MAY/JUNE 2016 # 05

the Translational Scientist

Swimming in Circles?

Mouse models can set us on the right path - but without human equivalents, it's easy to get lost in translation.

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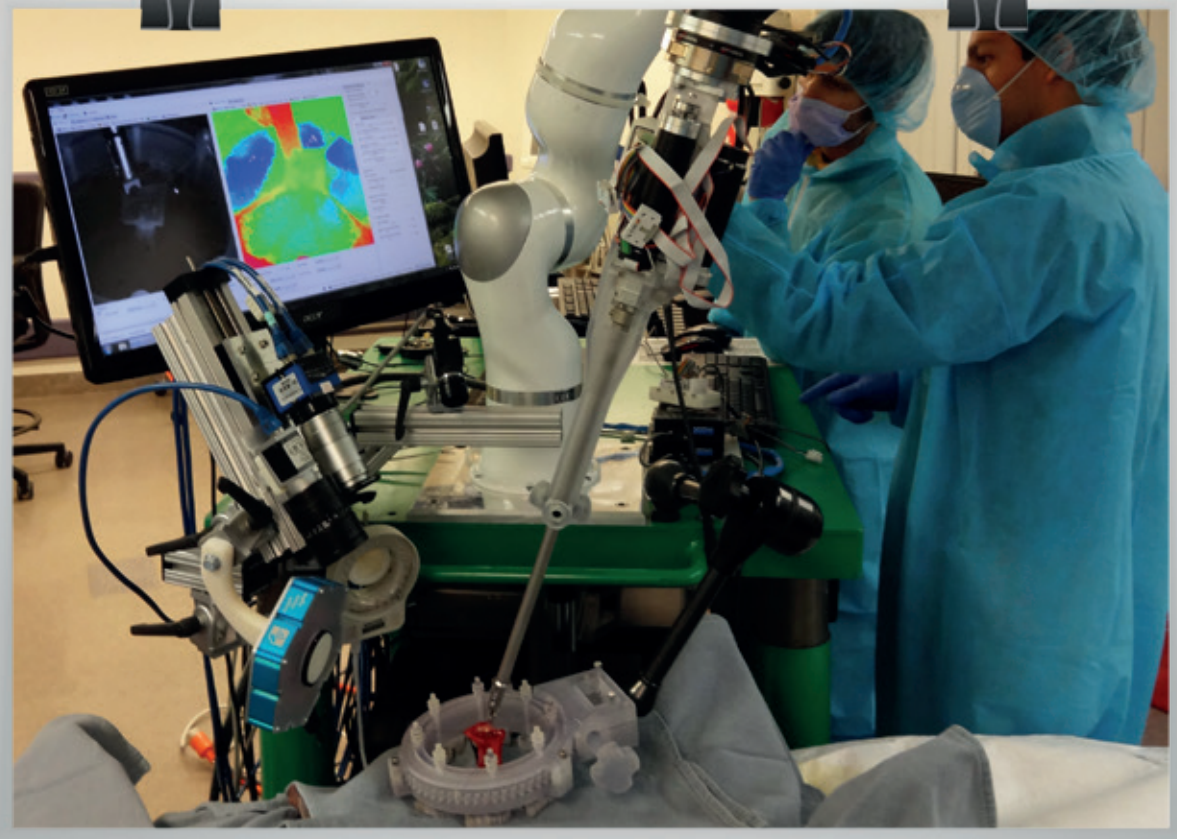

Azad Shademan and Ryan Decker during supervised autonomous in vivo bowel anastomosis, performed on pigs by the Smart Tissue Autonomous Robot (STAR). The robot outperformed even experienced surgeons in open bowel surgery.

Credit: Axel Krieger

Submit an image! Email charlotte.barker@texerepublishing.com

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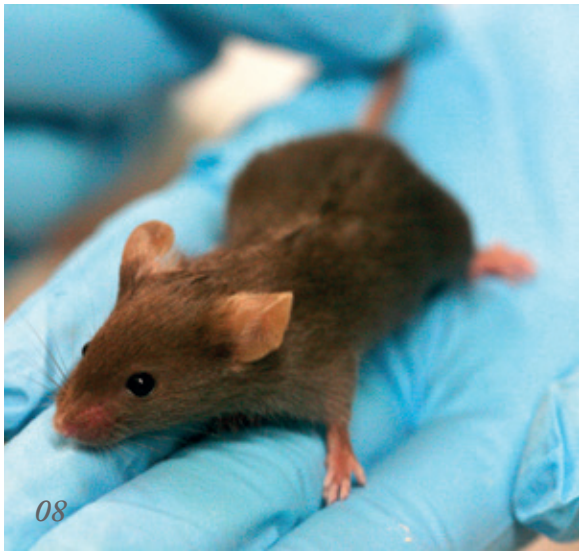

On The Cover

A mouse swims in a Morris water maze at Gladstone Institutes. Credit: Chris Goodfellow, Gladstone Institutes.

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the Translational Scientist

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&

the Analytical Scientist

Waseem Asghar

Meet the Winner

Waseem Asghar

Waseem Asghar, Assistant Professor at the Departments of Computer Engineering & Electrical Engineering, Computer Science, and Biological Sciences, Florida Atlantic University, USA, has been chosen as the winner of the 2016 Humanity in Science Award for "development of a new paper and flexible material-based diagnostic biosensing platform that could be used to remotely detect and determine treatment options for HIV, *E. coli*, *Staphylococcus aureus* and other pathogens."

Waseem will be presented with a humble prize of $25,000 during an all-expenses-paid trip to Analytica 2016 in Munich, and his work will feature in an upcoming issue of The Analytical Scientist.

Could it be you in 2017?

Analytical science has been at the heart of many scientific breakthroughs that have helped to improve people's lives worldwide. And yet analytical scientists rarely receive fanfare for their humble but life-changing work. The Humanity in Science Award was launched to recognize and reward analytical scientists who are changing lives for the better.

Has your own work had a positive impact on people's health and wellbeing? Details of the 2017 Humanity in Science Award will be announced soon.

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Fly Me to the Moon (and Beyond)

Cancer biology is advancing fast – but does America's "moonshot" to cure cancer fit the bill?

At the American Association for Cancer Research (AACR) Annual Meeting in New Orleans last month, one topic was on everyone's lips – President Obama's "Cancer Moonshot". Announced in January, the $1 billion initiative will be headed by Vice President Joe Biden, who lost his son, Beau, to a brain tumor in 2015. Biden addressed the AACR crowd on the closing day, and touched on the promise of immunotherapy, the need for open data sharing, and new ways of conducting clinical trials. Biden's comments were met with a positive reception from researchers, who he described as "one of the most valuable resources the country has."

However, in a special panel discussion on "Maximizing Cancer Cures," scientists were more tentative. The moderator asked attendees to indicate whether they thought that "moonshot" was a well-chosen word – the majority felt that it was not.

Some believe that curing cancer – like putting a man on the moon – is an engineering problem. They argue that the central issue is translating knowledge into medical advances. But most cancer researchers believe there's still a lot of basic biology to work through, not least because every cancer (and every tumor) is different. As NCI Acting Director Doug Lowry said in the same panel discussion, it is important not to put all our eggs (or research dollars) in one basket. There may be huge strides being made in cancer biology, but there could be completely new approaches out there, awaiting discovery (or, like immunotherapy, re-discovery). Indeed, judging by new research that looks set to overturn long-held beliefs about metastasis (see page 24), even the things we think we know may turn out to be only a small part of a greater puzzle.

Perhaps it's not a moonshot that we need, to make progress in cancer research, but a wider space program – a Starship Enterprise committed to exploring the solar system and beyond. Certainly, we need to drive existing science forward to help patients in the short term, but we must also keep searching for the therapies of the future. Targeting both near and distant spheres of interest is likely to be the only way to "cure cancer as we know it" – and will cost a lot more than one billion dollars.

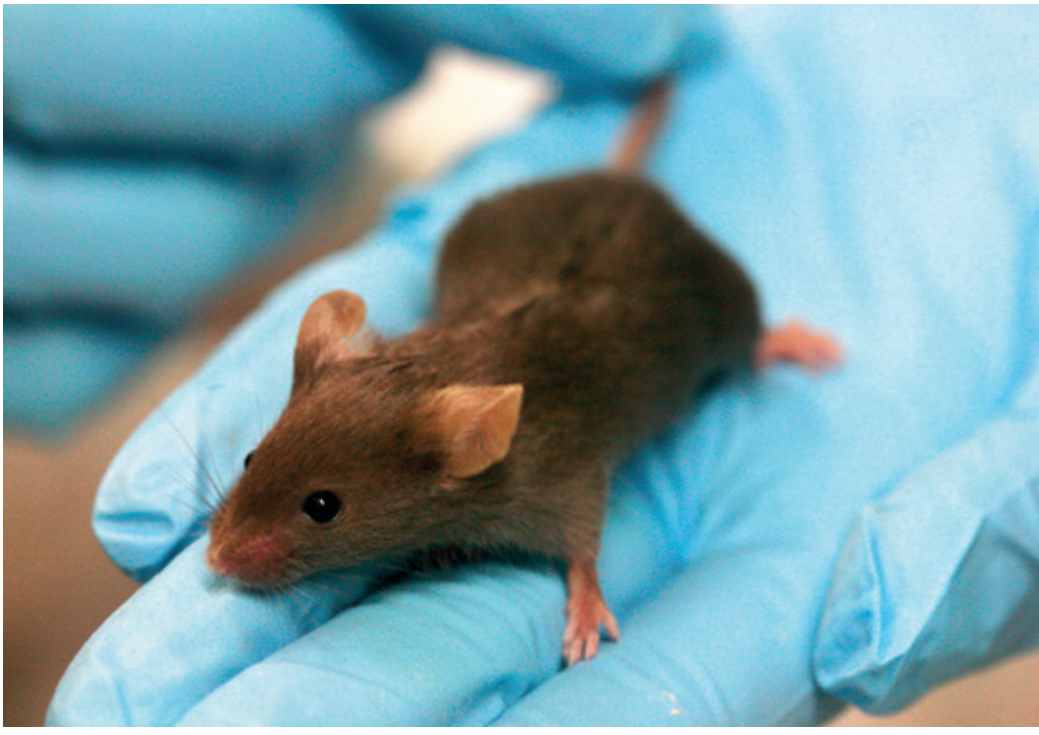
Whether or not AACR attendees agree on the feasibility of a moonshot, there was a sense of optimism at the conference. From cutting-edge cancer genomics to amazing clinical trial results for CAR T-cell therapies, real progress is being made. No doubt, skeptics and believers alike will be watching closely to find out if the VP's plans really can accelerate new advances into the clinic. Judging by the standing ovation he received, the majority will be rooting for him.

Charlotte Barker
Editor

Upfront

Reporting on research, personalities, policies and partnerships that are shaping translational science.

We welcome information on interesting collaborations or research that has really caught your eye, in a good or bad way. Email: edit@texerepublishing.com



Mucky Pups

New findings have revealed that "dirty" mice can actually recapitulate the human adult immune system better than lab mice

Mouse models are clearly important in translational research, and we rely on pre-clinical mouse studies to determine whether treatments are safe enough for human testing. But despite our reliance on the humble mouse, clinical results don't always match up with our expectations. A solution to boosting accuracy might lie with researchers investigating mice immune systems.

"In our new studies, we aimed to improve on the mouse model by exploring the impact that natural exposure to normal house microbes have on the immune response (1)," says Stephen Jameson, co-lead researcher and Professor in the Center for Immunology at the University of Minnesota.

The researchers, led by Jameson and David Masopust, tested the T-cell populations of lab mice, free-living barn mice, and pet-store mice and found that lab mice display an immune system closer to that of a newborn human, while the free-living and pet-store mice more closely resembled the immune systems of adult humans.

But were the immunological differences innate or environmental? To find out, the researchers co-habited lab mice with pet-store mice before re-testing the T-cell populations. After 15 days of mixing with the their less fastidious furry friends, the percentage of CD44 cytotoxic T cells increased in lab mice, bringing their immune systems closer to that of adult humans.

Though the results indicate that lab mice in "dirty" conditions may be better suited to pre-clinical studies, Jameson doesn't believe the existing

model should be completely replaced. "Valuable studies will continue to involve the current approach of maintaining mice in barrier facilities; for example, immunodeficient mice must be kept in lab conditions to survive," he says, "but on the other hand, since our research showed that 'dirty' mice have many immunological features in common with adult humans, we propose that using these animals will be a much closer approximation to the response of the human immune system, with clear implications for testing drugs and other therapeutics for their impact on the immune response."

Jameson also acknowledges that the new findings only scratch the surface of potential research directions with dirty mice, and has planned further detailed studies. "We will address three main areas with the new mouse model: immune response to cancer immunotherapy, the incidence of allergic and asthmatic disease, and the generation/control of autoimmune diseases," says Jameson. *WA*

Reference

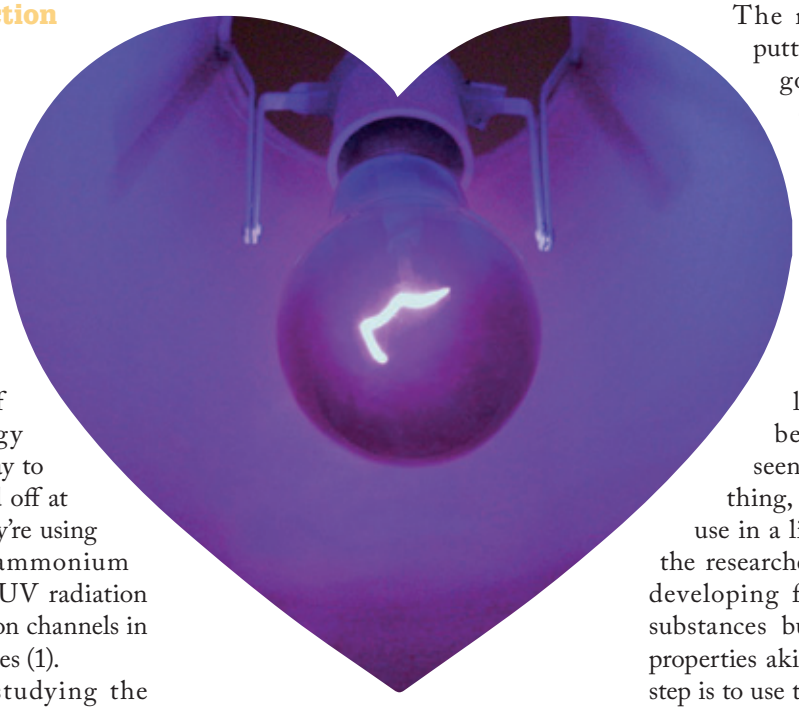
1. *LK Beura et al., "Normalizing the environment recapitulates adult immune traits in laboratory mice", Nature, 532, 512–516 (2016). PMID: 27096360.*

Light Hearted

UV radiation can control cardiomyocyte function – with intriguing therapeutic possibilities

The ability to easily and reversibly control excitable biological tissue has potential for many biomedical applications. Now, researchers from the Moscow Institute of Physics and Technology (MIPT) have found a way to turn cell function on and off at the flick of a switch. They're using azobenzene trimethylammonium bromide (azoTAB) and UV radiation to control voltage-gated ion channels in cultured rat cardiomyocytes (1).

"Initially, we were studying the behavior of excitation waves in heart tissue models, which can induce lethal arrhythmia," says Konstantin Agladze, lead researcher and Head of the MIPT Biophysics of Excitable Systems Laboratory. Having identified the cells responsible for the excitation waves, the team set out to find a way to control them. Their research led to azoTAB, a compound based on azobenzene - which can be used as a 'photo-switch' when two of its rings are connected.



UV radiation changes the shape – and consequently the activity – of azoTAB. In visible light azoTAB potentiates the K+ current, while suppressing Na^+ and $Ca2^+$ currents, which blocks contractions. When exposed to UV light, azoTAB changes form, resulting in normal function of K+, Na^+ and $Ca2^+$ currents, so that the cell can once again contract. "We also have some preliminary data showing that azoTAB makes photocontrol of neural cells possible," adds Agladze.

The researchers have been putting the photo-switch to good use by controlling cardiomyocyte rhythm across a sheet of cells in vitro, which could pave the way to potential treatments. The idea of turning off dangerous arrhythmia simply by applying light is certainly appealing. However, it's likely to be many years before the technology is seen in the clinic – for one thing, azoTAB is too toxic for use in a living heart. To that end, the researchers are working towards developing far less toxic chemical substances but with photo-sensitive properties akin to azoTAB. "Our next step is to use these non-toxic analogs to experiment on whole animal hearts," says Agladze. *WA*

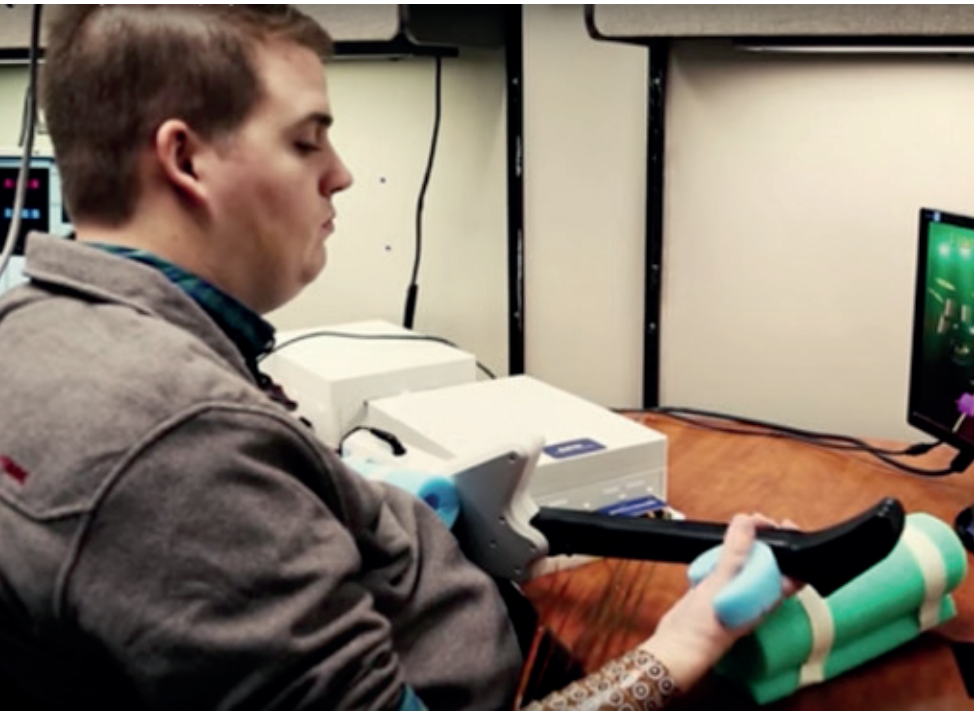
Reference

1. *SR Frolova et al., "Photocontrol of voltage-gated ion channel activity by azobenzene trimethylammonuium bromide in neonatal rat cardiomyocytes", PLoS One, 11 (2016). PMID: 27015602.*

Mind Over Matter

Neural implants and smart software give a paralyzed patient the gift of movement

Ian Burkhart, a 24-year-old quadriplegic, has been able to regain direct control over his right arm, using a system that circumvents his damaged spinal cord with a neural implant, a software interface, and a special sleeve (1). The neural implant in Burkhart's motor cortex reads synaptic impulses that are converted by algorithms into stimulating electrode signals for Burkhart's arm, which responds with muscle contractions – and the desired movement. The results have been impressive; trials started back in 2014 and now Burkhart can swipe a credit card and even play video games. To learn more, we spoke with Chad Bouton, first author of the new paper in Nature and Division Leader of Neurotechnology and Analytics at The Feinstein Institute for Medical Research.



