

the Translational Scientist

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APRIL 11-13, 2016 | Renaissance Long Beach Hotel, Long Beach, CA

5th INTERNATIONAL SYMPOSIUM ON HIGHER ORDER STRUCTURE OF PROTEIN THERAPEUTICS

ABSTRACT SUBMISSION DEADLINE:

January 15, 2016 *oral presentation*

March 11, 2016 *poster presentation*

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TOPICS TO BE DISCUSSED:

- Beyond mAbs: New Challenges for Novel Frameworks
- Biological Consequences of HOS (safety, efficacy, PK) and Impact to CQA
- HOS Development: Process/product development/commercialization/post-approval
- HOS Emerging and Novel Technologies
- HOS Fundamentals: Why Structure Matters
- HOS Protein Therapeutics Discovery, Candidate Selection and Engineering
- Merging Experimental and Computational Approaches to HOS Characterization

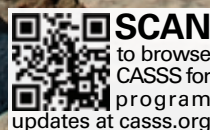
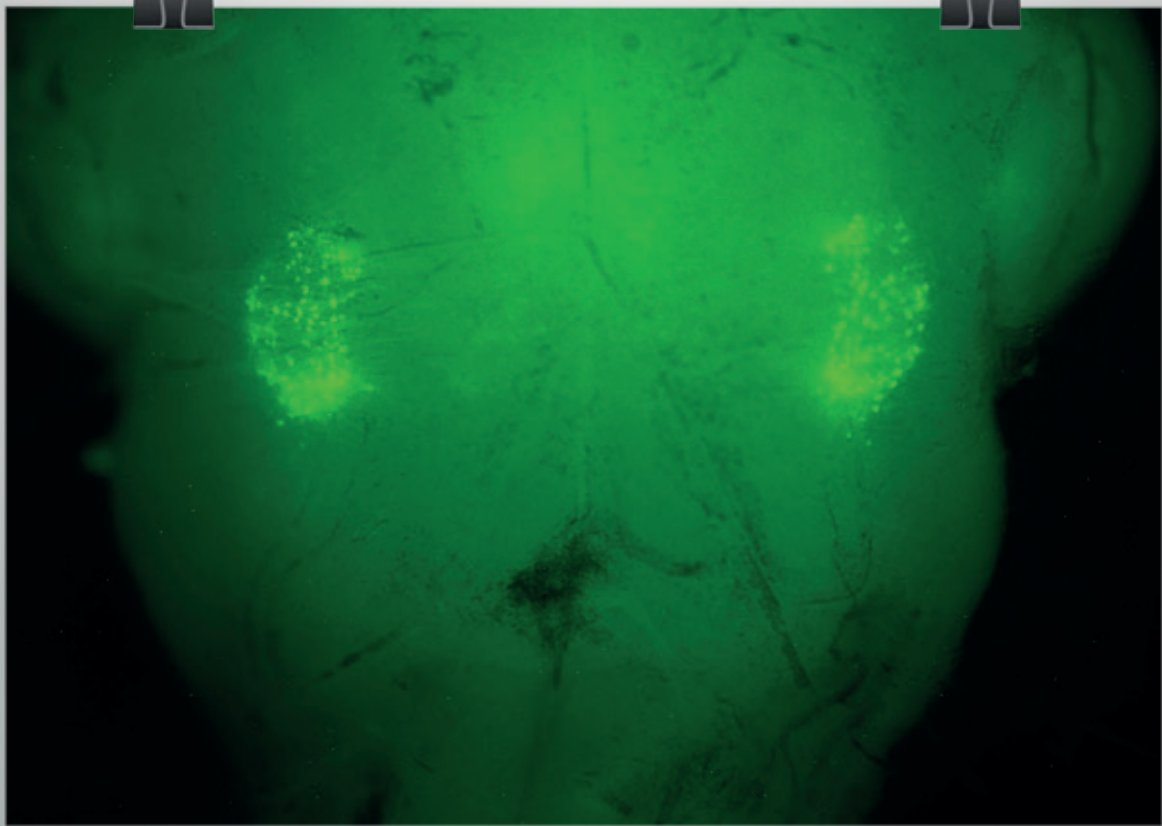


