapy is simply using toxic substances to try to kill the tumour before poisoning the patient. Dr Yasufuku knew there must be a better way.

How About Making Tiny Holes?

In earlier days of surgery, many surgeons found ways to avoid large incisions, turning to various lighted scopes to perform 'minimally invasive surgery'. Perhaps first to jump on this band wagon were the gynaecologists, who eschewed a long, midline incision (or even the 'bikini cut' transverse incision) in favour of small puncture wounds through which they could insert laparoscopy equipment. Several incisions no larger than a centimetre could be placed to allow lighted scopes and manipulators to be used to perform significant surgical operations on women. This surgery was often termed 'band aid surgery', because all you had covering your small incisions when you went home were band aids. General surgeons soon got on board, avoiding a long incision under the right ribs to take out a gall bladder, for example, and replacing it with a few small poke holes through which a laparoscopic cholecystectomy could be accomplished. Now, hernia repair, colon resection, appendectomy and other major surgical procedures can be accomplished through the laparoscope. Similarly, minimally invasive surgery for lung cancer became available via Video Assisted Thoracoscopic Surgery (VATS) in the 1990s. However the adoption by thoracic surgeons has been slow due to counter-intuitive hand movements to manipulate the instruments and 2-dimensional images with limited magnification. In order to solve these problems, Dr Yasufuku along with colleagues at Toronto General Hospital made history in Canada in 2012.

In October of 2012, Dr Yasufuku used a da Vinci robotic surgical apparatus – multiple remotely controlled arms manipulating instruments placed through small incisions in the patient's body – to remove part of the lung of a 78-year old man with lung cancer. Instead of suffering through a 20- to 30-centimetre thoracotomy incision, along with spreading apart of ribs and the attendant post-operative pain, the patient had four small 1-centimetre incisions in his chest that left almost no scarring. In fact, the one 'big' incision that was necessary was a 3-centimetre incision only needed so as to extract the excised portion of the lung. The patient was up and eating breakfast the next day and discharged from hospital soon after in virtually no pain or discomfort.

Photothermal ablation



Endoscopic excitation of dissociated porphysomes using navigation bronchoscopy

Bronchoscopic fluorescence visualization by porphysome of the target tumor

Bronchoscopic laser photothermal ablation

At follow-up visit he was cancer free. Since that first surgery, Dr Yasufuku has performed many other lung cancer surgeries using this minimally invasive technique. Now, not only patients with, say, gynaecologic problems can benefit from band aid surgery.

But this minimally invasive lung cancer procedure solves only one problem, that of a large and painful incision. There is still the question of how to recognise and localise tumours before you cut into – or at least poke holes in – the patient's chest. To be able to place the multiple small incisions for minimally invasive surgery, the surgeon needs a precise location of the tumour. Besides that, what if the tumour is too close to vital structures to excise, or the patient is too fragile for even minimally invasive surgery? Is there a way to get to the tumour and localise it better with our current diagnostic methods? Better than that, can you get to the tumour and destroy it without classic surgery at all? Dr Yasufuku had ideas about this.

First, Getting into the Chest Without a Scalpel

One way to get into a patient's chest without going between or through the ribs is to use the doorway Mother Nature gave us. Certainly pulmonologists use bronchoscopes all the time – flexible tubes that allow the physician to visualise the inside of the trachea and bronchi, almost all the way down into the alveoli themselves. Dr Yasufuku and fellow researchers considered this a great avenue down which to pursue lung tumours. The only problem is how do you actually see the tumour when your view is inside the airway tubes? In other words, if your lighted tube and camera is inside a bronchus, how do you visualise a tumour outside the bronchus, out in the lung tissue itself?