How did you end up bringing paralyzed limbs back to life?

My background is in electrical engineering and engineering mechanics. I hadn't really planned on going into the medical field until an opportunity came up to work in a medical technology R&D group at Battelle about 10 years ago. I was able to get involved with some of the very first patients that had been implanted with microchip electrode arrays in the motor area of the brain, and that's when I fell in love with neurotechnology and the nervous system.

How are neural impulses linked with physical movements?

We actually treated it a bit like learning a language; we used the process of association. We showed on-screen images of a hand moving, doing very specific finger and wrist movements. The patient watched those movements and we recorded the brain activity, then we attempted to link those together. Special software that we've developed learnt the brain activity, then built a decoder, with correction inputs from us if needed. The more interaction there was, the better it became at recognizing the patterns and associating them with different movements. The participant started to make improvements as well, and refined their thought patterns, so the machine and patient were actually learning together. It really was an amazing process to watch.

What has the reaction been like so far?

We've received a tremendous amount of positive feedback. The scientific community recognizes that there's still a lot of work to be done – namely, refinement of the technology – before it reaches the market one day. But it really is an important step forward and I think it is motivating researchers to work even harder – and even encouraging young people to get involved in the field.

What's next?

The current study has approval for up to five participants so there are plans for future patients, and there has been an extension of study for the current participant, which is great news. We're also thinking about other types of research studies we can do in spinal cord injury, and eventually our investigation may branch into more complex avenues, such as patients with stroke or brain injury.

Reference

1. *CE Bouton et al., "Restoring cortical control of functional movement in a human with quadriplegia", Nature (2016, Epub ahead of print). PMID: 27074513.*

One Step Forward...

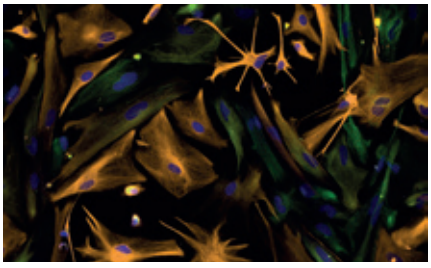
Stem cells restore function to damaged spinal cords in rats

Researchers in Japan and the USA have used stem cell grafts to regenerate the corticospinal tract (a bundle of specialized nerve cells that are crucial for motor function) in rats with severed spinal cords (1), pointing the way toward new treatment options.

"I'm a physician–scientist and work on the most clinically relevant aspects of neural regeneration. In humans, the most important motor system for voluntary control is the corticospinal tract, so we concentrated our work there," says co-lead researcher Mark Tuszynski, Director of the Translational Neuroscience Institute at the UC San Diego School of Medicine.

In the study, neural progenitor cells taken from rat embryos were induced to differentiate along a caudal (spinal) pathway, and implanted in the severed spinal cords of adult rats. The results show robust corticospinal axon regeneration, functional synapse formation, and improved forelimb function after grafts were placed into the sites of injury in rodents. The researchers then tried the same experiment using cells derived from human neural stem cells, yielding similar results.

Previous animal studies have demonstrated some success in using stem cells to regenerate the spinal cord after injury, but this is the first study to show regeneration of corticospinal axons in large, clinically relevant lesion sites. And though the results represent an important step forward, there is still work to be done before human trials can be considered. Tuszynski explains, "We are currently preparing a paper describing corticospinal regeneration in larger animals.



Credit: Carol Ibe, Eugene Major, National Institute of Neurological Disorders and Stroke, National Institutes of Health

We must scale these methods up to systems that better recapitulate the complexity of the human spinal cord. Clinical trials are still a few years off." While large animal trials are ongoing, the team is also working on identifying the precise stem cell type that should be advanced towards possible human clinical trials. *WA*

Reference

1. *K Kadoya et al., "Spinal cord reconstitution with homologous neural grafts enables robust corticospinal regeneration", Nat Med (2016, Epub ahead of print). PMID: 27019328.*

Bioprinting in the Palm of Your Hand

BioPen could allow surgeons to "draw" live cells onto damaged bone

A handheld 3D bioprinter, called BioPen, is able to deposit a hydrogel scaffold containing adipose stem cells (1). "For decades, we and others around the world have discovered and developed amazing new materials, but they weren't amenable to fabrication. Now, 3D printing allows us to utilize them surgically," says Gordon Wallace, lead researcher and Executive Research Director of the Australian Research Council Center of Excellence for Electromaterials Science.

The researchers used a gelatin-methacrylamide/hyaluronic acid-methacrylate (GelMa/HAMa) hydrogel (or "bio-ink"), mixed with human adipose stem cells taken from fat. The mixture was loaded into the BioPen, and solidified at the point of extrusion by a UV light. One week after the mixture was extruded from the BioPen, over 97 percent of the stem cells were still viable.

The hydrogel–cell combination used in this study was specifically formulated for use in cartilage injuries, commonly seen in knee joints. However, different mixtures could be used to replace other tissues. "There is a need to customize for the task at hand. Each cell type presents a unique set of challenges in BioPen implementation. Each may require a customized bio-ink but this is readily achievable," says Wallace.

The device still has several limitations and the team are already hard at work on a next-generation BioPen. "We are conscious of the fact that regulatory issues need to be addressed in parallel so this does not become the rate determining factor," says Wallace, "We will start animal trials soon, so we have a way to go to reach the clinical stage. Our ongoing challenges involve the refinement and customization of bio-ink and the on-pen light-induced curing system."

Not everyone has embraced the concept, but Wallace is philosophical, "As with many developments at the research frontier there are supporters and detractors. Both are important – the detractors help us identify deficiencies that we will rectify to ensure our supporters (many in the clinical field) can implement these advances as soon as possible." *WA*

Reference

1. *CD O'Connell et al., "Development of the BioPen: a handheld device for surgical printing of adipose stem cells at a chondral wound site", Biofabrication, 8 (2016). PMID: 27004561.*

Zika Research Gathers Pace

The CDC confirms the Zika–microcephaly link, and in vitro tissue models offer further insight

After weighing up the latest evidence, scientists from the Centers for Disease Control and Prevention (CDC) have declared that there is a "causal relationship" between Zika infection during pregnancy and birth defects. The researchers detail their conclusions in a special report in the New England Journal of Medicine (1).

In a related press release, the CDC retained a note of caution, "The report notes that no single piece of evidence provides conclusive proof that Zika virus infection is a cause of microcephaly and other fetal brain defects. Rather, increasing evidence from a number of recently published studies and a careful evaluation using established scientific criteria supports the authors' conclusions" (2).

Wanting to add weight to the overall body of evidence, a team of Brazilian scientists recently published work examining the effects of the virus on human neural stem cells grown as neurospheres and brain organoids (3). Neurospheres are simple clusters of free-floating neural stem cells that recapitulate the early characteristics of neurogenesis, while brain organoids are more complex bundles of neural tissue, possessing many features of the first trimester fetal brain. The researchers' observed Zika virus particles on the cell surface and in mitochondria and vesicles of the neural cells. All of the Zika-infected neurospheres suffered cell death to some degree, suggesting that the virus could impair early brain formation. Cell death was also observed in brain organoids infected with Zika, stunting their growth by 40 percent, compared with mock-infected cells. In comparison, brain organoids infected with dengue virus, which is not associated with birth defects, showed few ill effects.

In vitro tissue models like brain organoids are proving very useful in Zika research; they are cheaper, faster and less complex than working with rat or mouse models. Another study investigating Zika's effects on neurospheres and brain organoids has shed light on how the virus causes cell death (4). The researchers, based at University of California, San Diego, discovered that Zika infections lead to upregulation of toll-like receptor 3 (TLR3), an immune receptor that triggers the cells' self-destruct mechanisms. The researchers identified a number of TLR3-related genes responsible for the upregulation *(NTN1, EPHA3, ADGRB3, EPHB2, SLITRK5,* and *GRIK2)*, but further investigation is needed to determine their precise role. Importantly, they found that the stunted growth of Zika-infected neurospheres and brain organoids could be tempered by adding TLR3 inhibitors into the culture, which indicates that specialized TLR3 inhibitors could reduce the impact of Zika on fetal brain development. *WA*

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1. *SA Rasmussen et al., "Zika virus and birth defects – reviewing the evidence for causality", N Engl J Med (2016, Epub ahead of print). PMID: 27074377.*
2. *Centers for Disease Control and Prevention, "CDC concludes Zika causes microcephaly and other birth defects", (2016). Available at: http://1.usa.gov/1SaYTYV. Accessed May 6, 2016.*
3. *PP Garcez et al., "Zika virus impairs growth in human neurospheres and brain organoids", Science (2016, Epub ahead of print). PMID: 27064148.*
4. *J Dang et al., "Zika virus depletes neural progenitors in human cerebral organoids through activation of the innate immune receptor TLR3", Cell Stem Cell (2016, Epub ahead of print).*

Timeline:

1952:
- Zika first detected in humans

May 2015:
- Zika virus confirmed as the cause of an outbreak in Brazil

September 2015:
- Increase reported in the number of infants born with microcephaly in Zika virus-affected areas

November 2015:
- Zika virus isolated in a newborn baby with microcephaly

World Health Organization

February 2016:
- WHO declares microcephaly a public health emergency
- Brazilian scientists sequence the Zika virus genome
- Zika virus detected in the amniotic fluid of fetuses with microcephaly

April 2016:
- CDC concludes that Zika causes microcephaly and other birth defects
- Brazilian scientists demonstrate Zika reducing viability and growth in neurospheres and brain organoids
- Structure of thermally stable Zika virus uncovered

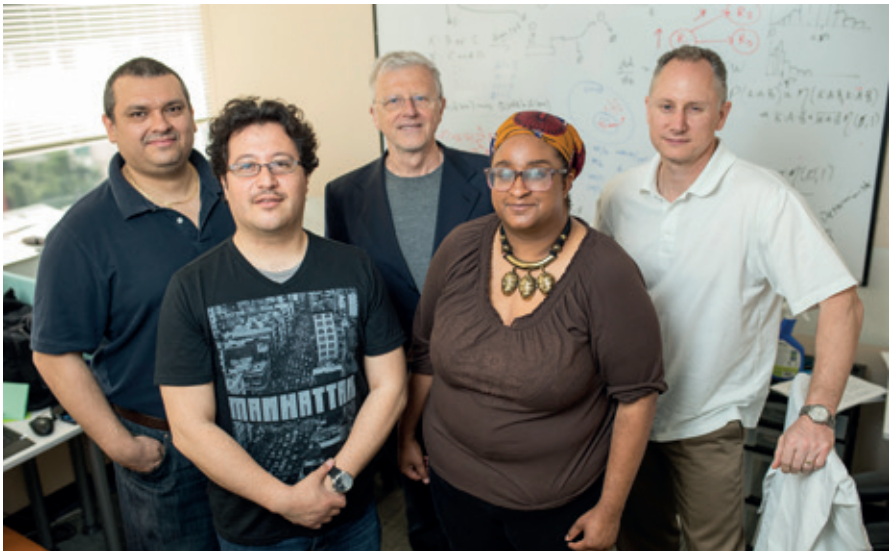
May 2016:
- Zika mechanism of action in cell death shown – a clue to possible drug targets?

Cancer Static Shock

Are the metrics used to measure cancer drug efficacy inherently flawed?

When it comes to anticancer drug development, the difficulties of translating in vitro efficacy into clinical success are well known. But what if the metrics scientists use to measure a drug's effect on cancer cell growth in vitro are inherently flawed?

A group of researchers from the Department of Cancer Biology at Vanderbilt University School of Medicine, US, believe that in vitro cell proliferation assays suffer from a number of biases (1). In response, they have developed a new metric, called "drug induced proliferation (DIP) rate". Darren Tyson, Assistant Professor of Cancer Biology and lead author of the study, tells us more.



From left: Vanderbilt researchers Leonard Harris, Carlos Lopez, Vito Quaranta, Keisha Hardeman, and Darren Tyson (photo by John Russell).

In what ways are current protocols flawed?

The use of a single measurement of cell number is widely employed across the scientific literature. Since it is based on a single time point measurement, we refer to this type of metric as "static". Static metrics are flawed in multiple ways. Firstly, because cells grow exponentially, an untreated population will rapidly outgrow a drug-treated population. Perhaps more critically, the ratio of control to drug-treated cells will steadily increase over time, creating the illusion that a drug's effectiveness is increasing over time. This is an example of what we call "time-dependent bias". Another source of time-dependent bias in static metrics is that many drugs exhibit a lag time before their effect stabilizes within a cell population. This stabilization delay can cause drugs to appear more or less potent or effective than they actually are, which means ineffective compounds wmay be being improperly passed through the drug discovery pipeline or, conversely, effective drugs may be discontinued prematurely.

How does your proposed DIP rate metric differ?

The DIP rate quantifies the growth of a cell population, or more precisely, the rate of change of a cell population size over time. Since the most important characteristic of a cancer drug is whether it can halt or reverse tumor growth, DIP rate is a natural and accurate metric: on a plot of cell population doublings (log2 cell counts) vs time, it appears as the slope of a line. As such, it is independent of time, once any delays in drug action have been accounted for. When developing the DIP rate metric, our biggest challenge was to determine when, after drug addition, a proliferation rate has stabilized. To support high-throughput drug screens, we had to develop reliable computational methods that could determine, in an automated fashion, when this occurs. The software is written in the R programming language and for academic applications can be obtained as free, open source software (2).

What are your next steps?

We want to measure DIP rates in large panels of cancer cell lines and search for novel molecular biomarkers of drug sensitivity, in addition to investigating DIP rate metric predictions of tumor cell responses in vivo. Both are translational tools for precision medicine.

We view the DIP rate as a metric of cell fitness in a particular environment, which extends beyond oncology. The DIP rate can act as a common currency, whether studying, for example, the influence of different drugs across a variety of cell lines, the effects of altering the microenvironment of stem cells, or the variation that exists at the single-cell level within a cell population (clonal heterogeneity, competition, or evolution).

References

1. *LA Harris et al., "An unbiased metric of antiproliferative drug effect in vitro", Nat Methods (2016, Epub ahead of print). PMID: 27135974.*
2. *D Tyson, LA Harris, "Code for Harris et al.,Nature Methods 2016." Available at: http://bit.ly/23E8DOC*

In My View

In this opinion section, experts from across the world share a single strongly-held view or key idea.

Submissions are welcome. Articles should be short, focused, personal and passionate, and may deal with any aspect of translational science. They should be under 600 words in length and written in the first person.

Contact the editors at edit@texerepublishing.com

Riding a New Wave of Translation

Is the overwhelming focus on genomics and transcriptomics in translational science really the best way to help patients?

By Valeria Ossovskaya, CEO and co-founder, BioCrypton, USA.

Clinicians, patients and the FDA are pressing biotech and pharma scientists to deliver more thoughtful and comprehensive translational strategies that can help to answer multidimensional clinical questions. This process is constantly hungry for innovation.

Next generation sequencing (NGS) has taken academic centers, biotech hubs, clinics, and investors by storm. Faster, better, more efficient sequencing opened promising translational avenues for complex diseases, genetic disorders and the microbiome. The speed and thoroughness with which NGS penetrated biotech, academia and pharma was amazing to witness. With massive promotion and impressive investments, NGS has become the defining trend of translational science – along with its promise to characterize human diseases and transfer the power of genomic research into the clinic.

The illusion that we see the landscape objectively is a powerful one, so it can be hard to accept that, while genomics tech is greatly contributing to the field, it has a lot of limitations. Moreover, genomics has yet to deliver on all its promises. Ultimately, genomics only gives us a partial solution to more fundamental questions about highly heterogeneous and fast evolving cancers, infections, autoimmune and viral diseases, and strategies to treat them. For example, neither genomics nor transcriptomics can deliver a complete and comprehensive answer to how a tumor will differentiate, proliferate, metastasize and adapt to therapy. Supported by generous funding, genomics has created millions of "me too" methods and companies, which cannot truly be called "innovation". In my view, it's time to think outside of the genomics box. If we really want to see a new wave of innovation, intelligent thinking should not be fragmented or driven by trends in technology.

What is the next step for translational science? The human proteome is far larger and more comprehensive than the genome. Moreover, the proteome is ultra-sensitive to intra- and extra-cellular stimuli and environmental factors. This gives us a significantly wider window of opportunity than genomics when it comes to designing translational strategies to resolve critical questions about patient stratification, complex diseases and response to therapeutic agents. Multiplexed and multidisciplinary technologies for robust exploration of the proteome in blood and other liquid biopsies are becoming powerful tools for translational science, molecular diagnostics and clinical trials.

It is more challenging to work with the proteome than with the genome or transcriptome. The proteome requires more sophisticated strategies, complex techniques and highly skilled scientists. That is where modern biotech, engineering and information technologies come in. The comprehensive integration of molecular biology with nanotechnology and powerful algorithms, search engines, and big data management systems can lead to outstanding opportunities that I believe will address translational science questions and personalize medicine needs in a more efficient and thoughtful manner than all previous efforts taken together.

Who will accelerate this next phase

of translational science? I think it unlikely that old-fashioned, conservative institutions like the NIH will contribute a great deal to this process – conceptually novel, high-risk projects are not members of the "NIH club". Instead, I foresee a key role for biotech hubs like Silicon Valley, which constantly integrate new angles of science with information technologies and high-tech inventions. In Silicon Valley, we aren't afraid to take a risk, and to mix and match different approaches to find better solutions for high unmet medical needs. Innovation is our religion here, and I believe cutting-edge high-tech hubs and startup ecosystems will be the forerunners in the next wave of transformation in translational science.

Perfect Partners?

When it comes to industry–academia partnerships, it's quality – not quantity – that counts.

By Abhay Pandit, PhD, Director, SFI Centre for Research in Medical Devices (CÚRAM), Ireland.

After seven years in the medical device industry and over 12 years in academia, I believe that both sectors have their own unique strengths. But I also believe that it is only by joining forces that we will find the fastest route to the clinic.

I know from my own experience that the skills and knowledge I gained in industry (high-level project management and an understanding of what it takes to get a product onto the market) would have been difficult to acquire in academia. But on the other hand, academia has afforded me the opportunity to do "blue sky" research that would not be possible in industry.

I now head Ireland's 50 million euro Science Foundation Ireland-funded Centre for Research in Medical Devices (CÚRAM), which works at the interface of industry and academia. Our funds are matched by investments from industry, to help solve some of the most pressing translational problems. It's not only about developing new technologies; it's also about adding know-how to existing technologies. For example, in a surprisingly large number of medical device products, the mechanisms of action are unclear. We can fill in the gaps, providing a high level of detail on the mechanisms and limitations, which allows the industry to develop the next generation of products.

In principle, industry–academia partnerships should be easy. Both groups are so well positioned to work with each other. Together they form an ecosystem where great science is used to create great products more efficiently. As bioengineers and scientists, that's what we all strive towards.

But in reality, forming these partnerships is no easy task, and it is all too easy to undermine them. We shouldn't fall into the trap of creating partnerships just for the sake of contract research. If that's the aim, contract labs can often do a better job than we could in academia. Real collaborations demand a two-way street.

Three kinds of projects I've seen work best with industry–academia partnerships are blue-sky projects, critical-path projects, and projects to develop standards. Industry can be reluctant to fund a blue-sky project, even if it's a good idea, because resources are often tied up with first-generation products. Academia can help by de-risking new projects, giving industry the confidence to invest.

If a medical device/biotech company has a critical-path project that's almost in their pipeline, multiple questions must be answered. How does this cytokine work? If this device is implanted, what is an acceptable response? What is the mechanism of action? And that's where academia can help provide robust and efficient data, resulting in a streamlined pipeline for the product, and ultimately helping it to reach patients quicker.