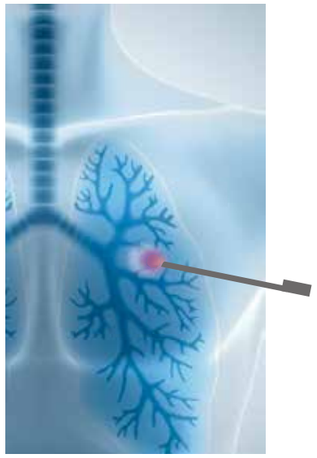
Because of modern medical imaging techniques and more astute medical strategies, doctors are now seeing increasing number of patients with small, non-palpable lung tumours that are extremely difficult to biopsy or find during surgery. Surgical resection is the standard of care and provides the best survival benefit for patients with early stage lung cancer, but if you cannot find the tumour, you cannot remove it. As well, some patients are not physically fit enough for surgery. That is where Dr Yasufuku and his group are getting really creative and jumping into the world of nanoparticles and looking at the manipulation of matter on a molecular scale. Dr Yasufuku and his group in Toronto have been developing innovative methods to solve these problems by application of new nanotechnology, the porphysome and the ICG lyposome, to their minimally invasive surgery techniques. Both the porphysome and the ICG lyposome were developed at the University of Toronto.

Porphysome? What's a Porphysome?

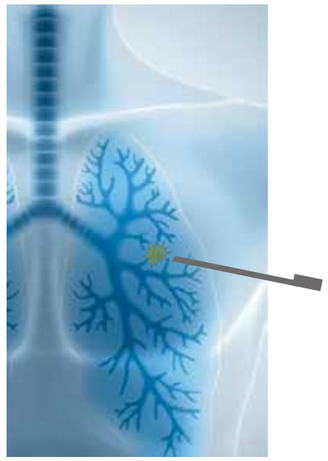
The porphysome is a first-of-its-kind, multifunctional, all-organic lipid nanoparticle discovered by Dr Yasufuku's colleague Dr Gang Zheng. The traditional role of lipids in this arena has been limited mainly to the use of liposomes – spherical nanoparticles consisting of a lipid bilayer – to deliver chemotherapy drugs. The clinical success of these liposomes has been based on their high biocompatibility, biodegradation and clearance properties, as well as their high therapeutic efficacy or contrast enhancement capabilities. The organic characteristics of lipids allow them to avoid eliciting an undesired immune response and help guarantee safe excretion from the body. A number of drug-loaded liposomes have been approved or are in clinical trial for various cancers. Unfortunately, the role of these lipid preparations is simply that of a passive carrier used to increase the circulation time of chemotherapy drugs and prevent interaction of the drugs with blood cells.

But now, with the synthesis of porphyrin-lipid – a porphyrin structure conjugated to a phospholipid – and the discovery of porphysome nanovesicles by Dr Zheng, the role of phospholipids in supramolecular structures has shifted from a simple support to an intrinsic imaging and therapeutic agent. Porphyrins are a group of aromatic organic compounds that are often pigmented

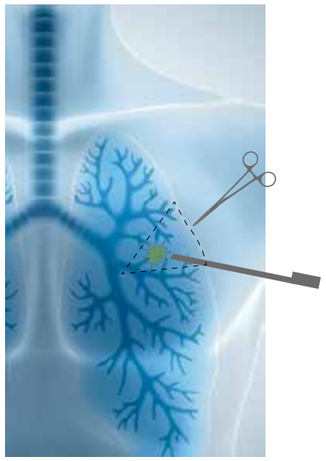
Intraoperative localization



Thoracoscopic excitation of dissolved porphysomes in the target tumor



Thoracoscopic fluorescence visualization of the target tumor



Minimally Invasive limited resection under fluorescence guidance

The use of nanoparticles, particularly porphysomes and ICG lyposome, is a quantum leap in our ability to fight lung cancer.

– the word porphyrin comes from the Greek word for purple. One well-known porphyrin is heme, the pigmented portion of red blood cells and component of haemoglobin. And because of the complex molecular structure of porphyrins, they can be used for a variety of purposes, including even carrying a metal ion attached to the porphyrin molecule by chelation. So a combination of porphyrin molecules of varying design with phospholipids – essentially tucking a specially designed porphyrin into a liposome – gives cancer researchers the ability to do wonderful things on a molecular scale. Porphysomes can be designed to accumulate into tumours and to emit bright fluorescence and near-infrared (laser) light to preferentially heat the tumour tissue. Porphysomes can be built containing metal molecules to allow for intraoperative localisation of early-stage lung cancer during minimally invasive surgery. Properly designed porphysomes could allow ultra-minimally invasive photothermal therapy of lung cancer in patients that are not surgical candidates. Porphysomes light up the tumour so the surgeon can snake a laser down a bronchoscope and zap it without making an incision at all. The possibilities are endless for Dr Yasufuku and his team.