"Academia can also create great assessment systems and testing tools to be implemented in industry."

Academia can also create great assessment systems and testing tools to be implemented in industry – such as in vitro 3D models – pushing development forward more efficiently. There have been some very nice tools developed in academia over the years, which have been underutilized by the industry.

There are certainly more academic–industry partnerships now than ever before, but I would like to see a smaller number of more impactful collaborations. I would rather work with five companies on well-funded, large-scale projects, than 20 companies with a small budget.

So how can we, as academics, cultivate better collaborations with industry? Contrary to what some may think, the medical device industry or pharma do not have infinitely deep pockets, and so

companies invest in the projects that they believe will yield the greatest reward. But it's also important that projects make best use of the skillsets of both teams.

No institute or company is too big to do it wrong, and if you've never worked in an external partnership before, the smartest and safest avenue is to start with a small collaboration and ramp it up; successful partnerships don't happen overnight. From my experience, deeper, more meaningful collaborations only come with long-term cultivation, which starts with establishing credibility.

Are industry partnerships for everyone? No. But they do play a crucial part in cultivating a forward-thinking, efficient translational ecosystem.

On the Spot Diagnosis

Mobile devices promise a new future of point-of-care diagnostics for all

By Laura Lechuga, Professor, Nanobiosensors and Bioanalytical Applications Group, Catalan Institute of Nanoscience and Nanotechnology (ICN2), Spain

Reality always surpasses fiction. Take, for example, the cult science fiction movie "Gattaca." In the film, the police identified citizens using instant genetic analysis on a mobile device. No one would have believed that it could actually happen. Years later, the fiction is turning into reality thanks to the latest advances in point-of-care (POC) devices, nanobiosensors, microfluidics, lab-on-chip and cellphone technologies. The idea of using your own smartphone as an instantaneous diagnostic device by just adding a few drops of your blood, saliva, urine, or tears onto a biochip, is getting closer to reality every day – and it is a concept I find fascinating.

Such is their utility that POC technologies are applicable across a broad range of healthcare contexts – from preventive medicine to advanced personalized and precision medicine. Importantly, they open a window of hope for economically disadvantaged countries and low-resource environments, where most of the population does not have access to hospitals or clinical labs (but do have a cell phone).

POC devices can enable quick, simple and cost-effective identification of many diseases at a very early stage. They can identify conditions such as cancer, diabetes, stroke, pneumonia, hepatitis A, HIV, malaria or tuberculosis, including drug-resistant strains, among many other pathologies. They can provide sensitive detection of diseases and screen metabolic disorders and infectious diseases, or support adherence to treatments.

A typical POC device will identify and quantify disease biomarkers in bodily fluids due to a nanoscale biomolecular interaction between the target biomarker and its specific bioreceptor (on a biochip). How does it work? The procedure is generally very simple: the patient extracts the biochip (specific for one disease or for a panel of them) from a sealed package, aggregates the sample (a few drops of a bodily fluid), and inserts the biochip in the mobile POC device. A specific biomolecular interaction will occur, resulting in a physical or chemical change whose detection enables identification of the disease. Measurements typically take a few seconds or minutes. The data can be read and processed using a dedicated app, which will diagnose the medical condition, suggest the right treatment or connect the patient with their doctor or directly with emergency services, if required.

In my view, the ideal POC device should be disposable, require no external power source, be able to deliver the result in less than five minutes, allow for the analysis of several biomarkers in the same fluid sample, and should cost less than US$1. Academic research groups, industry, governments and policymakers are aware of these major and rapid technological developments. Although the enabling technologies exist, the main challenge is the integration and connection of all of these in compelling, portable POC platforms.

Notwithstanding the technological barriers and challenges, global market estimates will grow from US$1.6 billion in 2013 to $5.6 billion in 2019 (according to Transparency Market Research), which is driving significant commercial interest and major competition. Also, several important prizes, such as the Qualcomm Tricorder Xprize (US$10 million) (1), the UK Longitude Prize (£10 million), or the EU "Horizon Prize for better use of antibiotics" (€1 million), the latter two aimed at solving the problem of global antibiotic resistance, indicate why we need revolutionary diagnostic tools.

I anticipate a frantic struggle among the major players to develop the first POC smartphone device for routine use in our daily lives. Such mobile health monitoring tools will open the door to a new world where preventive healthcare and truly personalized medicine are routine. The technology is already here. But are we ready for the next diagnostic revolution that places healthcare into the palm of your hand?

Reference

1. *M Schubert et al., "Where no healthcare device has gone before", The Pathologist, 6, 18–30 (2015). thepathologist.com/issues/0615/301*

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LOST IN TRANSLATION?

the Translational Scientist

Scientists from opposite ends of the translational spectrum have teamed up to help solve a pressing problem in Alzheimer's research. By creating a human equivalent to the water mazes used in rodent studies, they hope to allow easier comparison of data from animal and human studies.

The importance of collaboration when it comes to successful translation is undeniable, and yet partnerships between basic and clinical researchers remain relatively uncommon. There is often little dialogue between the scientists working with mouse models of a disease and the clinicians who attempt to apply the findings to human patients. Without clear lines of communication between these two groups, how do we know if their methods – and conclusions – are comparable?

A recent project brought together scientists from across the divide in a collaborative effort to create a (virtual) human version of the classic rodent test of navigational learning – the Morris water maze (1). Here, we speak with study collaborators Kate Possin, Steve Finkbeiner, and Pascal Sanchez (see profiles on page 21) to find out how harmonizing cognitive tests could speed up clinical translation in Alzheimer's.

Why does translation from animal models to humans pose so many problems?

Steve Finkbeiner: There are a lot of different ways to answer this question. One is that there is a limit to the extent that you can model a human disease in mice; there are many differences in the way mice and humans metabolize drugs, and translating between two different species is never going to be easy. But another factor could be the way that tests are carried out or how data are analyzed. With this project, we wanted to identify and try to minimize those differences.

Pascal Sanchez: We could spend hours talking about the translational challenge in moving from mouse models to humans. In the past, maybe people have put too much faith in animal models. I think we need to take a more nuanced approach. Animal models are useful, but I believe we need to start thinking about them in a different way, rather than trying to translate the discovery of a given drug in a mouse directly to humans.

I think another problem is that the media likes papers that make a big splash. They want a huge discovery in mice to immediately translate to humans. In reality, we all know that animal models only recapitulate some aspects of the disease. It's a much more complex picture in humans.

How did the collaboration begin?

SF: The genesis of our journey was a large philanthropic gift in 2009. We could have spent the money doing more basic research, but this particular donor had two sons at risk of neurodegenerative disease, and it seemed like a good opportunity to focus on moving our discoveries closer to the clinic. Part of that strategy involved partnering with pharma, which took me on a very steep learning curve over the obstacles for clinical translation. There is a perception amongst companies that doing drug development in neurologic diseases is incredibly risky, partly because animal models often fail to predict outcomes in clinical trials.

Then we got a gift from another philanthropist, who had already been supporting the Memory and Aging Center (where Kate works), and was keen to foster collaboration. We were looking for important aspects that would bring our two groups together – and help make the translational pipeline more predictable – which led us to Kate's work.

Kate Possin: My group was looking into testing navigational impairment in patients with Alzheimer's disease. We wanted to find a way to measure navigational learning, which you obviously can't do by using pencil and paper tests. You need real-world paradigms. You could do something like lead patients around the hallways of a hospital to see if they find their way, but that is not a very controlled environment. A standard test for cognitive impairment studies in rodents – and therefore studying mouse models of neurological diseases – is the Morris water maze. To that end, we had been working with computer programmers at Microsoft Research to develop a virtual water maze that could be applied to human patients.

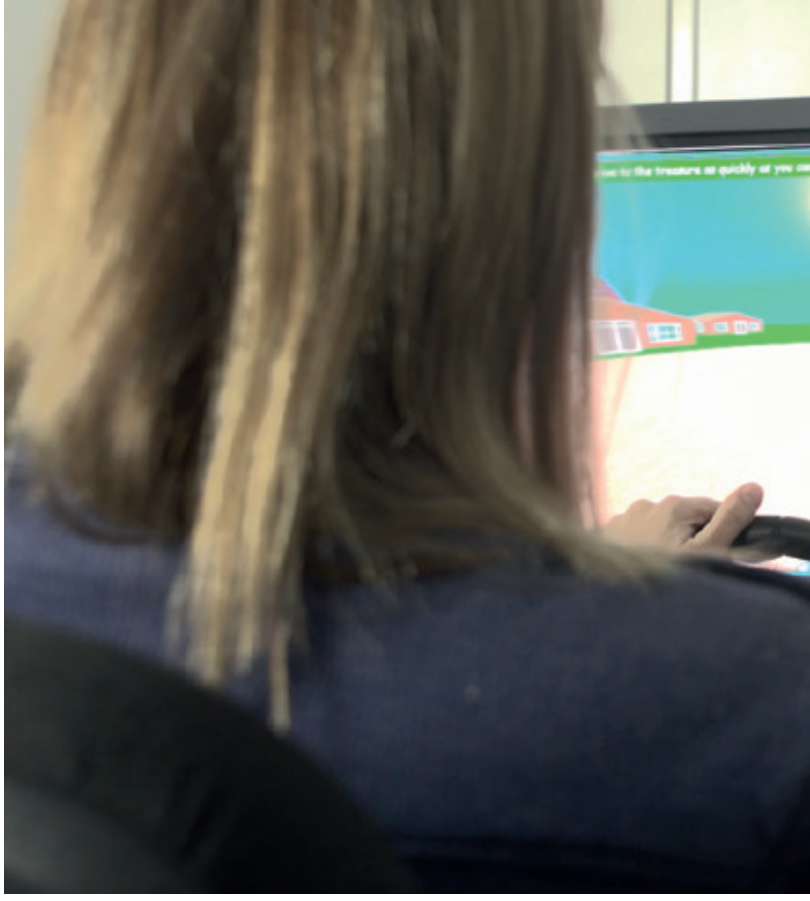
SF: We were really lucky to find that Kate was already thinking about these questions, and looking into innovative work on the water maze. That led the way for Pascal and others – who had done some beautiful work in mouse models – to generate comparable data sets.

PS: The goal was relatively simple: to create a test in which we could really engage the hippocampus in both humans and mice. Ultimately, that would enable us to better predict efficacy across species – and therefore improve the chances that a treatment that works in mice will also work in humans.

Why is navigational impairment important in Alzheimer's disease?

KP: In humans with Alzheimer's disease, getting lost is one of the early symptoms, because remembering how to get somewhere relies on the hippocampus – the first area of the brain to show damage from Alzheimer's pathology. To test a treatment for Alzheimer's disease you ideally want to target patients in the earliest stages, so you need a cognitive measure that is sensitive to early cognitive changes. Another reason navigational impairment is so compelling is that there is a huge body of research on the anatomy of navigation learning from years of rodent studies. Being able to measure the navigational impairment caused by Alzheimer's disease in both species gives us the potential to directly compare results across species, including studies of drug efficacy.

Human analogs of the Morris maze have been developed in the past, but there are a number of problems. Protocols vary and don't always match well with the mouse version; it was unclear which performance measures were most sensitive; and the statistical analyses used often fell short. In this study, we wanted to address those limitations.



"There is a perception amongst companies that doing drug development in neurologic diseases is incredibly risky."

What were the challenges in working across disciplines?

PS: As with the start of any new collaboration, it took a little time just to understand each other. We're all in the fields of neurology and neuroscience, but making analogies between the mouse and human tests wasn't easy. The way we measured different aspects of performance was quite different, so it took time to harmonize. Now, if we started on a different project together, it would be much faster because we understand each other better.

SF: At Gladstone, we actually tried many years ago to meet regularly with the Memory and Aging Centre to come up with a common language, but it failed. We had several meetings to reach that goal, but it was like our groups were from Mars and Venus!

I think one of the key reasons why our collaboration worked where others had failed was down to the people involved – Kate and Pascal are highly motivated. And the philanthropists' support was also crucial, because it enabled us to work at an interface where grant funding is very scarce; agencies are generally devoted to either pre-clinical or clinical work, but not the translational link between the two. In essence, the philanthropic funding helped form the glue to stick us together.

What were the first steps?

KP: After meeting Steve, I got a tour of the lab where they evaluate navigation learning in rodents. I talked with the staff who carried out the tests to make sure I really understood how it was administered in mice.

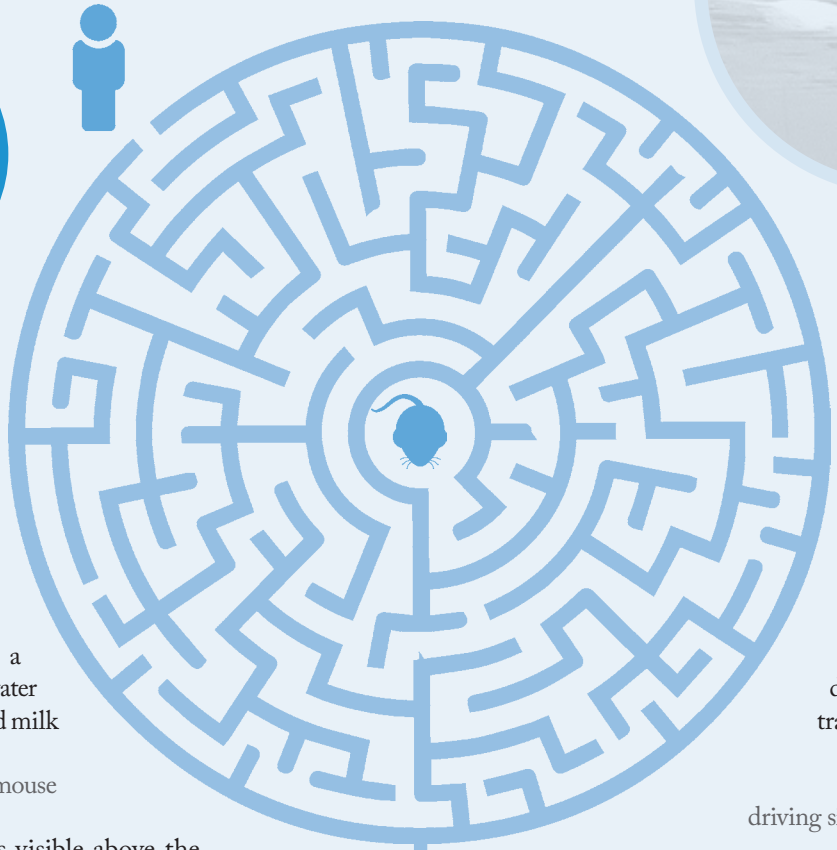
The Collaborators

Kate Possin is an assistant professor of neuropsychology at the Memory and Aging Center within the University of California, San Francisco (UCSF) Department of Neurology. Her work focuses on understanding the neurological basis of cognitive deficit, including developing new tools to measure cognition in patients with neurodegenerative diseases such as Alzheimer's.

Steve Finkbeiner is a professor at UCSF and Director of the Taube/Koret Center for Neurodegenerative Disease Research at the Gladstone Institutes. His lab strives to understand the molecular mechanisms involved in learning, memory and neurodegeneration, while the Taube/Koret Center aims to take discoveries from the lab and develop them into viable drug candidates that can be taken forward by industry partners.

Pascal Sanchez is a neuroscientist at the Gladstone Institutes. His aims are to discover and develop new drugs for neurodegenerative diseases in collaboration with industry partners, validate animal models of disease, and develop translatable cognitive tests.

The Morris Maze: Mouse vs Human



Mouse

Devised 30 years ago by Richard Morris, the Morris water navigation task (Morris maze) measures spatial learning and memory in rodents.

The mouse is placed in a circular pool, filled with water made opaque by powdered milk or nontoxic paint.

To escape the water, the mouse must locate a platform.

At first, the platform is visible above the water -the animal learns that swimming to the platform means escape.

Now the water level is raised so the platform is hidden – the animal must navigate to it from memory.

Visual cues (e.g. posters) are arranged around the room outside the pool, so the mouse can 'triangulate' its position.

Human

Previous Morris maze analogs in humans have included real-space, 2D and virtual environments, but protocols vary and do not always correlate between species.

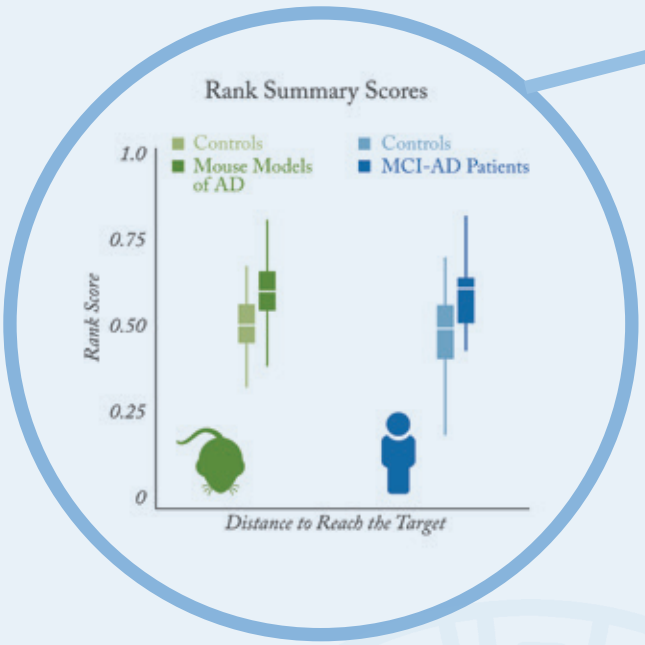
The authors set out to discover how well the test translates between animals and humans.

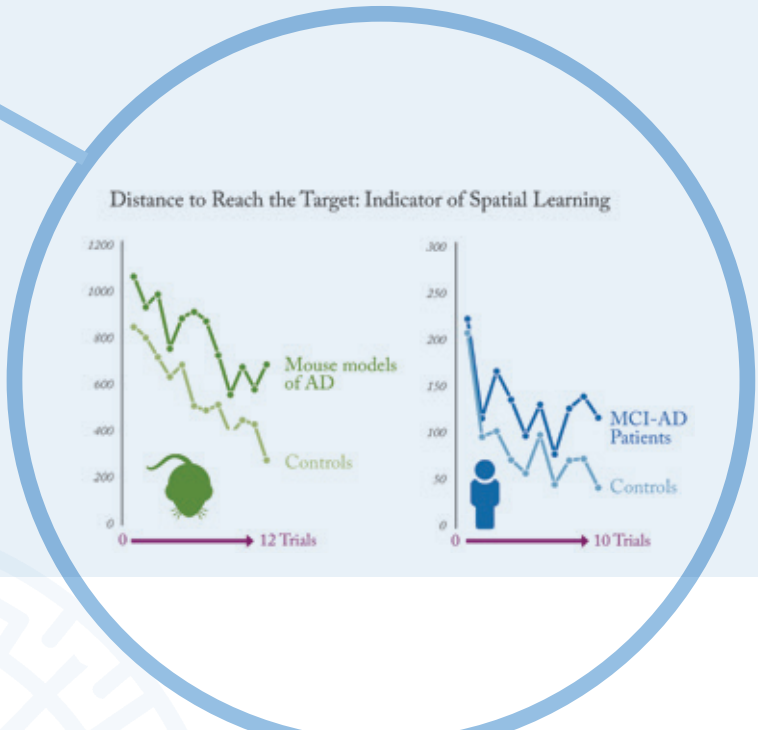
They developed a simple driving simulation, displayed on a computer monitor.

The goal for participants is to find "buried treasure" in a circular area.

The subject is first asked to drive to a visible target, with no other cues present.

Then, the target is hidden and trees, houses and other structures are added in the distance, to provide visual cues.





The other important part of the early collaboration was regularly meeting with people from Steve's group – which Pascal joined soon after – to talk informally about how we measure cognition across species and where there were links. As Pascal and Steve mentioned, we had a lot of really interesting conversations and spent time learning each other's languages, before we honed in on this particular project.

SF: I remember Kate's first visit. She was surprised at how often we use negative reinforcement for the mice in the tests, because of course that isn't something we do in humans. You can't put people into a swimming pool and make them swim around until they find a platform.

How comparable were the tests?

KP: The human and rodent maze tests do seem to be comparable. Early-stage Alzheimer's patients and transgenic mice expressing amyloid precursor protein showed a similar level of impairment on the test, compared with healthy controls.

The biggest difference is that in the mouse model, there's a set of trials where the mouse has to learn to swim over to a visible platform. In human tests, we can simply tell the patient to drive to the target, so even patients with a high degree of impairment can do it, while the mice suffering neurodegeneration struggled with this task.

PS: The task is more complicated for mice. We cannot tell them what they have to do, so they have to discover it by themselves. The incentive to perform is also different. For mice, it is a stressful situation. You put them in water and they have to escape. Human subjects are in comfortable chairs, at room temperature. They want to perform well, but it's not the same as the stress in the mouse model, so the incentive to perform is different.

One thing that I learnt while working with clinicians is that it's not as easy to tweak the experiments in humans as it is with mice models. But we have been thinking about ideas to increase motivation in human subjects. For example, they could be given bitcoins, which they will lose if they fail to perform to a certain level.

That is a huge advantage of clinicians and basic researchers working with each other: coming together to identify a disparity and figuring out the best way to adjust for it. Something that could have easily been adjusted in the animal model might require a complicated work-around in human tests or be missed completely – and that's why collaboration is so important.

What can others learn from your work?

PS: Our goal was to provide not just a description of our results, but real-world recommendations for other researchers in the field. The Morris water maze has been extensively used in mice and rats, but unfortunately people are not necessarily using it correctly or analyzing the data in the right way. So in our recent paper, we provide a number of recommendations on the best way to use the test in humans and animals, including appropriate sample sizes to be able to detect disease-related differences.

KP: When I was showing the data in human patients to Pascal and the others, I was very concerned because it looked so messy. There was a lot of trial-by-trial variability, which led to concerns over the way these types of data are typically analyzed. Then Pascal showed me the mouse data, and it turned out to be just as messy. I thought I was doing something wrong with the humans, but actually we were getting very similar data.

We then wanted to find the cleanest possible way to analyze the data, so we worked with a couple of statisticians to explore different analyses that might be appropriate and powerful enough to detect group differences and, in future, maybe even drug effects. Typically, Morris water maze analyses use repeated-measures ANOVA, but as we – and others – have pointed out, this method violates some key statistical assumptions. We present a rank summary score method that avoids those problems but is still powerful. It also happens to be the easiest one to apply, so it doesn't require a strong statistical background.

What have you learnt from cross-disciplinary collaboration?

KP: We achieved results and solved problems that we might not have done if we worked separately. It was a great experience that not only benefitted the project, but also helped us all personally.

SF: I'd add that it is tough! It really did feel like we were speaking separate languages, and made me realize that our respective worlds are pretty different. It takes real commitment from the groups involved to work together on an important problem.

PS: It's hard, but it's also extremely rewarding. We would all like our discoveries to be translated and collaborations like this help us to be more involved in that process. These types of projects shouldn't be outliers and, given that the UCSF Memory and Aging Centre is right next door to the Gladstone Institutes, we have no excuse! We have several collaborative projects ongoing, and hope to set up more in the future.

KP: To continue the Morris maze work, we hope to explore whether the test can be used to measure drug efficacy. Another important piece of research would be to compare the test to other cognitive measures that are typically used in Alzheimer's drug trials, such as the Alzheimer's Disease Assessment Scale for Cognition (ADAS-Cog).

Reference

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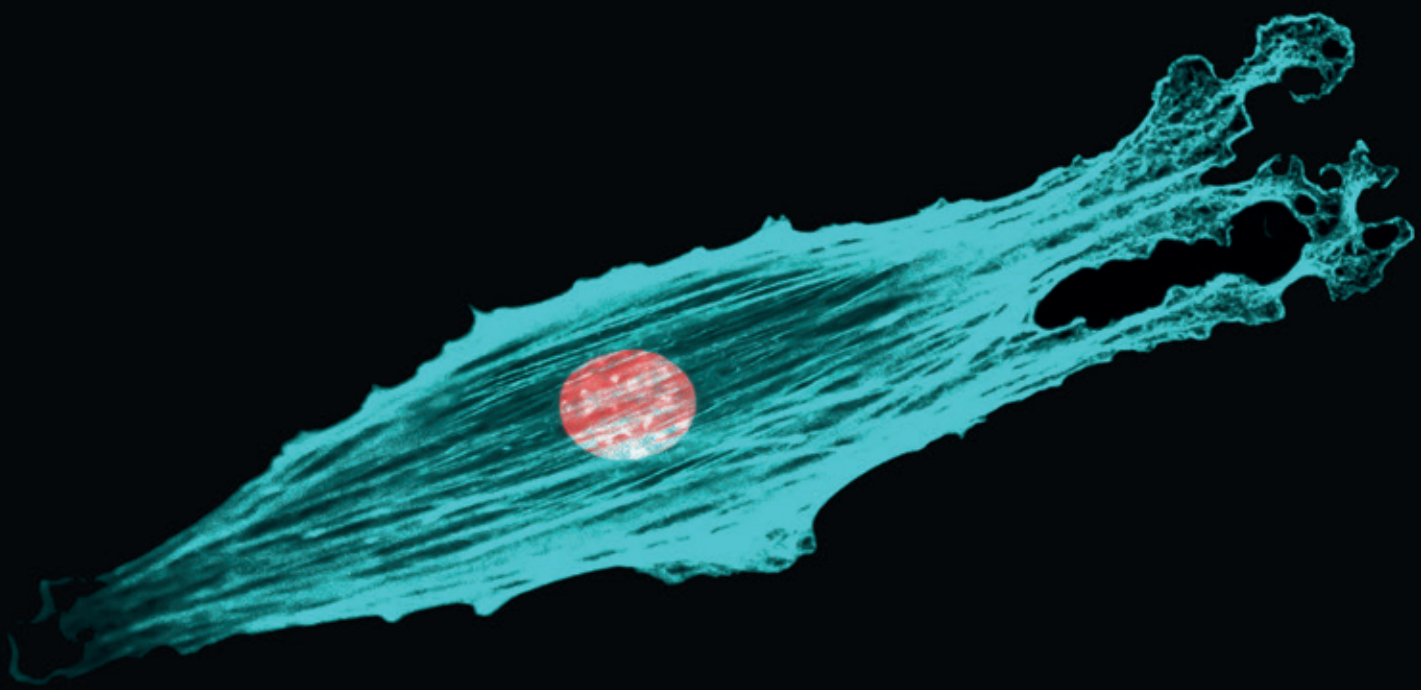

Cells on the (Invasion) Front Lines

Long thought to be a key component of the metastatic process, researchers are now questioning long-held beliefs on the role of the epithelial-to-mesenchymal transition.

By Michael Schubert

The diagnosis and treatment of cancer has moved ahead by leaps and bounds – except when it comes to metastatic disease. Cancer that has spread beyond its origins remains the leading cause of death from the disease, according to the World Health Organization, and although it's a major focus of research, we know little more about its mechanisms today than we did a decade or more ago. This is especially true when it comes to the epithelial-to-mesenchymal transition (EMT), a key component of the metastatic process… or is it?

The debate over EMT's significance is a fierce and ongoing one, largely due to the difficulties inherent in observing and understanding it. Why are researchers so convinced of EMT's role in metastasis? It's well known that mesenchymal cells are more capable of escaping the primary tumor, and of taking up residence in distant sites. But the evidence against EMT-driven metastasis is mounting, too – most cells in metastatic lesions exhibit epithelial, not mesenchymal, characteristics. Some scientists refer to the reverse process, mesenchymal-to-epithelial transition (MET) to explain this behavior, but others aren't so sure. At the root of the confusion is a lack of evidence. Until the entire metastatic process – local invasion, intravasation, circulation, arrest and extravasation, proliferation, and angiogenesis – is observed in mesenchymal cells, the role of EMT in metastasis remains an open question.

Despite the debate, many researchers simply take EMT's role in metastasis as read. Searching the PubMed database for "EMT and metastasis" brings up 3,675 publications, and even Wikipedia – the first port of call for most non-experts without access to peer-reviewed articles – boldly states, "EMT and MET form the initiation and completion of the invasion–metastasis cascade." There's little indication of doubt, and yet, recent studies are threatening to completely overhaul the research community's view of EMT and metastasis. The quest to understand EMT's role is more intense than ever, thanks to groundbreaking new data from research groups whose conclusions go against the grain.

PROCEED WITH CAUTION

Two recent studies on EMT may revise the field's understanding of the process – but it's important to keep in mind the limitations.

By Shyamala Maheswaran

For many years, cancer researchers have believed that metastasis relies on the transition of tumor cells from an epithelial to a mesenchymal phenotype. Even after tumor analysis revealed that the cells of secondary cancers exhibit epithelial characteristics, this was ascribed to a reversal of the transition – from mesenchymal back to epithelial phenotype. Why has this belief persisted so strongly despite uncertainty and debate – and why have the recent papers by Fischer et al. (see page 28, "Tracking the Transition") and Zheng et al. (see page 29, "The PDAC Key") had such an impact on the research landscape?

EMT is an embryonic process required for proper development. It has been observed in tissue culture upon expression of various transcription factors, and following treatment with different cytokines. In vitro, EMT is associated with increased cell migration and invasion. In many cases, the increased invasion observed in vitro translates into increased metastasis in mouse tumor models. But clinical evidence supporting EMT in human tumors has been somewhat limited, due to the difficulty in distinguishing mesenchymally transformed cancer cells from reactive fibroblasts within a tumor. This has led to some debate regarding the importance of EMT in tumor dissemination in the clinical setting. That's where the two new studies may shed light.

Pros and cons

EMT is reversible; it's currently believed that epithelial cells transition into a mesenchymal state, then revert to the epithelial state upon reaching the distal site. The plasticity and transient nature of EMT has made it difficult to follow these cells from the time they transition to a mesenchymal state, through invasion into the blood, and to the point of colonization at distal sites. The two studies reported in Nature are particularly interesting because they both addressed this problem, albeit using very different approaches. Fischer et al. used green fluorescent protein (GFP) expression as a proxy for mesenchymal transition and traced lineage-switched epithelial tumor cells from inception to metastatic colonization in two different mouse mammary tumor models. Zheng et al. knocked down the EMT-inducing transcription factors Snail and Twist in the pancreatic epithelium of mice so that they could monitor the consequences of EMT in the metastatic dissemination of pancreatic tumor cells. These approaches allowed definitive, real-time monitoring of the tumor cells and concluded that EMT is dispensable for metastatic colonization, but plays a role in drug resistance.

"These approaches allowed definitive, real-time monitoring of the tumor cells and concluded that EMT is dispensable for metastatic colonization, but plays a role in drug resistance."

That doesn't mean that these studies are without limitations (1). First, EMT relies on a complex signaling network that involves multiple transcription factors and signaling proteins, in some instances with redundant functions. Whether lineage-tracing studies with single genes can accurately mimic this complex process is unclear. Second, cancer progression involves a continually evolving genomic and epigenetic landscape, so it's unlikely that mouse tumor models driven by only a few oncogenic events fully recapitulate this process. The studies certainly show that EMT is dispensable for metastasis, but readers must recognize the limitations of the mouse models.

Interestingly, in the mouse model generated by Fischer et al., epithelial tumor cells that switch to a mesenchymal state are permanently marked with GFP expression, and illustrate that a small subset of such cells do indeed spontaneously transition into a mesenchymal state (although it isn't required to drive overt metastasis). The prevalence of green cells following drug treatment suggests that cells with a history of EMT, regardless of their current state, are more resistant to drugs. The mechanism by which EMT increases cell survival under adverse conditions is not yet known – but perhaps our new understanding of EMT will provide a springboard for further research.

Old theories have been challenged – now what?

The new studies tell a very different story compared with the prevailing narrative. Why? No one can say for certain, but the EMT models used for in vitro research represent powerful induction of the transition by EMT-inducing transcription factors and cytokines. Spontaneous EMT in clinical specimens might be much more subtle, and could account for – or at least contribute to – the discrepancy between these two studies and those that have previously been reported. Another consideration is that EMT relies on the activation of complex and sometimes redundant signaling modules, an aspect not reflected by the mouse models used in the Nature studies. Although those models do show that EMT is dispensable for metastasis, the findings need to be evaluated within the context of the complexity of tumor progression, which involves an ever-evolving genomic and epigenetic landscape.

EMT is an attractive concept to define the process of metastasis: it involves loss of cell-cell interaction and gain of cell motility. But there are other cellular mechanisms of tumor dissemination, like collective epithelial cell migration or tumor microemboli, that may drive the spread of cancer. And metastasis isn't the end of the story – EMT is also emerging as an important contributor to drug resistance, a phenomenon supported by the findings from both Nature papers. In my own recent work, my colleagues and I demonstrated drug-induced shifts in the epithelial and mesenchymal tumor populations of breast cancer patients. So although the new findings raise questions about EMT's role in metastasis, they also show that the transition does occur in tumors – and not without a purpose, as cells that switch lineage are more resistant to drugs. It's now critical to gain further insight into the molecular nature of this process, so that we can use that information to research better treatments and more accurate prognoses.

As the field moves toward a more complete understanding of the tropism exhibited by tumor cells shed into blood and the role of EMT in drug resistance, I have one word of caution for researchers and clinicians alike. It's important to carefully evaluate what we learn about metastasis from cell culture and mouse models against both human clinical samples derived from repeat biopsies or tumor cells circulating in the blood and freshly established tumor cell cultures. By keeping an open mind to both new information and the limitations of pioneering studies, we can ensure that we're able to focus on the "big picture" of how cancer metastasis happens and what we can do to combat it.

Shyamala Maheswaran is Associate Professor of Surgery at Harvard Medical School and Assistant Molecular Biologist at the Center for Cancer Research, Massachusetts General Hospital, Boston, USA.

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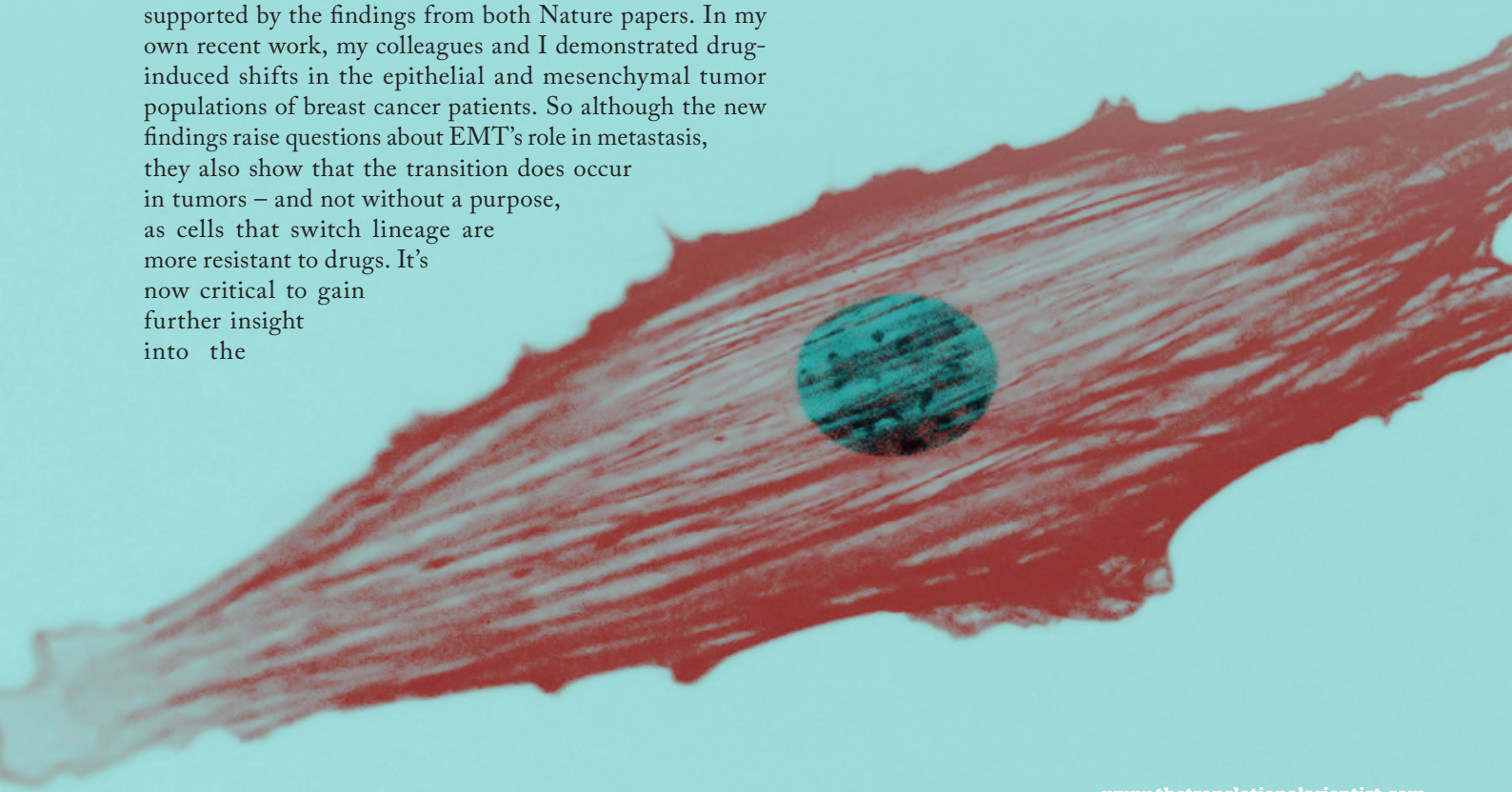

TRACKING THE TRANSITION

A triple-transgenic mouse model allows researchers to trace the lineage of EMT tumor cells, and reveals the transition's surprising lack of significance in metastasis

At Weill Cornell Medical College in New York City, Dingcheng Gao's research group studies cell and developmental biology. Recently, he and his colleagues published a paper outlining their research into EMT (1). They identified a key difficulty with our understanding to date: namely, that there's no way to track transient and reversible EMT phenotypes in living organisms. Without that ability, we can't find out whether or not cells are indeed undergoing EMT to initiate metastasis, then undergoing MET to return to an epithelial phenotype.

So Gao and his colleagues generated a triple-transgenic mouse model known as *MMTV-PyMT/Rosa26-RFP-GFP/Fsp1-cre*, or tri-PyMT. The mouse has three special attributes: an oncogene (PyMT or, in some cases, Neu) driven by the *MMTV* promoter; a recombinase (Cre) driven by the mesenchymal-specific *Fsp1* promoter; and two fluorescent proteins, red and green, each under separate control. The fluorescent proteins combine to form an irreversible color switch system – so once a cell has undergone EMT (and acquired green fluorescence), it's incapable of reverting to red fluorescence. This means that it's easy to see which cells have made the transition from epithelial to mesenchymal, even after they have transitioned back to epithelial characteristics.