Add ICG Lyposome and Start Working

Beyond the fascinating porphysome, the Toronto researchers also designed the ICG lyposome. This is a novel lipid-based liposomal nanoparticle encapsulating the commercially available fluorescent dye indocyanine green plus an X-ray contrast agent. This combination would allow precise imaging using both CT and near-infrared techniques to help guide the surgeon during minimally invasive thoracic surgery. The ability to 'see' even very small tumours, either during surgery or during bronchoscopy, would be a great advantage for surgeons like Dr Yasufuku. And with these two exciting nanoparticle additions to their armamentarium, the group at Toronto is planning a couple of studies to use both porphysomes and ICG lyposome to fight lung cancer.

First, Dr Yasufuku has a grant to construct an image-guided localisation platform for minimally invasive lung surgery. He plans to develop a new system in which the surgeon would get intraoperative real-time information on blood vessel anatomy as well as tumour location in the lung by lighting up the tumour with ICG lyposome. That way the tumour will show up on CT and near-infrared imaging so the surgeon can do his minimally invasive magic with precision. This project includes putting the ICG lyposome together with the minimally invasive techniques and various scanning devices and perfecting it in preclinical animal models for ultimate use on human cancer patients. In other words, use animals first to make sure the nanoparticles, the X-ray machines and the surgical system all coordinate and everything runs without a hitch.

Perhaps even more exciting, Dr Yasufuku wants to skip the surgery altogether and work on an 'ultra-minimally invasive' treatment for lung cancer, even less invasive than his band aid surgery. He wants to use infrared light delivered to the target tumour though a bronchoscope to destroy the malignant tissue by local heating, i.e. photothermal therapy. Specially designed porphysomes will be used to localise the target tumour by fluorescence imaging and to enhance the local absorption of the laser light, something porphyrins are good at. The project also includes perfecting the technique in preclinical animal models and then moving into human clinical trials, since it is something that has never been done in humans. The first-in-human trials will be performed in the Guided Therapeutic Operating Room (GTx OR) built in the Toronto General Hospital. The project team in Toronto already has all the expertise: Dr Yasufuku knows how to wield a bronchoscope, his colleague Dr Brian Wilson is an expert in fluorescence endoscopy, Dr Robert Weersink can work the X-ray machines, Dr Zheng wrote the book on the nanoparticles and Drs Wilson and Weersink are all about photothermal laser ablation. This team has been working together for years now and nobody else is more prepared to take this giant leap into the world of nanoparticles and minimalist surgery to defeat a major killer, lung cancer.

Meet the researcher

Dr Kazuhiro Yasufuku, MD, PhD
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Dr Kazuhiro Yasufuku is a thoracic surgeon and research scientist. He received his MD in 1992 from the School of Medicine of Chiba University, Japan. In 2002 he also received a PhD from the Graduate School of Medicine there. After a residency in Thoracic Surgery in Japan and fellowships in Thoracic Surgery and Transplantation in both Canada and Japan, Dr Yasufuku moved to Toronto, where he is currently the Director of Endoscopy at the University Health Network (UHN) as well as the Director of the Interventional Thoracic Surgery Program at the Division of Thoracic Surgery, University of Toronto. He is also the clinical lead of Thoracic Surgery within the Guided Therapeutics program at UHN and leads the Thoracic Robotic Surgery Program at UHN. He is a Scientist at the Latner Thoracic Surgery Research Laboratory, Toronto General Research Institute, UHN. He has authored or co-authored over 150 peer-reviewed journal articles, as well as book chapters and other publications.

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PHOTODYNAMIC THERAPY: AN ILLUMINATING APPROACH TO CANCER TREATMENT

Professor Eli Glatstein and his colleagues at the University of Pennsylvania have applied photodynamic therapy for the treatment of a variety of types of cancer. Here, we illustrate the rationale behind this technique and discuss malignant pleural mesothelioma, a type of cancer where encouraging results have been shown, and highlight some of the key results achieved by this research group in their groundbreaking work.

Photodynamic therapy – what is it and how does it work?

Photodynamic therapy is a method to kill cancer cells selectively using light at a specific wavelength, in a way that reduces damage to other tissues. This binary therapy combines the use of a photosensitising drug and a source of non-ionising radiation (in this case, visible light!) to kill cancer cells at the site of a solid tumour. The patient is administered a photosensitising drug, which can accumulate in tumour cells, while having little impact on healthy cells elsewhere in the body. This is in contrast with traditional chemotherapeutics, which can produce toxic effects throughout the body due to their inherent toxic properties. Once the tumour has been loaded with photosensitiser, it is then irradiated with light of a specific wavelength (consistent with the characteristics of the sensitizing agent) that causes the photosensitiser in the cells of the tumour to initiate the formation of reactive singlet oxygen (which is denoted as 1O2). This process results in the death of the exposed tumour cells. The photosensitiser causes the formation of reactive singlet oxygen only in the presence of a specific wavelength of light, meaning that the technique is highly targeted to cancerous tissues that both absorb the photosensitiser and are exposed to the light.

Photodynamic therapy causes the death of tumour cells in a variety of ways. These include initiation of apoptosis, which is a complex biochemical cell death program, and necrosis, which is a traumatic form of cell death caused by direct damage to cellular components. Other anti-tumour mechanisms involve damage to tumour vasculature, which impairs the tumour blood supply. Photodynamic therapy can also produce a local inflammatory reaction, which can result in an immune response directed against the tumour. Importantly, however, none of these mechanisms directly target DNA in the tumour cells. This is in contrast with conventional ionising external beam radiotherapy and conventional chemotherapeutics, which cause tumour cell death by initiating breaks in DNA. While the disruption of DNA is effective in killing cancer cells, it can also cause secondary cancers in off-target tissues by facilitating mutations in DNA, which adversely affect cell proliferation and mortality. Other advantages of photodynamic therapy include the ability to use it in chemoresistant or radioresistant cancer. These forms of cancer are resistant to the effects of conventional chemotherapeutic drugs or conventional ionising external beam radiotherapy. However, the mechanisms underlying chemoresistance or radioresistance tend not to interfere in the destructive potential of photodynamic therapy in solid tumours and so the approach can be used in these types of cancer. Photodynamic therapy does not elicit treatment resistance in exposed cells and can be used repeatedly on the same tumour site, without any change in the effectiveness of the therapy. Finally, photodynamic therapy does not typically interfere with other treatment modalities such as chemotherapy or radiotherapy, in terms of changing their effectiveness or compounding their side effects, and so it is highly amenable to use in combination treatment approaches.

Frequently, this takes the form of surgical removal of a tumour with accompanying photodynamic therapy to target other small metastatic tumours or 'nodules' that might be present. The light required to activate commonly used photosensitisers cannot typically travel more than 5-10 mm into the exposed tissue. Consequently, photodynamic therapy is best suited for the treatment of superficially accessible cancers, such as skin cancer, or those accessible on the linings of organs or body cavities, such as the thoracic or abdominal cavities, as part of surgical interventions to remove cancers from these areas.

Malignant pleural mesothelioma

Malignant pleural mesothelioma is a rare cancer of the pleura, the membrane that lines the outside of the lungs. It is often caused by exposure to asbestos. Survival rates are very poor, and patients typically exhibit a median survival of about 12 months. Treatments include chemotherapy, radiotherapy and surgical resection. In particular, in patients for which surgery is an option, surgical removal of cancerous tissue can sometimes improve survival. However, this can involve removal of the entire lung, which has obvious implications for lung function and quality of life, but is considered to be the most effective method to ensure removal of as much of the cancerous tissue as possible. A surgical procedure called radical pleurectomy aims to remove as much of the cancerous pleura as possible while sparing the lung, but complete removal of the cancerous tissue is more difficult with this technique compared with removal of the entire lung. Both surgical approaches have attached benefits and risks. However, in many patients a process called punctate miliary seeding occurs, where the cancer spreads extensively as a series of small nodules over the surfaces of the thoracic cavity. Professor Glatstein explains the challenge that this poses to a surgeon: 'This condition is what medical students often refer to as the "peek and shriek" operation – surgeons open the chest or abdominal cavity, take one look at what they see and immediately close the patient up because there's no surgical procedure that can remove all these tiny tumour nodules that may number in the hundreds or even thousands. There is no known cure for "seeding" of the peritoneal or pleural surfaces.'