"We wanted to find direct evidence in vivo to prove the EMT/MET hypothesis in metastasis formation," explains Gao. "Therefore, we established the EMT lineage tracing model using a permanent fluorescent marker switch to trace the reversible EMT process." But the team were in for a surprise. The cancer cells of the mice, which developed primary breast tumors followed by spontaneous lung metastases, didn't show the expected results. In fact, they showed exactly the opposite: none of the secondary lesions changed color following the natural progression of lung metastasis. The lack of color switching indicates that *Fsp1*, the mesenchymal promoter designed to permit green fluorescence, was never activated – and thus, that the metastatic cells may never have undergone EMT. Furthermore, inhibiting EMT with the use of the microRNA miR-200 prevented red-to-green color switching – but had no effect on the ability of tumor cells to metastasize.

"Cancer cells are capable of metastasizing through other mechanisms, such as collective invasion and random dissemination," says Gao. He cites a recent report by Cheung et al. in which the authors traced the lineage of metastatic tumors and showed that seeding by cell clusters, rather than by single cells, can result in polyclonal metastases (2). Collective invasion is typical of carcinomas like those often found in the breast or lung, and challenges the belief that metastases arise from single "escaped" tumor cells that undergo EMT. But if EMT isn't the key player in cancer dissemination, then what is?

"We've observed that EMT is a relatively rare event in primary tumors," says Gao. "Even though EMT tumor cells gain some anti-apoptosis properties that may help them survive in circulation, these advantages are accompanied by a downside – a decreased ability to proliferate. In general, metastasis is a very inefficient process for tumor cells. In our experiments, the rare cells that had undergone EMT were easily outnumbered by the epithelial cells, not just in the primary tumor, but also in the circulation and metastatic lesions." So if EMT is costly for tumor cells and most metastatic cells show no obvious reliance despite its potential survival advantage, what is its purpose in the tumor?

> "Our results suggest that tumor cells that undergo EMT are more resistant to chemotherapy than non-EMT cells."

The second part of the Cornell paper offers an answer. Evidence from previous studies has suggested a link between EMT and chemoresistance – most notably in residual breast cancer, where the remaining tumor cells display mesenchymal characteristics (2). Gao and his team decided to investigate this link by treating their tri-PyMT mouse models with cyclophosphamide. Even during the initial treatment phase, green fluorescent (mesenchymal) cells were less proliferative – but also less apoptotic – than epithelial cells, indicating lower susceptibility to chemotherapy. But in metastatic lung tumors, the effect stood out even more. The mesenchymal cells outnumbered the epithelial population by almost three to one, and made notable contributions to five of the 17 total metastatic lesions (in contrast to untreated mice, where no lesions contained a significant mesenchymal cell population). "Post-EMT tumor cells showed a greater ability to survive chemo treatment," Gao summarizes. "This won them a better chance to develop into metastatic lesions."

"Our results suggest that tumor cells that undergo EMT are more resistant to chemotherapy than non-EMT cells. More importantly, we have observed a significant contribution of these EMT tumor cells to metastasis formation under chemotherapy conditions. Therefore, targeting EMT tumor cells may provide novel

therapeutic approaches to overcome chemoresistant metastasis." Gao thinks this is a vital piece of knowledge in the clinic. "Given that most patients with advanced-stage tumors are treated with chemotherapy, it's important to evaluate the EMT status of their tumors. Patients whose cells have undergone the transition would benefit from EMT-targeting therapy approaches." Of course, there's much still to be learned about the nature of metastasis. "One immediately attractive question," says Gao, "is whether the metastatic epithelial tumor cells differ in other characteristics from the majority of cells in the primary tumor. Characteristics like CK14 expression, multiple clonality, and other potential mechanisms in metastasis need to be further investigated." For his part, Gao and his laboratory are currently focused on developing novel strategies for targeting EMT tumor cells, with the hope of one day finding a way to overcome cancer chemoresistance.

Dingcheng Gao is Assistant Professor of Cell and Developmental Biology in Cardiothoracic Surgery at Weill Cornell Medical College, New York, USA.

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THE PDAC KEY

Pancreatic cancer cells don't seem to rely on EMT for metastasis – but it plays a key role in their ability to resist our best chemotherapy options

At the same time, hundreds of miles away in Houston, a group of researchers from the MD Anderson Cancer Center, Baylor College of Medicine and Rice University were collaborating on a closely related piece of work. Using specialized mouse models of pancreatic cancer with impaired EMT, Raghu Kalluri and his colleagues were investigating the transition's role in mediating metastasis and chemoresistance. The unexpected conclusion they reached mirrored the one from Dingcheng Gao's group – namely, that pancreatic cancer, like breast cancer, can metastasize without undergoing EMT (1).

The group began by creating transgenic mouse models of pancreatic ductal adenocarcinoma (PDAC) in which either Snail or Twist, two of the transcription factors responsible for inducing EMT, were knocked out. Deleting the *Snai1* and *Twist1* genes had no effect on the development or appearance of pancreatic tumors, but the researchers noted a significant decrease in cells undergoing EMT. Immunolabeling of the primary tumor showed far fewer epithelial cells that expressed either αSMA (a mesenchymal marker indicating EMT-positive status) or Zeb1 (another EMT-inducing transcription factor similar to Snail), and global gene expression profiling revealed a decrease in the expression of EMT-associated genes. What was increased, on the other hand, was the degree to which cancer cells proliferated when the transition was suppressed. With no change in the timing of tumorigenesis and local invasion, it's clear from these experiments that PDAC doesn't rely on EMT to initiate and progress.

But metastasis is at the heart of the question. Do these cancers rely on EMT in order to spread to distant areas of a mouse's – or a patient's – body? The researchers compared circulating tumor cells in control and EMT-suppressed mice and found that the numbers were unchanged. Histopathology and immunostaining in livers, lungs and spleens (the major target organs of metastasis) revealed approximately the same frequency of cancer spreading in both groups – and, when examined more closely, the metastases all proliferated at about the same rate and were largely negative for EMT-inducing factors Twist, Snail, Zeb1 and αSMA. The take-home message? Removing EMT from the equation doesn't affect the cells' ability or inclination to metastasize.

So it appears that the Texas group's pancreatic tumors behave much like the Cornell group's breast cancers. Is the same true of the cells' ability to survive chemotherapy? Previous studies have established a link between EMT and gemcitabine resistance in PDAC (2–4). "Gemcitabine works primarily on cancer cells that are dividing or proliferating. When cancer cells suspend their proliferation – such as when they launch an EMT program – then anti-proliferation drugs like gemcitabine do not target them well," says Kalluri (5). The next step, then, was to test sensitivity to the drug in cells with suppressed EMT. The researchers administered gemcitabine to control, Snail- and Twist-knockout mice and discovered that, with the EMT-inducing factors removed, the chemotherapy-treated animals showed improved histopathology and survival. This held true across different mouse models of pancreatic cancer, all of which

showed better responses to gemcitabine after EMT suppression – decreased tumor burden and proliferation, increased cancer cell death, and extended survival times.

"We found that EMT program suppressed drug transporter and concentrative proteins, which inadvertently protected these cancwer cells from anti-proliferative drugs such as gemcitabine," says Kalluri. "The correlation of decreased survival of pancreatic cancer patients with an increased EMT program is likely due to their impaired capacity to respond to chemotherapy, leading to overall poor prognosis and higher incidence of metastasis." (5)

Are there other possible explanations? The research still has gaps; it's possible that other EMT-inducing transcription factors are replacing Snail and Twist in knockout mice, or that EMT suppression from birth (as in the mouse models) has a different effect to EMT suppression only at or after the onset of disease. It doesn't look like the transition plays a significant role in PDAC metastasis – but in order to make that statement conclusively, more research, and probably more fierce debate amongst researchers, is needed.

But at the moment, the findings are fairly clear with respect to chemoresistance, and it seems clear that – by reducing proliferation and decreasing the expression of genes involved in transporting and concentrating drugs – the transition confers resistance to treatment and thus compromises patient survival. What does that mean for the clinic? Ultimately, that establishing a patient's EMT status may provide insight into the potential for treatment – and that although treatments targeting the transition may not prevent metastasis, could offer a way of enhancing the effectiveness of existing therapies.

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RESEARCH TIMELINE

1995
An overview of epithelio-mesenchymal transition
ED Hay

The EMT produces a mesenchymal tissue type in higher chordates. It's a central process for embrogenesis. But mesenchymal cells, unlike epithelial ones, can invade and migrate through the extracellular matrix – meaning that EMT has the potential to create invasive metastatic carcinoma cells. E-cadherin gene transfection can convert mesenchymal cells back to epithelial phenotype.

Acta Anat (Basel), 154, 8–20.

2007
Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype?
H Peinado et al.

Snail, Zeb and some basic helix-loop-helix (bHLH) factors induce EMT and repress E-cadherin expression. These changes are associated with tumor progression. As a result, further research into these EMT-inducing factors may ultimately have clinical implications, with the potential for targeted treatments that prevent EMT and restore E-cadherin expression.

Nat Rev Cancer, 7, 415–428.

2008
The epithelial–mesenchymal transition generates cells with properties of stem cells
SA Mani et al.

The induction of EMT in human mammary epithelial cells results in the acquisition of not only mesenchymal traits, but also properties associated with stem cells (like increased expression of stem-cell markers or the ability to form mammospheres). Stem-like cells and post-EMT cells exhibit similar behaviors and express similar markers, and post-EMT cells are more efficient at forming mammospheres, colonies and tumors.

Cell, 133, 704–715.

2010

EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer

A Singh, J Settleman

"EMT induction in cancer cells results in the acquisition of invasive and metastatic properties." The transition can also contribute to the emergence of cancer stem cells and drug resistance. It's possible that reversible epigenetic changes associated with chemoresistance may depend on the differentiation state of the tumor – and thus on cancer cells' stem cell-like characteristics or EMT status.

Oncogene, 29, 4741–4751.

2011

Cancer stem cells and epithelial-to-mesenchymal transition (EMT)-phenotypic cells: are they cousins or twins?

D Kong et al.

Cells that have undergone EMT share molecular characteristics with cancer stem cells and are associated with tumor aggressiveness and metastasis. "The acquisition of an EMT phenotype is a critical process for switching early stage carcinomas into invasive malignancies, which is often associated with the loss of epithelial differentiation and gain of mesenchymal phenotype."

Cancers (Basel), 3, 716–729.

2014

Twist1-induced dissemination preserves epithelial identity and requires E-cadherin

ER Shamir et al.

What are the minimum molecular events necessary to induce the dissemination of epithelial cells? Expression of EMT induction factor *Twist1* resulted in rapid dissemination, along with changes to extracellular compartment and cell–matrix (but not cell–cell) adhesion genes. The cells were unexpectedly able to disseminate with membrane-localized β-catenin and E-cadherin (whose knockdown strongly inhibited the process). Therefore, dissemination can occur without loss of the epithelial phenotype – indicating that cancer metastasis might also occur without EMT.

J Cell Biol, 204, 839–856.

Now

Epithelial-to-mesenchymal transition is not required for lung metastasis but contributes to chemoresistance

KR Fischer et al.

Nature, 527, 472–476.

Epithelial-to-mesenchymal transition is dispensable for metastasis but induces chemoresistance in pancreatic cancer

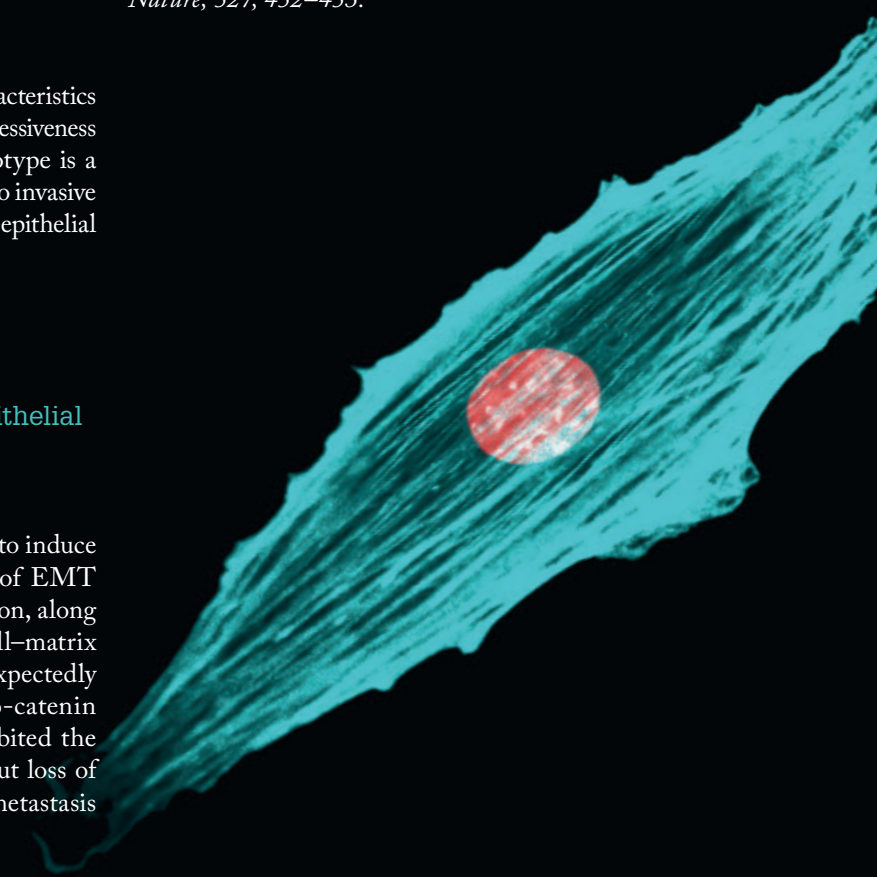
X Zheng et al.

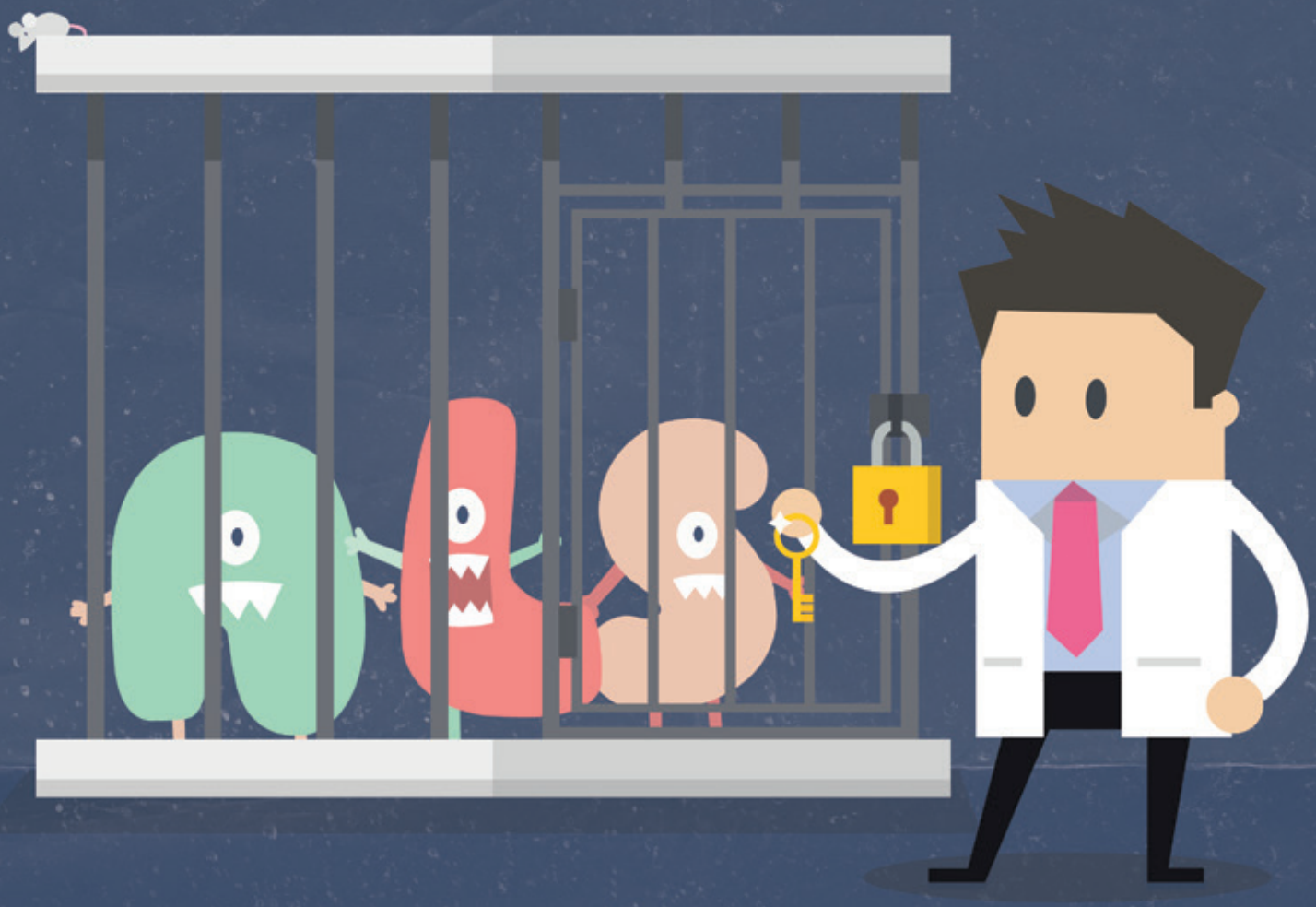
Nature, 527, 525–530.

Cell fate: Transition loses its invasive edge

S Maheswaran, DA Haber

Nature, 527, 452–453.





Arresting ALS

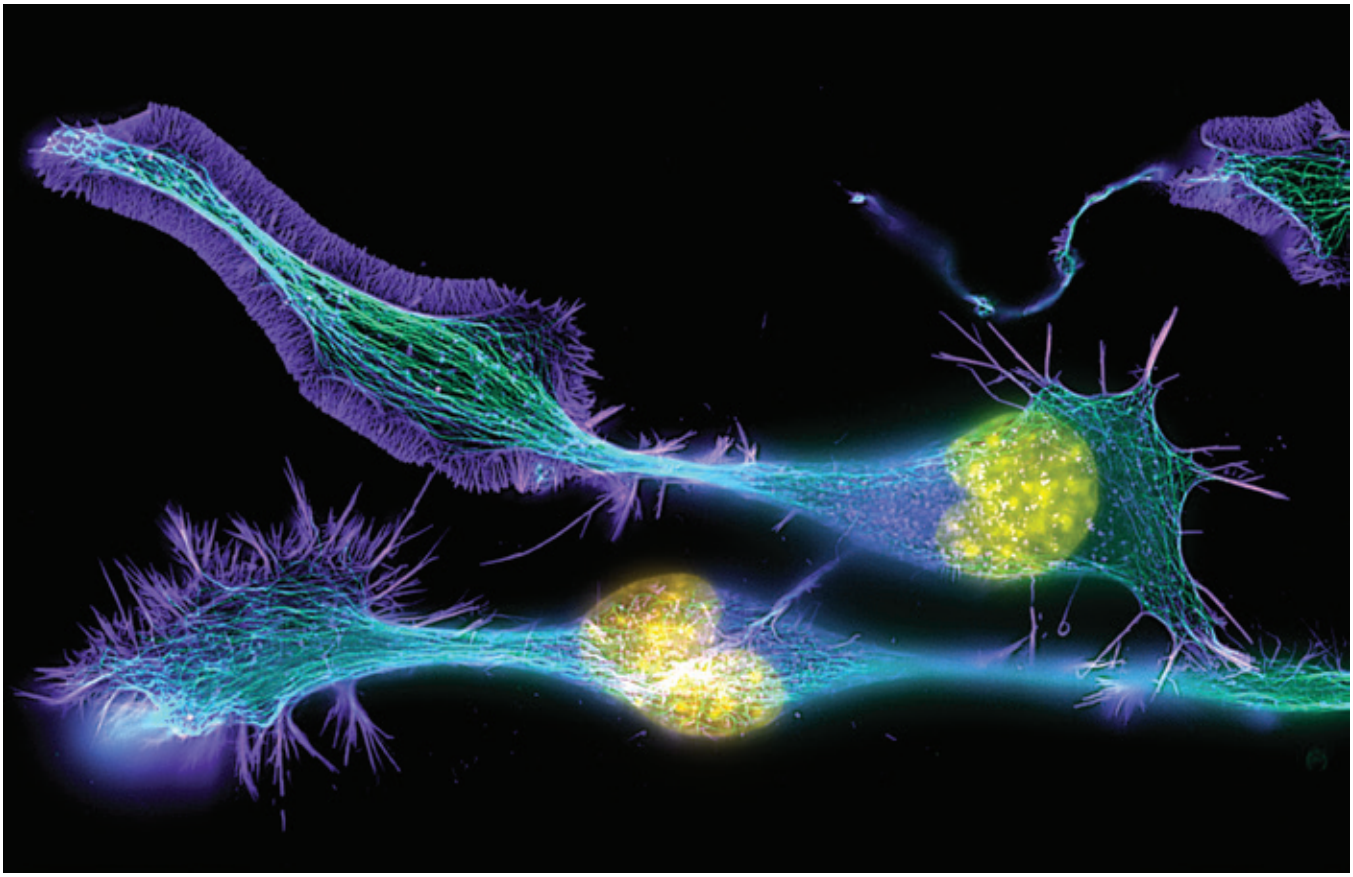
When it comes to prognosis, the mouse models I use to study amyotrophic lateral sclerosis (ALS) are sadly accurate – like their human counterparts with familial ALS, all mice with these mutations die young. After 20 years of trying – and failing – to find a treatment that could extend life, I was close to giving up, until a new drug candidate came along. Then something amazing happened – a mouse lived.