'We have some patients with malignant pleural mesothelioma and lung cancer presenting with a malignant pleural effusion without any hematogenous metastases who have survived 5 years or longer without suffering any relapse, which for these conditions is virtually unheard of'

Professor Glatstein's approach

Repeat encounters with malignant pleural mesothelioma patients who demonstrated punctate miliary seeding got Professor Glatstein thinking about solutions to this inoperable condition. Then a talk he attended about photodynamic therapy for skin cancer gave him the idea that photodynamic therapy could be used as a potential anti-cancer technique in the thoracic or abdominal cavity. 'I heard Tom Dougherty from Roswell Park talk about photodynamic therapy for skin cancers with photosensitisers and laser light (tuned to the wavelength of activation for the sensitiser). I realised that this potentially could solve the problem in conjunction with surgery since the laser light could be adapted to the various cavities, kill the tumour cells and (because it's still light in the visible light spectrum) essentially spare the lung or abdominal gut tissue due to the characteristics of light and the binary nature of the photosensitiser.' In short, Professor Glatstein recognised the value of photodynamic therapy in treating extensive areas of tumour nodules in the thoracic or abdominal cavity – something that would be virtually impossible with traditional surgical approaches. A new method to combat malignant pleural mesothelioma was born, specifically geared towards photodynamic adjuvant treatment during surgical procedures. Professor Glatstein and his collaborators, including Professor Keith Cengel who focuses on enhancing the sensitivity of cancer cells, began to apply photodynamic therapy in conjunction with surgically removing cancerous tissues in the pleura.

Results to date

The team has conducted several clinical trials to date, investigating the use of photodynamic therapy as a supplementary treatment during surgical removal of malignant pleural mesothelioma, with encouraging results. In 2011, they published the results of a study that investigated the clinical outcomes of patients with advanced malignant pleural mesothelioma who underwent either modified extrapleural pneumonectomy (surgical removal of the pleura, pericardium, diaphragm and the lung) or radical pleurectomy (a similar procedure which spares the lung) and who were treated with photodynamic therapy during the surgery. They reported that mean survival was significantly better in patients who underwent radical pleurectomy in conjunction with photodynamic therapy. Consequently, the team now focus exclusively on this less extensive technique, which has the advantages of preserving lung function and reducing patient morbidity. Another trial, for which the results were published in 2012, using the same lung sparing/photodynamic therapy approach demonstrated a median survival of 31.7 months for the entire patient cohort and a median survival of 36 months in patients with the most common subtype of malignant pleural mesothelioma, and the approach was safe and largely well tolerated. These results are encouraging, given that nearly all the enrolled patients had advanced malignant pleural mesothelioma (stage III/IV malignant pleural mesothelioma), and that median survival for this condition is typically about 12 months. Professor Glatstein is enthusiastic about the results they have achieved so far, and is encouraged to undertake future trials to optimise the therapy even further: 'we have some patients with malignant pleural mesothelioma and lung cancer presenting with a malignant pleural effusion without any hematogenous metastases who have survived 5 years or longer without suffering any relapse, which for these conditions is virtually unheard of. Obviously such cases are still the exception to the norm, but we are optimistic that we can improve the techniques and achieve even better outcomes.'

The team has also been working to optimise

the delivery system for the technique. The light is delivered to the pleural cavity using a micro lens apparatus with custom applicators. Achieving a uniform distribution of delivered light throughout the pleural cavity is very important in photodynamic therapy, to ensure that the technique is maximally effective. However, the highly variable and non-regular contours of the pleural cavity make it very difficult to assess and achieve this. The team has employed a navigation system consisting of an infrared camera, which can accurately map the contours of the pleural cavity. The navigation system both tracks the position of the laser in real time and maps the pleural cavity in detail. This combination assists the surgeon in knowing when the correct amount of light has been delivered, at the correct locations. This level of control helps to standardise treatments across patients, reduce variability in treatment outcomes and maximise the effectiveness of the treatment.