By Joe Beckman

Every day I receive emails from ALS patients and their loved ones, which is both gratifying and heart breaking. The time from onset of the disease to death can be just a few years, and patients are desperate for some hope. The possibility that my work might help these people is a large part of what has kept me going for over 20 years, despite all the setbacks and frustrations.

My ALS journey actually started with an interest in oxidative stress. I was studying the oxidant peroxynitrite, which mediates tyrosine nitration – a process you can find in stroke, diabetes, heart disease, neurodegenerative disorders and many other conditions. One of the major antioxidant defenses that prevents the formation of peroxynitrite and protects the body from oxidative stress is the protein superoxide dismutase (SOD). But SOD also has a dark side – we discovered that it can catalyze tyrosine nitration, speeding up oxidative damage.

In 1993, it was found that some inherited cases of ALS are caused by mutations in the SOD-producing gene *SOD1 (1)*. Our research group hypothesized that in these patients, SOD would catalyze tyrosine nitration and make the disease worse – a toxic gain-of-function. Soon after, we discovered that mutant SOD did exactly that (2), and ever since we've been focused on better understanding the role of SOD in ALS, and ultimately finding a therapy.



Developing mouse nerve cells. Credit: Torsten Wittmann, University of California, San Francisco

A cloud with a copper lining?

In many diseases – even intractable enigmas like Alzheimer's – therapies work in mouse models, but cannot be translated into humans. In ALS – a disease whose pathology has been cited since 1824 and named since 1874 (3) – there had never even been a single functionally effective therapy in a mouse model. To this day, only one drug – riluzole – has been approved for use in the treatment of ALS, and that came onto the market 20 years ago, and increases life expectancy by only 2–3 months (4).

Over the years, we made many discoveries that kept pushing our knowledge a little bit further, but nothing that we could pursue as a possible therapy. The *SOD1* mutant mice still died at around 130 days. Eventually, we decided to shift focus – if we couldn't slow the disease process, could we speed it up? If we know how to break something, we reasoned, we might get a better idea of how to fix it.

Copper and zinc are essential for the maturation of SOD, and in ALS mouse models a lack of copper binding causes accumulation of mutant, immature SOD. A paper published in 2007 revealed that if you overexpress the copper chaperone for SOD (a metalloprotein named CCS) in wild-type mice, the mice are perfectly fine. But if you overexpress CCS in an ALS mouse model, the mice start dying six or seven times faster (6). That was an interesting discovery, because all human ALS patients have comparatively high CCS relative to SOD.

A former student of mine – Blaine Roberts – visited our laboratory and talked about a compound being studied by the Florey Institute in Melbourne (where he is now head of metalloproteomics), called copper-ATSM (CuATSM). The compound is traditionally used in PET imaging but Peter Crouch's lab, alongside Roberts, had shown that it can improve locomotor function in ALS model mice (7). CuATSM delivers copper to the brain, and we suspected that it would counteract the copper deficiency seen in ALS mouse models.

A breakthrough

We acquired mice from the CCS study (6) – which developed ALS symptoms relatively slowly – and cross-bred them

with the standard *(SOD1-G93A)* ALS mouse model, resulting in mice that died in 8–14 days. The first hurdle we faced in testing the drug with this model was how to get the CuATSM into tiny four or five day old mice, who were already runts. Our solution came when we found that CuATSM is soluble in dimethyl sulfoxide (DMSO).

As we pipetted the CuATSM/DMSO solution onto the backs of the baby mice, it was absorbed through the skin within minutes, turning it bright red. Soon after, we watched the color fade as the solution was distributed into the subcutaneous fat, then subsequently to the brain and other organs via the bloodstream. Now the question became – would CuATSM affect disease progression?

"It was difficult to bite our tongues, because we saw these results just as the ice bucket challenge was becoming a global phenomenon and everyone was talking about ALS."

Straight away, the mice started to improve markedly. They gained weight, and a few days later, far from being at death's door, they showed no signs of disease. They began to develop quite normally, and to our surprise they passed the crucial 130-day mark, then 150 days, then 200. It was around day 230 when the first mouse became sick, but the others made it beyond their first birthday – unheard of in ALS research!

But our first response wasn't elation, instead it was "something has to be wrong". Our first thought was that the transgenes could have become inactivated, and only once we had ruled that out did we allow ourselves to get a little excited. Even then, we wanted to be really sure that the findings were airtight, so we immediately embarked on a series of very carefully controlled trials. It was difficult to bite our tongues, because we saw these results just as the ice bucket challenge was becoming a global phenomenon and everyone was talking about ALS (see "Putting ALS on Ice").

We spent a lot of time considering sample sizes, consulting with statisticians about the setup and animal specialists about breeding the mice. We also

Putting ALS On Ice

By Joe Beckman

The ice bucket campaign accelerated the momentum of ALS research; plus, it educated people about the disease and the challenges faced by those living with it. We have been fortunate to receive ongoing funding from the Department of Defense that has kept our lab going through some tough times, and the ice bucket challenge has given that opportunity to other researchers through the extraordinary generosity of so many people around the globe.

After the campaign, it was great to see a lot of undergraduates coming to my lab looking to work on ALS, all of whom were incredibly motivated and passionate. My hope is that the attention and funding generated by the campaign is the beginning of growing interest, and not just a temporary blip on a radar.

What a difference a year makes

Fiscal year ending Jan 31st 2014 - **$8.4 million** in contributions

Fiscal year ending Jan 31st 2015 - **$121.4 million** in contributions ($115 million from the ice bucket challenge)

How the money will be spent

67% Research.
20% Patient & community services.
9% Public and professional education.
2% Fundraising.
2% Processing fees.

Data from the ALS Association.

ALS Genes

Genotypes of familial ALS and their associated chromosomes

1	*ALS 10*	13	
2	*ALS 2* *ALS 19*	14	*ALS 9*
3	*ALS 17*	15	*ALS 5*
4		16	*ALS 6*
5	*ALS 21*	17	*ALS 18*
6	*ALS 11*	18	*ALS 3*
7		19	
8		20	*ALS 7* *ALS 8*
9	*ALS 4* *ALS 14* *ALS 16*	21	*ALS 1* *(SOD1)*
10	*ALS 12*	22	
11		x	*ALS 15*
12	*ALS 13* *ALS 20*	y	

blinded the study since the CuATSM/DMSO could stain the fur. We did that all to maximize the amount of good data we could obtain. Initially, we were worried about CuATSM toxicity but it soon became clear that there was no serious toxic effect on the mice, so we were able to increase the dose considerably. Ultimately, we settled on treating pups from the age of 5 days, with 30 mg/kg/dose of CuATSM twice daily, and the results surpassed all our expectations. We extended the lives of the standard *SOD1* mutant ALS mouse model by 25 percent, and the ALS mouse model with both *SOD1* mutation and overexpressed CCS by an amazing 500 percent (8). What's more, cessation of CuATSM caused the mice to develop ALS symptoms, and restarting therapy rescued them.

Trials and tribulations

We carried out a lot more experiments to reproduce certain elements of our findings, and compared our results with those of the drug's developers in Melbourne, so by the time the paper came out we felt confident in our results. Despite this, we were met with skepticism from some of the peer reviewers, who were concerned that the mouse model may not translate into human patients. Only 2–7 percent of all ALS patients have a *SOD1* mutation, and some consider this familial form a separate disease to sporadic ALS. Although it makes up the vast majority of cases, very little is known about the etiology of sporadic ALS – which is why most ALS researchers study familial forms. I believe there is a definite possibility that the drug might work in sporadic ALS patients – after all, the *SOD1* mutation amplifies traits of the wildtype protein. But even if the drug does only work in the *SOD1* mutation patients, it would be a much needed breakthrough for the disease.

The Melbourne group have taken the lead in developing the compound for Parkinson's disease and ALS. Their license was granted to a newly formed company called Procypra, based in the USA, which is developing CuATSM for treatment of Parkinson's and ALS.

Where to next?

The identification of new genes is driving the field forward – we need to find out what is making things go wrong before we can start to think about how to fix it. There are now over 20 different types of genetic mutations linked with ALS, each one opening up new avenues of research (see "ALS Genes" and "Research Roundup"). However, it's unfortunate that the *SOD1* gene has been neglected by researchers who feel that, after decades of study with very little progress, it's a dead end. I'm hopeful that our results will change that perception and encourage other researchers to start using this model again.

Right now, the team and I are working really hard on finding other variants of the CuATSM drug that will deliver enhanced results; the drug that we have right now works much better than the first 20 versions we tried, so there's definitely hope that we can keep improving. We're also advancing the mass spectrometry methods that we use to measure what's happening inside the motor neurons, to give us an even clearer view into the mechanisms of disease. Thanks to those efforts, we already have a pretty good idea about how SOD causes motor neurons to die, but we want to keep honing those ideas until we can address all the potential criticisms.

I'm really excited about the field right now. The ice bucket challenge put ALS in the spotlight and injected much-needed funding. Now we must continue to drive research forward until we have an effective treatment in the clinic. We understand so much more about the mechanisms of the disease, that it no longer seems like a hopeless task. Research has reduced the disease to something we can attack, and hopefully defeat.

Joe Beckman is the Principle Investigator, and Burgess and Elizabeth Jamieson Chair, in Healthspan Research at the Linus Pauling Institute, Oregon State University, OR, USA.

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Research Roundup

With a boost in funding and awareness from the ice bucket campaign, ALS research is moving fast. Here are just three of the latest advances.

Symptom relief

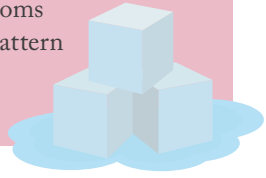
AB Science SA have recently announced the success of their phase 2/3 trials investigating the efficacy of a protein kinase inhibitor – designated masitinib – in improving the severity of disability according to the ALS functional rating scale (ALSFRS-R) of patients (9). The drug targets mast cells and macrophages through inhibition of certain kinases, and affects symptoms associated with CNS-related diseases, including ALS.

Role for retrovirus

Researchers from the NIH recently published a paper citing that human endogenous retrovirus-K (HERV-K) plays a role in sporadic ALS (10). The researchers found the virus expressed in cortical and spinal neurons in ALS patients, and discovered that HERV-K and its envelope proteins may contribute to neurodegeneration.

Misfolding mutants

A paper investigating the propagation of ALS lends weight to the idea that mutant SOD protein spreads via a prion-like mechanism. Mice which had a mutation in the *SOD1* gene but had not yet developed symptoms, were injected with material from the spinal cords of affected mice, containing misfolded, mutant SOD proteins. Mice who received the injection developed ALS symptoms within months, which spread in a pattern similar to patients with ALS (11).



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Toolbox

Key tools
New technology
Emerging techniques

Ghost in the Cell

New software couples computer programming with cell function using the ultimate coding language: DNA

By William Aryitey

A team of bioengineers at MIT have created a programming language for living cells (1), an advance that could allow even novices to design biological circuits.

The language is called Cello (derived from "cell logic"), and works by converting a text description of a desired computational operation to a DNA sequence. For example, cell circuits can be designed to sense environmental factors, and respond to them by upregulating specified genes.

Every living cell contains a genetic program, encoded in DNA, which controls cell functions via the interactions of a network of regulatory proteins (for example, repressors and activators). To describe how different interactions correlate to function, synthetic biologists use terminology from electrical engineering – for example, one set of interactions might function as a sensor, another as an oscillator.

Synthetic biologists have been using cells as "machines" for some time and have built up a library of genetic parts, such as sensors, biological clocks, and actuators. But the process of designing circuits has traditionally been laborious and required in-depth genetic knowledge. Cello makes the construction of genetic circuits accessible to anyone with basic computer programming skills.

The story behind Cello begins in the late 1980s and early 1990s with lead researcher Christopher Voigt's teenage hobby of computer programming. "I started off with no interest in biology at all. I wanted to study computer science but my dad suggested a broader degree, so I ended up majoring in chemical engineering," says Voigt, now a professor of biological engineering at MIT. At college he took no biology, focusing purely on physical science. "What led me to the biotechnology side of things was a bit of serendipity. After picking up some exam results in inorganic chemistry, I was staring at a bunch of comics around a professor's door. I didn't notice he was inside, but he spotted me. He called me into his office and started talking about his research in biophysics. By the end of our conversation he had offered me a job. That's how I got my start."

From there, Voigt became interested in protein engineering, which led him to synthetic biology. "To me, biology is the ultimate programming language. So when I started my own lab, the team's focus was on understanding that program," says Voigt.

Short circuit

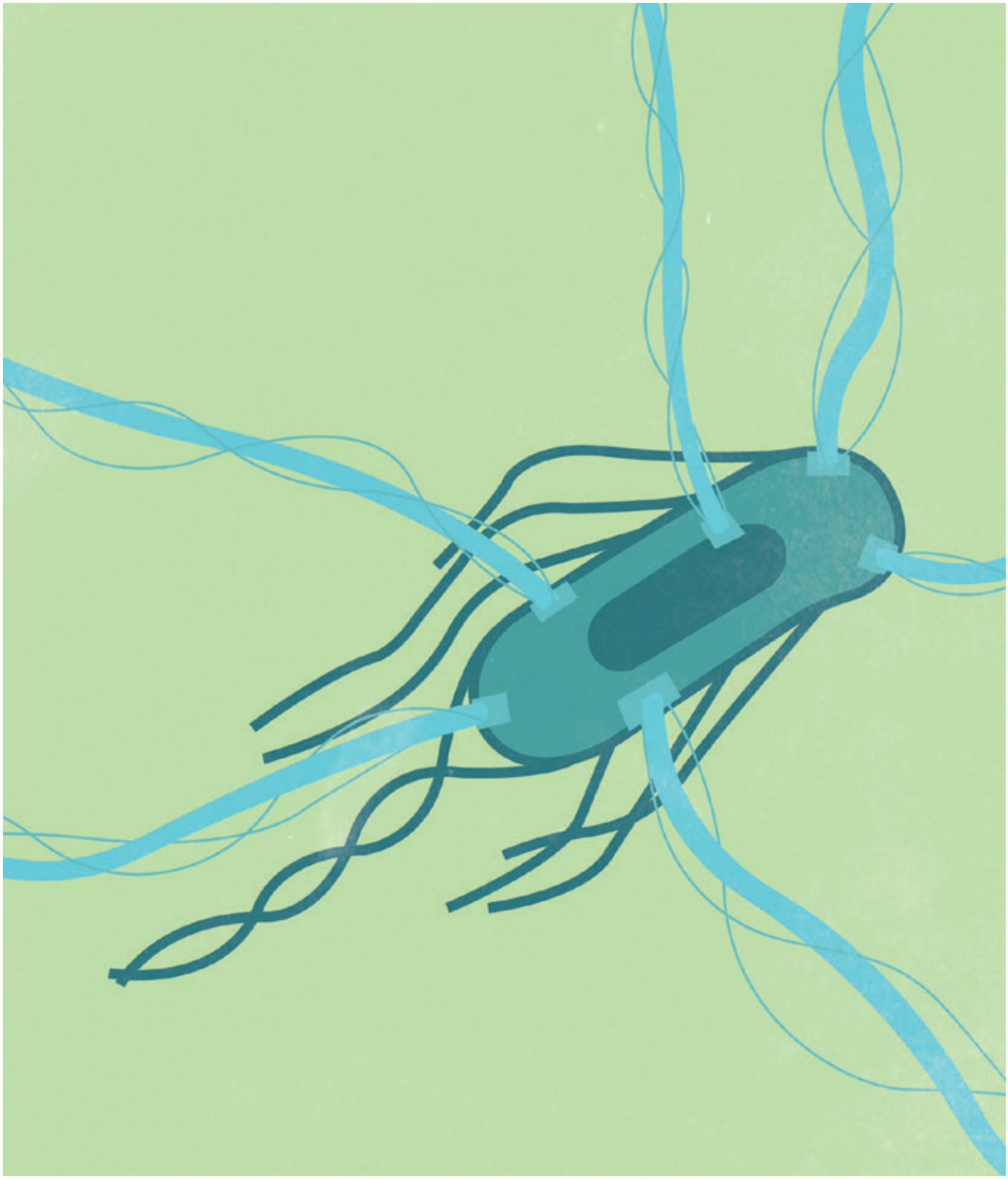
Writing Cello was relatively straightforward, says Voigt, but generating functional DNA proved more difficult. The challenge was to get the genetic components – called logic gates by analogy with electronic circuits – to operate in the same way no matter what context the programmer put them in.

At first, using a gate in certain contexts would cause circuit failure, and resolving those kinds of issues took a lot of good engineering, not to mention dogged determination. "Those problems were often because moving the gates in relation to the DNA would alter some of the biochemistry, which would then propagate through the entire circuit. So we had to figure out what was causing these issues, and then create fixes to each one systematically," says Voigt.

"To me, biology is the ultimate programming language. So when I started my own lab, the team's focus was on understanding that program."

Working with colleagues at Boston University and the National Institute of Standards and Technology, the team carried out a study, published in April 2016, using the system to successfully build biological circuits consisting of up to 12,000 base pairs. An impressive 45 out of 60 circuits designed as part of the study worked the first time they were tested.

It's a significant achievement, but the team aren't stopping there; they are continuing work to make the system more



Logic Gates

Logic gates are basic building blocks used in digital circuits, which usually have two inputs (A and B) and one output (Y) – all of which are Boolean (they can only be true or false). There are five basic types of logic gates, described below. Cello uses two-input and three-input NOT and NOR gates in the form of repressor proteins.