Future work

The team is highly interdisciplinary and involves physicists, biomedical scientists and physicians, who work together to develop the most effective methods to deliver photodynamic therapy. In addition to their work on applying photodynamic therapy to lung cancers they are also interested in conducting investigations into using the technique for early stage cancers of the anus and just recently completed a clinical trial of photodynamic therapy for early stage head and neck cancers. Professor Glatstein foresees a role for PDT also in bladder, prostate, breast and gastrointestinal cancers. Photodynamic therapy is set for a variety of developments, aimed to increase its effectiveness and reduce side effects. These include new photosensitising drugs, which could increase the depth of treatment penetration in tissue, reduce the length of time required to achieve a phototoxic effect and decrease the risk of any side effects. The team would also like to determine the effect of photodynamic therapy on the immune system of patients, given that there is some evidence for local immune responses in treated tumours. Combining photodynamic therapy with other treatment modalities, such as immunotherapy or gene therapy is also an area of interest for the team, to see if synergistic treatment outcomes are possible. The future is bright for photodynamic therapy.

‘We are optimistic that we can improve the techniques and achieve even better outcomes’

Meet the researcher

Professor Eli Glatstein
Professor of Radiation Oncology
Perelman School of Medicine
University of Pennsylvania
USA

Professor Eli Glatstein achieved his medical degree at Stanford Medical School, where he first became interested in radiation oncology. After completing his postgraduate studies, he spent 2 years working at the Gray Lab in the UK under Professor J.F. Fowler, after which he served on the Stanford Faculty for 5 years and then as the Chief of the Radiation Oncology Branch at the National Cancer Institute (NCI) for 15 years. In 1996, Professor Glatstein moved to Philadelphia to become Professor of Radiation Oncology at the University of Pennsylvania, where he continues to make advances in cancer radiation therapy.

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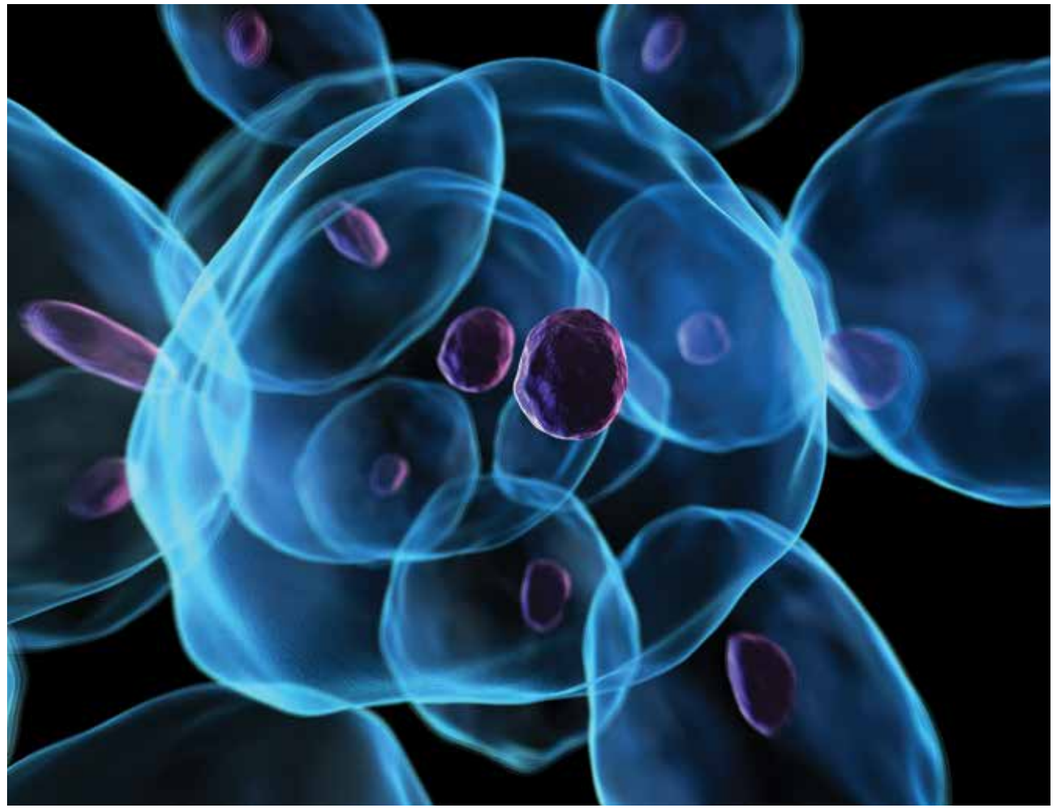
KEY COLLABORATORS