Type of gate	*Output is "On" (1) if…*	*Symbol*	*Result*
AND	*A and B are true*		*Input* | *Output* A B Y 0 0 0 0 1 0 1 0 0 1 1 1
OR	*A or B are true*		*Input* | *Output* *A B Y* 0 0 0 0 1 1 1 0 1 1 1 1
NOT	*A is not true*		*Input* | *Output* *A Y* 0 1 1 0
NAND	*A and B are not true*		*Input* | *Output* *A B Y* 0 0 1 0 1 1 1 0 1 1 1 0
NOR	*A or B are not true*		*Input* | *Output* *A B Y* 0 0 1 0 1 0 1 0 0 1 1 0

robust and flexible. "One big hurdle is the number of gates. Right now we can implement a maximum of 12 – and it gets really difficult after nine – but ideally we'd like 50 or 100 gates in a cell. That would really allow us to unleash the potential of biology," says Voigt, "The other challenge is moving to different organisms, which really expands what we can do. Each cell has its own idiosyncrasies and nuances to overcome, so we're trying to create a system that we don't need to rebuild from scratch for every organism."

Ex machina

Cello is based on Verilog, a programming language used for electronic circuits. It allows the user to select from dropdown lists of inputs (sensors) and outputs (actuators), and type in Verilog commands to specify how they should be logically connected. For example, one of the available inputs is the pTET promoter, which switches on or off transcription in response to the presence of the antibiotic tetracycline, while outputs include fluorescent proteins in various colors.

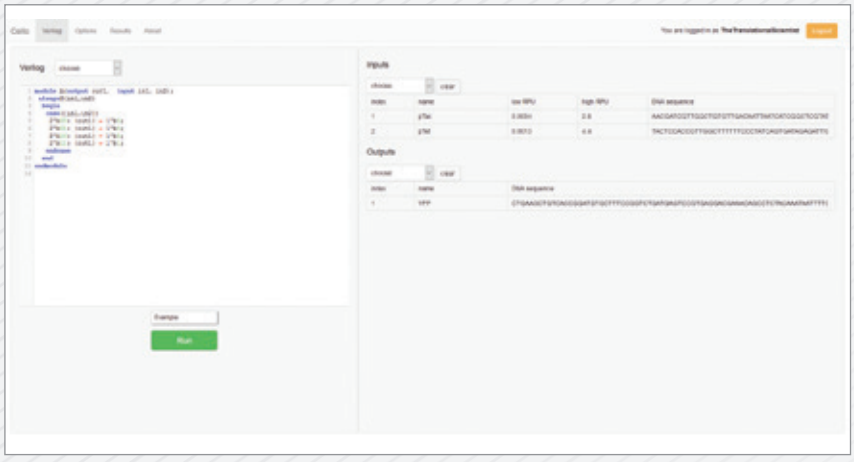
Having compiled your program, just click "run" and Cello automatically calculates the optimum DNA sequence for the circuit. To run the program in *E. coli*, the researchers synthesized the DNA and inserted it into two plasmids. One plasmid contains the circuit and sensors, while the other encodes the actuator.

"We wanted to implement basic software that would allow users without an understanding of biophysics or genetics to be able to use the logic gates. If a computer is handling that sort of information, it frees the designer up to think about the program they want to run, and not necessarily all the interactions required to implement it," says Voigt.

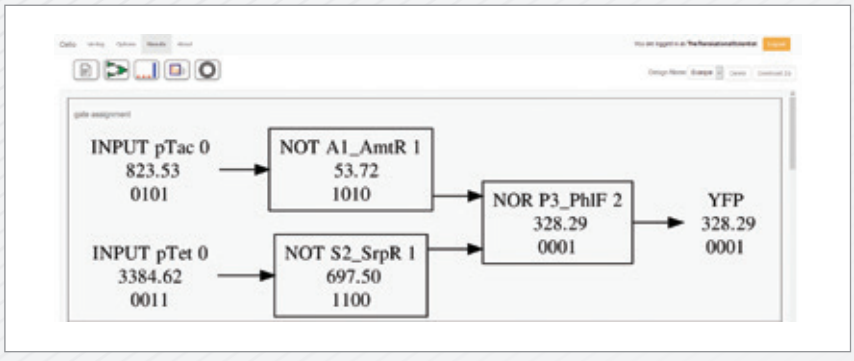
The team have made Cello freely available to try online (www.cellocad.org), so biologists and computer scientists alike can try their hand at programming a cell circuit. And the programming code is open

Here's One We Made Earlier

The Cello website – log in, choose inputs and outputs, then connect them using Verilog code (or, like us, use the demo program). Name your creation, and hit "run".



Below is the resulting circuit diagram. In this example, if both tetracycline and lactose are present, the cell will produce yellow fluorescent protein. The results include an optimal genetic layout and plasmid DNA sequence.



source, so anyone can upload new sensors, actuators and "user constraints files", which define the organism, gate technology, and valid operating conditions. "We wanted to make it freely available so people could be as creative as possible and not worry about the development involved," says Voigt.

Ctrl Alt Gamete

Synthetic biologists have proposed a range of potential applications for engineered cells, including everything from agriculture to chemical production. Amongst biomedical scientists, the potential for therapeutic applications has sparked interest. Engineered cells could be programmed to navigate to an area of disease and deliver drugs in doses dependent on time, location, or concentration. Another possible application is reprogramming the body's own microbiome to fight disease.

This research serves as an example of how approaching a field from a different angle, in this case tackling the genome from a computer programming perspective, can lead to advances. "It's a completely different mindset", agrees Voigt, "Instead of trying to unpeel biology and all its complexity, the idea is to simplify it all the way down. We pick out the elements that can be implemented by a computer and ignore everything else as much as possible."

For this reason, Voigt believes that the increasing sophistication of synthetic genomes won't necessarily teach us a great deal about the workings of genetics in nature. "It's sort of like how building an airplane doesn't teach us anything about how birds fly – the principles are totally different. As we've gotten better at creating modular systems that can be put together by a computer, we're getting further and further away from the messy, haphazard genome created by evolution. And in order to make our system designable, we had to implement fixes that look nothing like the genetics you see in biology. Somebody with an expert eye could go into our DNA and instantly recognize that it's not natural," says Voigt.

A new breed of bioengineers

With the launch of Cello, Voigt hopes that people with a penchant for computer science might be turned on to the fascinating potential of biological circuits and, like him, discover an interest they never knew they had. "The potential applications are huge and one reason people aren't getting involved is because it could take years to develop each individual circuit. We thought making it accessible was the best way to speed up the process," concludes Voigt.

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Translated

Celebrating success
Translation in action
Bench-to-bedside

In Good Company

What does it take to create a competitive companion diagnostic? Bharathi Vennapusa outlines her role in the development and approval of the ALK CDx – a fully-automated immunohistochemistry assay that identifies lung cancer patients who may be eligible for treatment with Pfizer's Xalkori (crizotinib).

How did you get involved in companion diagnostics development?

After training as a pathologist and specializing in molecular pathology, I decided I wanted a career that was neither entirely basic research nor clinical practice. I didn't know much about companion diagnostics at that time, but through a friend I learned about Ventana Medical Systems (now a member of the Roche Group), which was active in the field. Pathologists often don't consider careers in pharma, but I became inspired by the prospect after reading a journal article by Ventana's Chief Medical Officer, Eric Walk, which discussed the role of pathologists in the industry. I decided I wanted to get involved, so I joined Ventana as a pathologist in companion diagnostics.

Working in companion diagnostics allows me to get involved in research that can be translated into clinical practice. Certainly, our biomarker assays can be used for research, but our main goal is to develop assays that can be used in the clinic. We all have our own reasons for joining Ventana – some may have family members afflicted with cancer, for example – but we all share a real personal interest in improving the lives of cancer patients. In reality, companion diagnostics are the cornerstone of personalized healthcare; they are critical to finding the right treatment for the right patient.

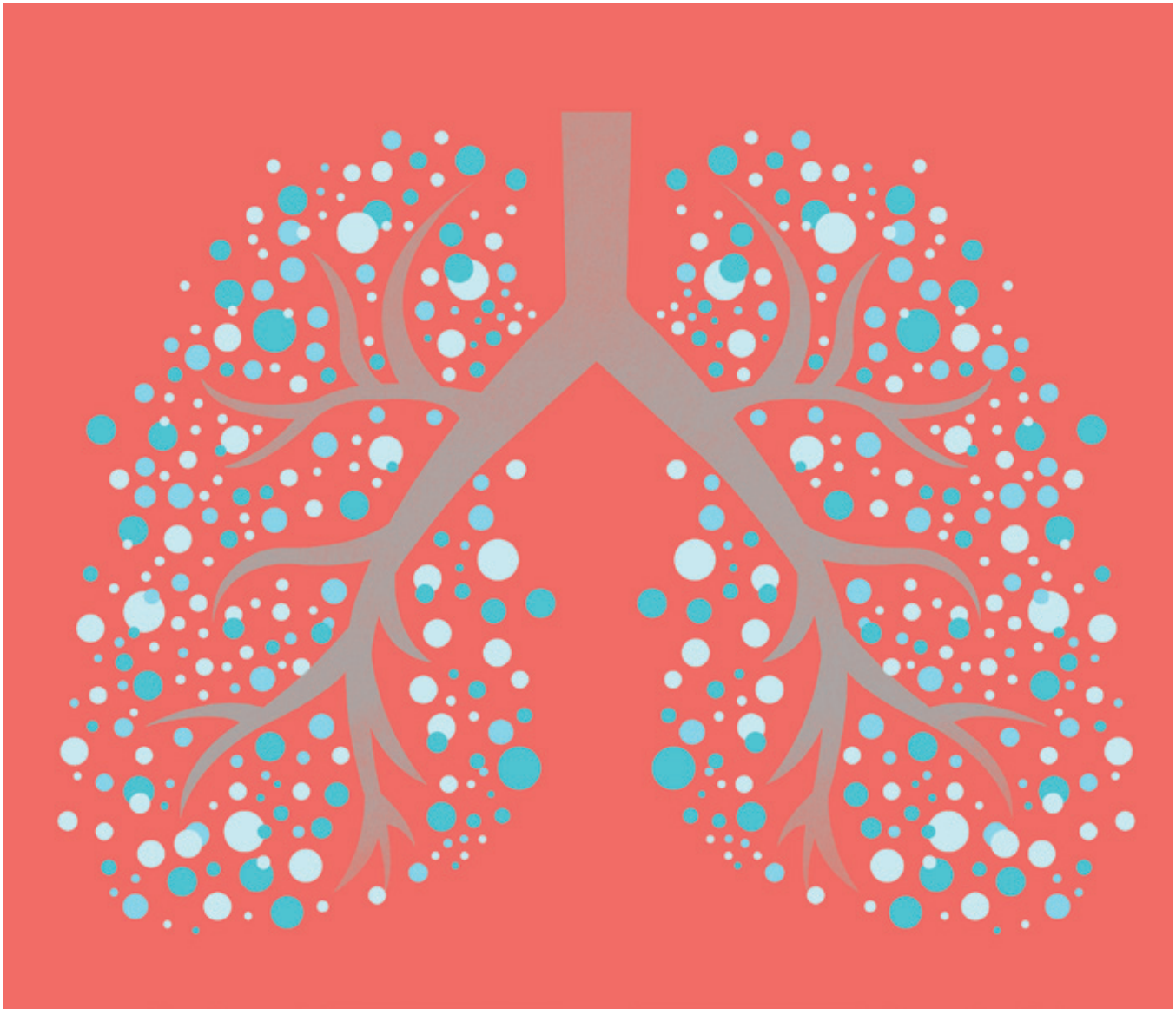
Why develop ALK CDx, given that a competing product was already available?

It's true that Abbott was already marketing the Vysis fluorescence in situ hybridization (FISH) assay as a companion diagnostic for Xalkori. But Pfizer wanted to develop an immunohistochemistry (IHC) assay, and so they approached us. From my perspective, having worked with both FISH and IHC assays, IHC has significant advantages. With IHC, the turnaround time is faster – patients can receive results in days as compared to weeks for FISH. Part of the reason that FISH is slower is because it's not fully automated – some manual work is required. To perform that manual work, specific training and a specialized microscope set up in a dark room are required. One can't just read the assay from one's own office. This contributes to FISH assays being more expensive. In contrast, the ALK CDx is more fully automated and can be validated and run in any lab that can perform IHC assays. It is less expensive, easier to interpret, and can be read by any trained pathologist with a regular microscope in a regular setting. IHC is also accessible to pathologists almost anywhere, including the EU, China, and the US. The assay was also validated by method comparison; in other words, we tested patient samples that had already been tested by FISH and we demonstrated very high concordance with the ALK CDx assay, so the quality of the data is the same as with the FISH assay. We've had great feedback from users; pathologists really like the assay and appreciate that they can interpret it themselves rather than via a technician.

How straightforward was the regulatory pathway?

We have found the FDA to be very helpful during product development, both with diagnostics and with drugs, and it was the same story for the ALK CDx assay. I think the encouraging data associated with new cancer immunotherapies is helping regulators rethink their strategy and guidance, which is also making them increasingly more collaborative – especially with regard to relevant companion diagnostics. Indeed, the FDA encourages diagnostic and drug companies to collaborate on strategies to exploit the many molecular markers that have been discovered. It's a regulatory attitude that is likely related to the many unmet medical needs in oncology; at present, only a minority of cancers are treated with targeted therapies. Regulatory support for the development of companion diagnostics will help get new targeted treatments to cancer patients sooner rather than later.

All the same, when ALK CDx was approved, we all felt like a great milestone had been achieved. Everyone was excited – not just the internal team, but the entire company – because developing a good, sensitive and specific assay takes a lot of work, and submitting documentation and answering the questions posed by the regulators can be stressful. The approval



was great in itself, but it also gave us confidence in our other development-stage companion diagnostics.

What challenges did you encounter?

One of the major challenges faced by companion diagnostics companies is the difficulty in procuring sufficient cancer tissue for product development. Validating the assay requires many tests and studies, which was particularly challenging because the prevalence of ALK+ lung cancer is about five percent. We had to screen thousands of patient samples to get sufficient numbers to support our ALK CDx assay development program, and it's not always easy to get good quality samples in these quantities.

Another challenge is that, although the ALK CDx assay is very easy to interpret, pathologists still need to be trained in its use so that they can appreciate the nuances of the assay, and understand its constraints. Essentially, we need to do everything in our power to prevent the risk of a wrong diagnosis. To that end, we developed an e-learning tool for the ALK CDx assay to walk pathologists through the challenges that they might encounter when interpreting the assay in real life. Such training is an area that we intend to continue to work hard on and constantly improve.

At a Glance

Product name: Ventana ALK (D5F3) CDx Assay

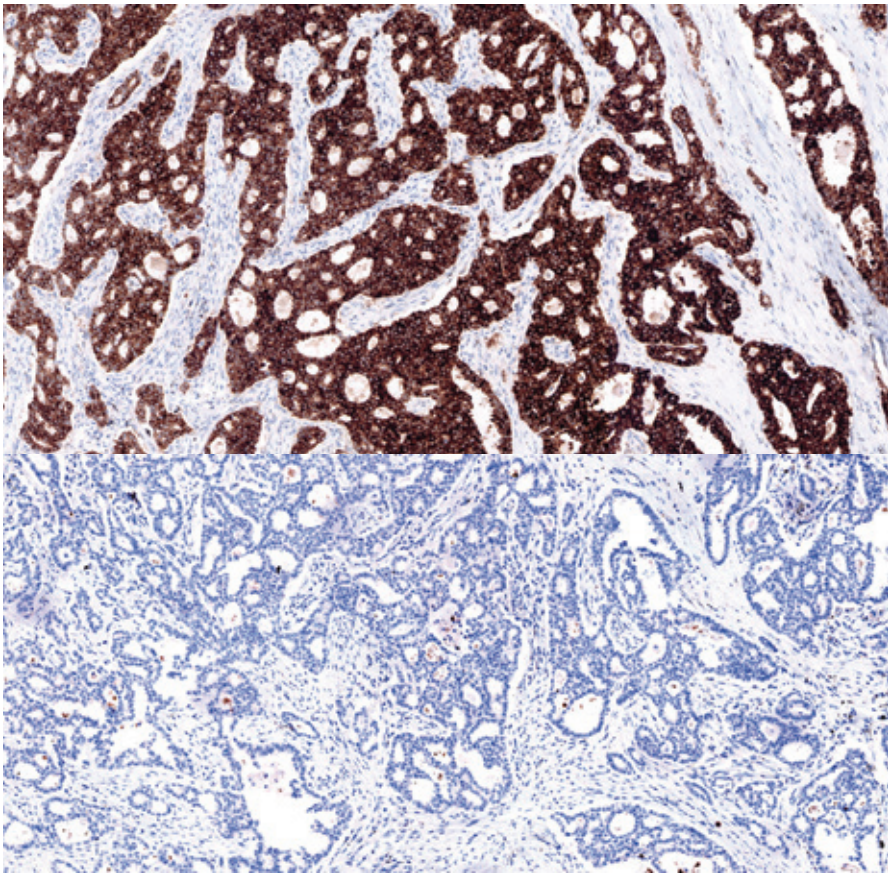
Brand name: ALK CDx

Developed by: Ventana (Roche), in collaboration with Pfizer

Marketed by: Ventana (Roche)

Product Description: Laboratory immunohistochemical test that identifies whether the anaplastic lymphoma kinase (ALK) protein is present in a non-small cell lung cancer tissue sample. A positive result indicates that the patient may be eligible for treatment with the Pfizer drug crizotinib (Xalkori).

Approval status: Approved in the USA (June 2015), Europe (October 2012), and China (Sep 2013).



Positive (top) and negative (bottom) case of lung tissue stained for ALK with Ventana ALK (D5F3) CDx Assay.

What changes would you like to see in the companion diagnostics industry?

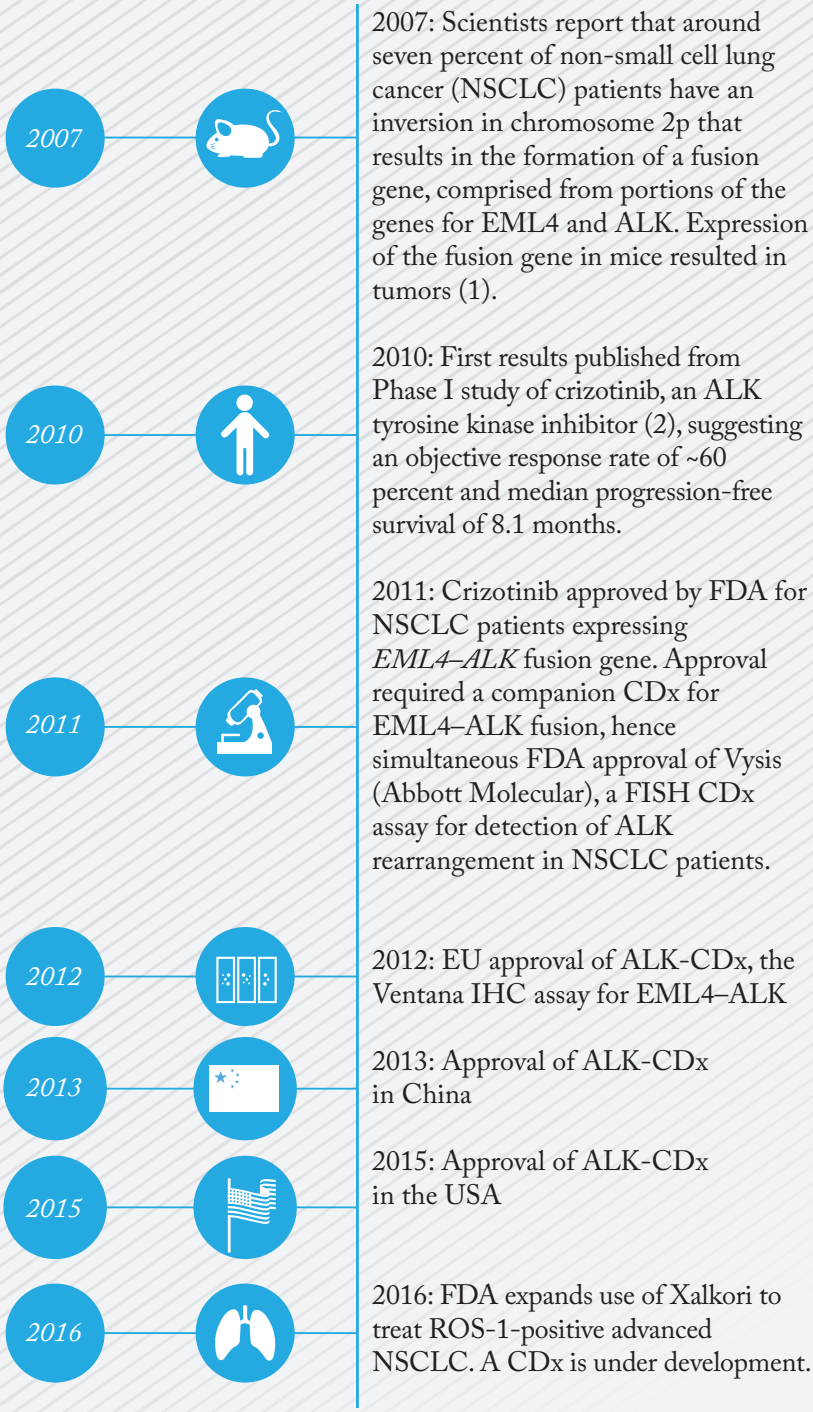
One of the greatest opportunities for change lies in the economics of companion diagnostics. At present, diagnostics are not always reimbursed – and when they are it is at a much lower rate than the related therapeutic. This holds back funding for diagnostics development. I'd really like payers to develop a better understanding of what we are doing. I'd also like the medical community to better appreciate what pathologists do. Pathologists are the ones enabling the diagnosis, and the tests pathologists do determine what treatment the patient will receive. Companion diagnostics essentially help the patient find the right treatment, which also means reducing the risk of exposing the patient to unnecessary treatment. Yet the funding for companion diagnostics development, and the incentives for commercialization, are relatively low. We need to educate key stakeholders about the value of these products – not just pathologists, but also payers, government bodies and private insurance companies.

I'd also like to see an honest dialogue between stakeholders, including the regulators, around the issue of obtaining sufficient cancer tissue to validate companion diagnostics. I feel that there is room for improvement in that area. In fact, communication in general is an area for constant improvement. We certainly have a close relationship with the regulatory bodies in the US, China, and Europe, but we want to improve and extend that further. Likewise, I also think we need to continue to grow our relationships with pharma companies and with independent pathologists. Getting feedback from experts outside the company – for example, on how we can improve training in assay interpretation – is critical. We've learned a lot of lessons from the ALK CDx assay, which we've already started implementing in the development of newer companion diagnostics.

Any thoughts on the future of companion diagnostics?

Over the last four years I've seen explosive growth in companion diagnostics

Timeline: Crizotinib and Companions



development. There were only one or two projects when I started, but now we are working on more than 10 such projects at any one time.

In all our companion diagnostics projects, especially the IHC-based assays, we expect to see more success, but also more assay complexity. For example, while some drugs may be safe and effective when prescribed on the basis of assaying a single biomarker, in the future we may need to base a prescription on two or more biomarkers, which implies presentation in a multiplex format. Accordingly, we are developing a multiplexing capability that can test for multiple markers on a single slide. This resource may also help address the difficulty in procuring sufficient tissue specimens from cancer patients, which is being exacerbated by the trend to use less invasive procedures. So if, as seems likely, diagnostics developers have much less tissue to work with in the future, next-generation technologies like multiplexing may be essential to be able to fully exploit what is available. In addition, we may need to develop digital pathology techniques, PCR, next generation sequencing, and bioinformatics tools to help decipher the data output.

By expanding the use of new, relevant technologies in companion diagnostics, by incorporating additional guidance from regulatory agencies, and by closely collaborating with regulators, drug developers and diagnostic companies, I believe society will quickly start to see the benefits of next generation companion diagnostics. And I am very excited to be part of this evolving story.

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In Perspective

The big picture
Global health
Populations

Cancer Control on a Shoestring

As cancer rates continue to climb, low and middle income countries are ramping up prevention and screening efforts.

By Rengaswamy Sankaranarayanan

The burden of cancer in low and middle income countries (LMICs) is significant and growing year by year, accounting for around 70 percent of all cancer deaths worldwide. Cancer is increasing in LMICs for two main reasons. One is simple demography – populations are aging and when people live longer they are more likely to develop cancer. But risk factors are also changing. Incidence of cervical cancer is slowly falling in some countries, while breast cancer rates are way up – likely a consequence of changing patterns of reproduction. So-called "Western" lifestyles with limited physical activity and high levels of processed food are also coming into play.

In high-income countries, most cancer patients now survive for years after diagnosis, whereas in LMICs, less than a third of patients with cancer survive (1). Some cancers with a poor prognosis, such as lung, esophagus, stomach, and liver cancers, are more common in LMICs (2). And patients in LMICs are diagnosed much later, on average, than those in high-income countries.

As a clinical oncologist practicing in India during the 1980s, most of my patients came to me too late, when there was little to offer beyond palliative care. Those experiences made me determined to improve cancer control in India and other LMICs. Since the early 1990s, I have been pursuing that goal at WHO's International Agency for Research on Cancer (IARC).

The IARC provides a platform for collaboration at an international level; individual countries and researchers can benefit from each other's experiences, which is particularly important when we need to make the best use of limited resources. Working across so many countries gives us an overview of the global situation – and the chance to really influence public health policy and implementation.

My work focuses on early detection interventions related to major cancers, such as breast, cervix, colorectal and oral cancers, most of which are increasing in incidence. Some cancers lack good treatment options, but in many cases well established interventions exist but are not available or affordable for LMICs. I'm interested in learning how we can rapidly scale-up interventions and make them feasible in health services with limited resources.

Recently, the WHO has spearheaded a major focus on controlling non-communicable diseases, including cancer, diabetes, cardiovascular disease, and stroke. In 2012, WHO member states agreed on the goal of reducing premature death from non-communicable diseases by 25 percent by 2025, starting from a 2008 baseline. The United Nations (UN) Sustainable Development Goals for 2030 set a target to reduce premature deaths from non-communicable diseases by a third. A substantial number of those premature deaths are a result of cancer. It is an important opportunity for cancer control.

"In low and middle income countries, less than a third of patients with cancer survive."

Two decades ago, such targets would have been impossible. Today, we see increasing awareness among government authorities about cancer prevention programs, and the picture is now far more optimistic than when I started out in this field.

A tale of two vaccines

Human papilloma virus (HPV) vaccination programs have been a great example of this new energy. HPV

A breast cancer awareness program in India.

vaccination has already been rolled out in 80 countries (including 35 LMICs), with another 25 countries currently carrying out pilot studies. In stark contrast, the first anticancer vaccine (against hepatitis B) was first approved in the early 1980s, but took 20 years to be widely adopted. Only after it became a Gavi-eligible vaccine in 2000 – dramatically reducing the cost to health systems – did hepatitis B vaccination take off; by 2004, half of LMICs had introduced the vaccine. On the other hand, the HPV vaccine was made Gavi-eligible in 2013, six years after it was introduced.

South and Central American countries, where rates of cervical cancer have historically been some of the highest in the world, have been particularly quick to implement vaccination programs. In continental South America, almost all countries have introduced a national HPV vaccination program, and Central America is not far behind. However, other regions have faced barriers to implementation. More women die of cervical cancer in Asia than anywhere else in the world, but HPV vaccination has not yet gained momentum in the region. India has seen substantial misinformation about the safety of the vaccine, and any plans to introduce the vaccine have been put on hold. Japan introduced the vaccine and saw good uptake initially, but media reports of unfounded links between vaccination, and long-term pain and numbness in very few vaccinated girls pushed the government to withdraw their recommendation.

These experiences highlight the importance of public education. Malaysia is a notable exception within Asia, and provides a model for other countries to follow. Here, a comprehensive four-year education campaign prepared the public, schools and religious establishment for the introduction of the vaccine, and contributed to a very high rate of coverage.

Cancer Control Around the World

Lung

No national cancer control strategy is complete without an effective tobacco control program. The WHO Framework Convention on Tobacco Control is the first global public health treaty on the subject – signed by 180 countries – and provides a framework for countries without specific drug control policies. We know what works in tobacco control – sustained funding of comprehensive programs, tax increases on tobacco products, smoke-free policies and aggressive media campaigns. Now, we need to empower nations to implement these measures. Amongst all these interventions, taxation is probably the most effective. A ten percent increase in price reduces consumption of cigarettes by five percent in LMICs (3).

Breast

Breast cancer is the number one cancer of women in most countries. In developing countries, incidence is increasing by 1–3 percent per annum. At the moment, we lack specific prevention measures for breast cancer, but screening and breast awareness programs can help detect tumors at an earlier stage. Regular, systematic self-examination is often promoted, but a Chinese study involving over 266,000 women found no significant impact on mortality (4), and scientists are still addressing the question of whether a systematic clinical breast examination screening of asymptomatic women is more effective than general breast awareness. The greatest unmet need is in sub-Saharan Africa, where around 70 percent of women with breast cancer present with tumors larger than 5 cm (5).

Colorectal

Colorectal cancer is increasing at a rate of 1–2 percent per year in many developing countries. A pilot trial introducing fecal occult blood testing into primary care services in a province of Thailand showed significant detection rates for colorectal cancer (6) and led the Thai government to expand the scheme to another five provinces.

Liver

Thanks to investments in national immunization programs, and improvements in cold chain and capacity, there was a substantial improvement in LMIC hepatitis B vaccination rates between 2000 and 2012. Thailand was one of the first countries to incorporate hepatitis B vaccination in the national immunization program, in a phased introduction starting with pilot trials from 1988 onwards, and a recent study confirms that children born after the vaccine became standard are significantly less likely to be carriers (7). A 69 percent reduction in liver cancer in vaccinated young people have been reported from Taiwan, which introduced hepatitis B vaccination during 1984–86 (8)

Oral

Most cases are associated with tobacco and alcohol use so, as with lung cancer, substance control is vital. A 34 percent reduction in oral cancer mortality was seen following regular screening among users of tobacco or alcohol or both in a randomized trial in India (9). However, only two regions currently have oral cancer screening programs: Cuba and Taiwan.

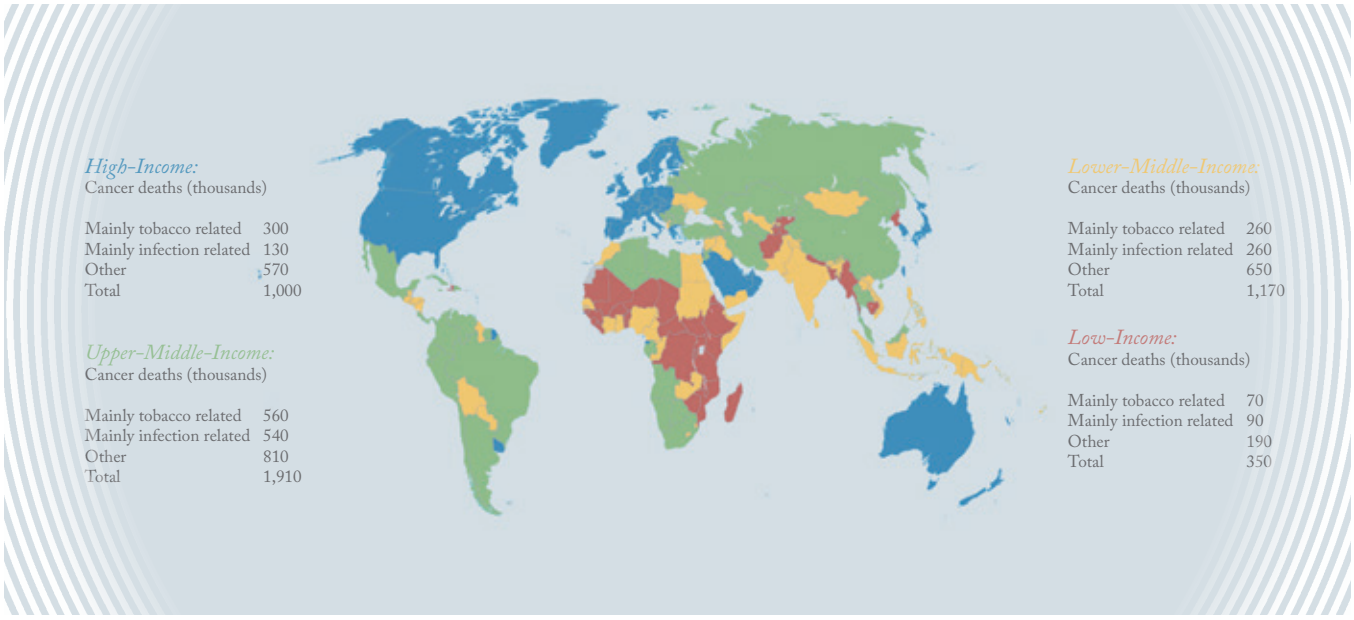
A balancing act

There is still much to do. Over the next few years, IARC will be working to help increase the incorporation of HPV vaccines into national immunization programs. We also hope to see countries introducing cervical cancer screening and treatment for pre-cancer within their services, as well as increasing access to early diagnosis and treatment of breast cancer. Looking a little further ahead, we hope to see the introduction of more early detection programs in the primary healthcare services for colorectal cancer.

What is needed to make it happen? A stable budget to fund long-term programs is a prerequisite, along with adequate equipment and infrastructure, but it's important to remember that money alone is not enough. One of the biggest bottlenecks at the moment in areas like sub-Saharan Africa is a lack of human resources. Not just doctors and nurses, but pathologists, epidemiologists, surgeons and technicians. Investments in hospitals and equipment are useless unless you also fund recruitment and training of healthcare workers.

Screening programs require a considerable investment in infrastructure and human resources. Cancer screening is something you have to do repeatedly, and by definition involves apparently healthy people. The introduction of a new screening program should go hand in hand with good educational awareness campaigns, to encourage participation. It is also important to apply a high level of quality assurance, by assessing false-positive tests and over-diagnosis, to make sure the program isn't doing more harm than good. Evidence from successful programs in Europe and Australia suggests that a screening program takes at least 15–20 years to reach the target level of participation and start to show results.

On the other hand, we must remember that funding is a delicate balancing act. Prevention and screening are



Cancer mortality before age 70 years, by World Bank income groupings, 2012.

vital to reduce mortality in the long term, but for immediate impact, it's important to invest in better diagnosis and treatment too. You do not need to have sophisticated infrastructure and approaches to detect and treat disease early; even with very basic interventions, you can make considerable inroads. The WHO has identified the "best buys" for LMICs in non-communicable disease prevention, including some specific to cancer. Tobacco control interventions, hepatitis B vaccination and some form of screening for precancerous cervical lesions are all interventions with impressive cost-effectiveness.

Having worked in clinical oncology, I am a passionate advocate for improving cancer services, but I recognize that there are many other healthcare needs and, increasingly, cancer control is being incorporated into wider non-communicable disease programs. We live in a changing world, with shifting priorities, and we have to be pragmatic about our place within those priorities.

Overall, cancer control in LMICs is improving, albeit slowly. Increased awareness and willingness from governments to introduce new interventions and improve existing services has led to huge strides over the past two decades, and I am hopeful for a future when everyone at risk of, or diagnosed with, cancer can expect a good standard of care.

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Oncogenetic Pioneer

Sitting Down With… Robert A. Weinberg, founding member of the Whitehead Institute for Biomedical Research and Professor of Biology at the Massachusetts Institute of Technology, USA.

Congratulations on receiving the American Association for Cancer Research (AACR) Lifetime Achievement Award.

Thank you - it is very flattering, although it does make it sound like I am being put out to pasture! I certainly have no plans to retire in the foreseeable future.

What has been the overarching theme of your work?

We are trying to determine the molecular and genetic determinants of various steps in the process of going from a fully normal to highly oncogenic cell, including the acquired ability of a cell to disseminate and create metastases. From a young age, I liked to take things apart and find out how the mechanism inside works. My research is just another manifestation of that – trying to peer inside cancer's complex machinery.

Did you know early on where that curiosity would lead you?

I had no idea what I wanted to be. I started out as a pre-medical student but then I learned that doctors have to stay up all night to deal with patients, and decided medicine wasn't for me – I need my sleep!

I now teach an Introduction to Biology course for undergraduates but, as I tell the class in my first lecture, when I took the same course in 1961 I got a D. As an undergraduate I didn't enjoy biology at first, but I came to love it. In 1963 I took a genetics course here at MIT, which laid out the principles of molecular biology. Suddenly, it dawned on me that we might be able to understand the full complexity of the biosphere by studying DNA, RNA, and proteins. That was a revelation to me.

Once you had discovered your passion for biology, what drew you towards cancer research?

I am not one of those people who plan out their lives; I just put one foot in front of the next. Working in cancer research was really just a series of fortuitous accidents. I was interested in studying mRNA, and tumor viruses were a tool to do that. I ended up sharing a lab with David Baltimore, who had just discovered reverse transcriptase, and began to work on RNA tumor viruses that could infect and transform cells. Over time, my interests evolved and I ended up studying the cellular genes that control cancer. My main ambition is simply to do interesting things.

What led to your discovery of the first human oncogene?

We were working with retroviruses and found that if we transferred the DNA produced by reverse transcription in an infected cell into a naïve cell, the naïve cell would start producing retrovirus particles. We then transferred the reverse-transcribed genome of a Harvey sarcoma virus into a naïve cell and found that it transformed the cells in the same way that an infection would. Next, we transferred the genomic DNA of a Harvey sarcoma virus-infected cell and found that this too would transform a naïve cell. This indicated that one could detect a single copy transforming element through transfection followed by assay of foci of transformed cells. At that point, it occurred to me that we might be able to find cellular oncogenes that arise not through infection, but through mutagenesis. I was influenced by the work of Bruce Ames, who showed that many chemical carcinogens are also mutagens. I reasoned that the genomes of chemically transformed cells might carry mutant genes, responsible for the aberrant behavior of the cells. In 1979, we showed that the genome of a cell transformed by a chemical carcinogen contained oncogenic information – the first discovery of an oncogene in a non-virus-transformed cell, ostensibly a cellular transforming gene.

What projects are going on in your lab today?

In 2003 we started to work with genes involved in the cell-biological program termed the epithelial–mesenchymal transition (EMT) and found that in primary carcinoma cells, such genes could impart the ability of these cells to physically disseminate and seed metastasis; that discovery governs our research agenda to this day. We're interested in how activating the EMT program in a poorly invasive and poorly metastatic epithelial cancer cell can transform it into a powerful cancer-initiating cell.

What are the main roadblocks in the field right now?

There are both scientific and policy roadblocks. The epigenetics of cancer cell biology is a major scientific challenge right now. There has been a focus on the genomes of cancer cells but it is becoming clear that their behavior is governed in large part by non-genetic elements. These epigenetic transcriptional circuits are still poorly understood.

There is also the funding issue, which means that many young people no longer view a career in preclinical cancer research as a viable option. In 10–15 years we are going to need the best and brightest young researchers to continue to move basic cancer research forward, but those people are being driven from the field. If we are to reverse that trend, the funding climate has to change dramatically.

What about President Obama's "Cancer Moonshot"?

The question is whether the extra funding will be invested in innovative research that offers significant steps forward over the long term, or whether it will be directed to strategies that are already well-tested and well-funded. My preference would be for the money to be used for funding young researchers, but I fear that is not going to happen.

Where are the most exciting advances?

Tumor immunology. It's an entirely new paradigm that allows us to eliminate cancer cells by unchaining the immune system. I can only look at this field from a distance – but still can say it's very exciting!