Professor Keith Cengel, University of Pennsylvania

THE EUROPEAN ASSOCIATION FOR CANCER RESEARCH

The European Association for Cancer Research, or EACR, was established in 1968 by a team of scientists who wanted to enable communication among cancer researchers across Europe. Today, the EACR coordinates scientific meetings and high quality training courses, and facilitates communication and collaboration amongst its membership community of nearly 10,000 members from 87 countries. The organisation continues to raise the profile of cancer research in Europe and addresses the need for sustained political and economic support. Here, we have had the pleasure of speaking to **Professor Richard Marais,** President of the EACR, who tells us all about the EACR's role in the advancement of cancer research in Europe and beyond.



Firstly, could you please give us a brief introduction to the EACR, and tell us a little about its history, aims and objectives?

The EACR was established in 1968 – we are looking forward to celebrating our 50th anniversary in 2018! We now have almost 10,000 members around the world, ranging from first year PhD students to Nobel Prize winners. Our objective is simple: to advance cancer research for the public benefit, from basic research to prevention, treatment and care. We aim to achieve that by delivering top-quality scientific meetings and conferences available to our members at reduced rates, and by providing our members with opportunities to apply for bursaries, travel fellowships and sponsorship for their own meetings.

How does the EACR actively facilitate communication and collaboration between cancer researchers from different institutions and different countries?

All of our members receive a fortnightly email bulletin, providing news of our meetings and networking opportunities. Through our extremely highly rated Conference Series, we offer our members the chance to hear from, and meet in person, some of the top speakers in their field of expertise. These meetings are kept deliberately small and intimate. Our next meeting is Goodbye Flat Biology in Berlin from 02-05 October 2016. Our fortnightly bulletin also contains job updates, details of funding opportunities and a summary of forthcoming conferences from other organisations.

In July 2016 we have our Biennial Congress – EACR24 – this year held in my home city of Manchester, UK, from 09–12 July 2016. Our Congress is a fantastic opportunity for our members to come together, learn from each other, and take their experience back to their home institutions.

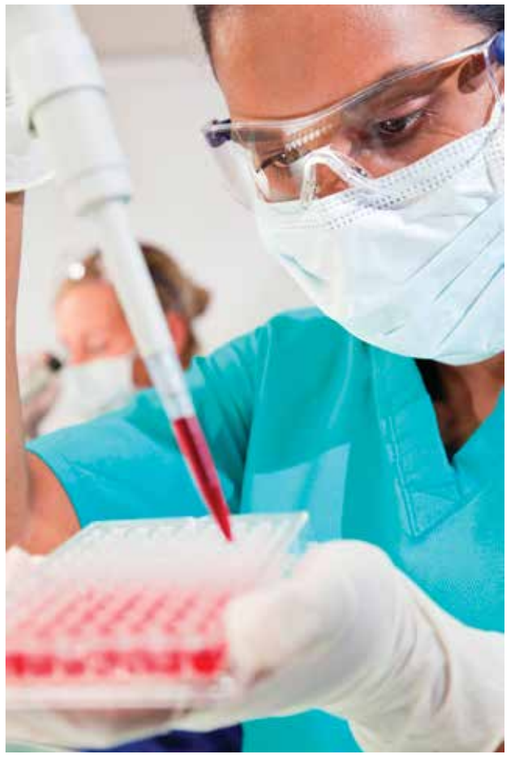
In what other ways does the EACR help to advance cancer research, and what funding does the EACR provide?

We are proud of our Travel Fellowship Programme, which has been providing opportunities for early-career scientists to advance their research since 1976. Now co-sponsored by Worldwide Cancer Research, the Programme supports the development of researchers through visits to centres of excellence or participation in specialised practical workshops and courses. This valuable opportunity is exclusively available to early-career EACR members. We have supported researchers from 37 countries with over half a million euros of funding.

We also have a generous bursary programme to enable researchers to apply for travel funding and a free registration at our conferences. For example, we were delighted to award funding to enable more than 40 researchers to attend EACR24 in Manchester.

What are the greatest challenges that currently shape Europe's cancer research agenda?

We recently carried out a survey of our members, and asked them this very question! We can therefore tell you with some authority that by



far and away the biggest challenge our researchers face is the shortage of funding and the constant need to search and apply for grants. The short-term nature of many grants also makes it difficult for researchers to take on ambitious research projects and is almost certainly affecting the quality of the research that is being conducted. It is also having a big impact on people's careers, because it makes it difficult to formulate long-term plans.

(In the same survey, we were delighted to learn that 98% of respondents would recommend EACR membership to others!)

The EACR head office is based in the UK – if the UK votes to leave the EU in June of this year, will this negatively affect the EACR's activities?

Cancer knows no boundaries and our members work together without regard to nationality. Although our headquarters are in the UK, we operate throughout Europe and will continue to do so for the foreseeable future. Our aim and our determination is to support our members in achieving our mission.

Does the EACR collaborate with other cancer research organisations such as ECCO and Cancer Research UK, and if so, in what way?

Collaboration is a way of life for us and our members. We continue to work with ECCO on European oncopolicy and have recently entered into an exciting new partnership with ESMO; the European Society of Medical Oncologists. There is a natural link between our associations and we are keen to work together to enable even greater cooperation and exchange of ideas between basic and translational researchers and their clinical colleagues. To that end, we will partner with ESMO in their 2017 Congress to be held in Madrid and again at their Congress in 2019. We are delighted that, coupled with our own Congresses in 2016 and 2018, this gives our members the opportunity to attend a major cancer conference each year.

In terms of partnerships with cancer charities, we have a strong relationship with Worldwide Cancer Research and are very grateful for the support we receive from them for our Travel Fellowships each year.

Please tell us a little about the EACR's position on public engagement and knowledge dissemination?

Last year, the EACR joined with the EORTC and ESMO on the CAREFOR project – the Clinical Academic Cancer Research Forum. CAREFOR's purpose is to communicate the role and benefits of independent clinical research, as well as bringing about improvements in the clinical trials landscape. Further developments will be announced as the project progresses.

We are always keen to tell the public about the ground-breaking work carried out by our researchers; this is something we will focus on in our next strategic plan.

Finally, can you please share your thoughts on the future of cancer research in Europe, and the ongoing role of the EACR in that future?

If we think back over the life of the EACR, cancer research has made enormous strides in the diagnosis, treatment and prevention of cancer. The advances are currently occurring at breath-taking pace, so we are in no doubt that the advances over the next 50 years will be equally enormous. EACR will continue to support these developments by playing a key role in bringing people together so that they can share research outcomes, develop collaborations and continue to work together to advance improvements in cancer patient care.

EACR
European Association for Cancer Research

www.eacr.org

Cancer Society of America

Our Mission: To provide relief to those who suffer from cancer, and to support cancer research groups to find cures to save lives.

FIGHT CANCER. FIND CURES. SAVE LIVES!

- Cancer is the second cause of death in the United States.
- 1 in every 4 persons dies from cancer in USA!
- Cancer death rate in the USA has increased nearly four times since 1900!
- About 40% of people in the USA will be diagnosed with cancer at some point during their life span!
- Approximately 1500 people are diagnosed with some sort of cancer in the USA every day!
- Breast, prostate, colorectal, and lung cancers are the most popular types of cancer.

Did you know that 1/3 of cancer deaths could be avoided through awareness and prevention?

www.CancerSocietyOfAmerica.org

DONATE TODAY: www.cancersocietyofamerica.org/donate-charity-research.html