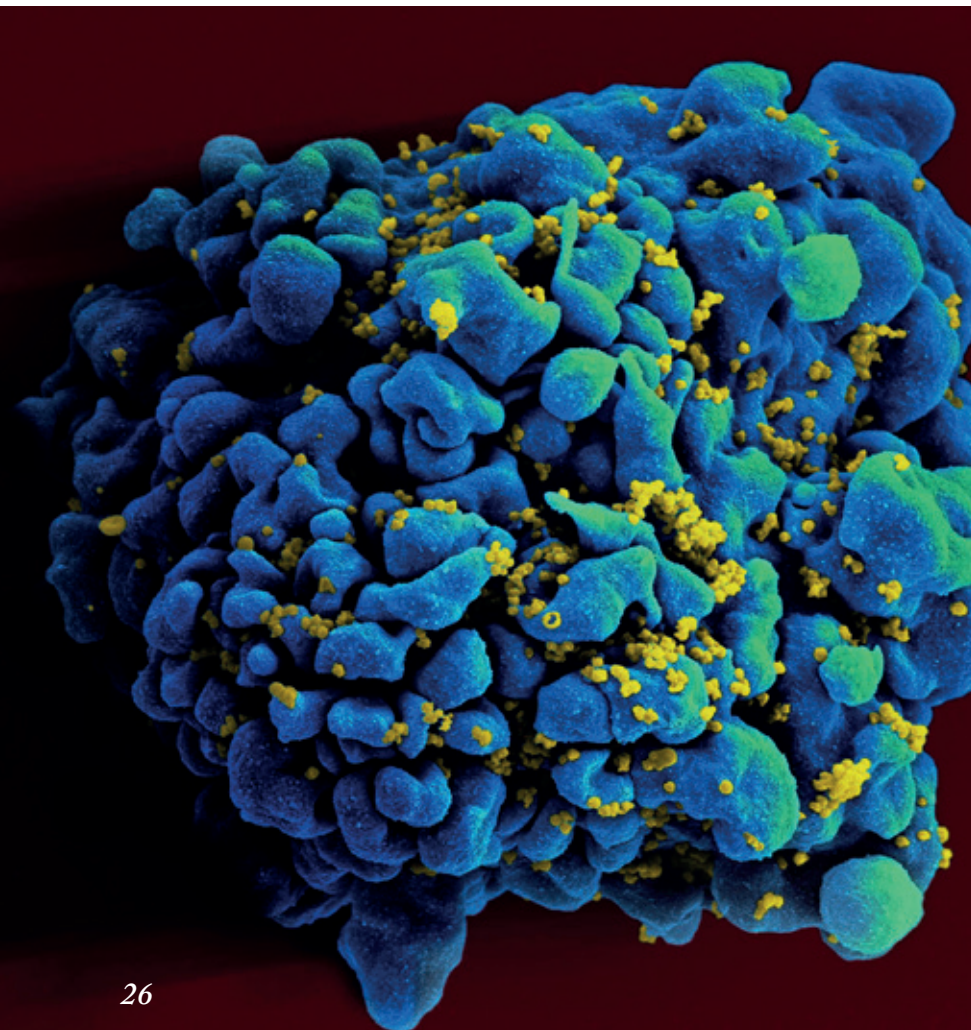
Image of the Month



On each side of the brain stem, a florescent green marker illuminates the two networks of 200 neurons that control the sighing reflex, in an image from researchers at UCLA and Stanford. Sighing maintains normal lung function by re-inflating collapsed alveoli. Identifying the neurons responsible not only provides useful information on the link between brain and behavior, but could be of therapeutic use to help people whose sighing reflex goes into overdrive due to anxiety or psychiatric disorders.

Credit: Krasnow lab/Stanford.

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Only Half the Battle,
By Charlotte Barker

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is an essential link in the
translational chain missing?*

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Editor - Charlotte Barker
charlotte.barker@texerepublishing.com

Associate Editor - William Aryitey
william.aryitey@texerepublishing.com

Editorial Director - Fedra Pavlou
fedra.pavlou@texerepublishing.com

Content Director - Rich Whitworth
rich.whitworth@texerepublishing.com

Publisher - Chris Breslin
chris.breslin@texerepublishing.com

Senior Designer - Marc Bird
marc.bird@texerepublishing.com

Junior Designer - Emily Strefford-Johnson
emily.johnson@texerepublishing.com

Digital Content Manager - David Roberts
david.roberts@texerepublishing.com

Mac Operator Web/Print - Peter Bartley
peter.bartley@texerepublishing.com

Tablet Producer - Abygail Bradley
abygail.bradley@texerepublishing.com

Audience Insight Manager - Tracey Nicholls
tracey.nicholls@texerepublishing.com

Traffic and Audience Associate - Lindsey Vickers
lindsey.vickers@texerepublishing.com

Traffic and Audience Associate - Jody Fryett
jody.fryett@texerepublishing.com

Apprentice, Social Media / Analytics - Ben Holah
ben.holah@texerepublishing.com

Events and Office Administrator
- Alice Daniels-Wright
alice.danielswright@texerepublishing.com

Financial Controller - Phil Dale
phil.dale@texerepublishing.com

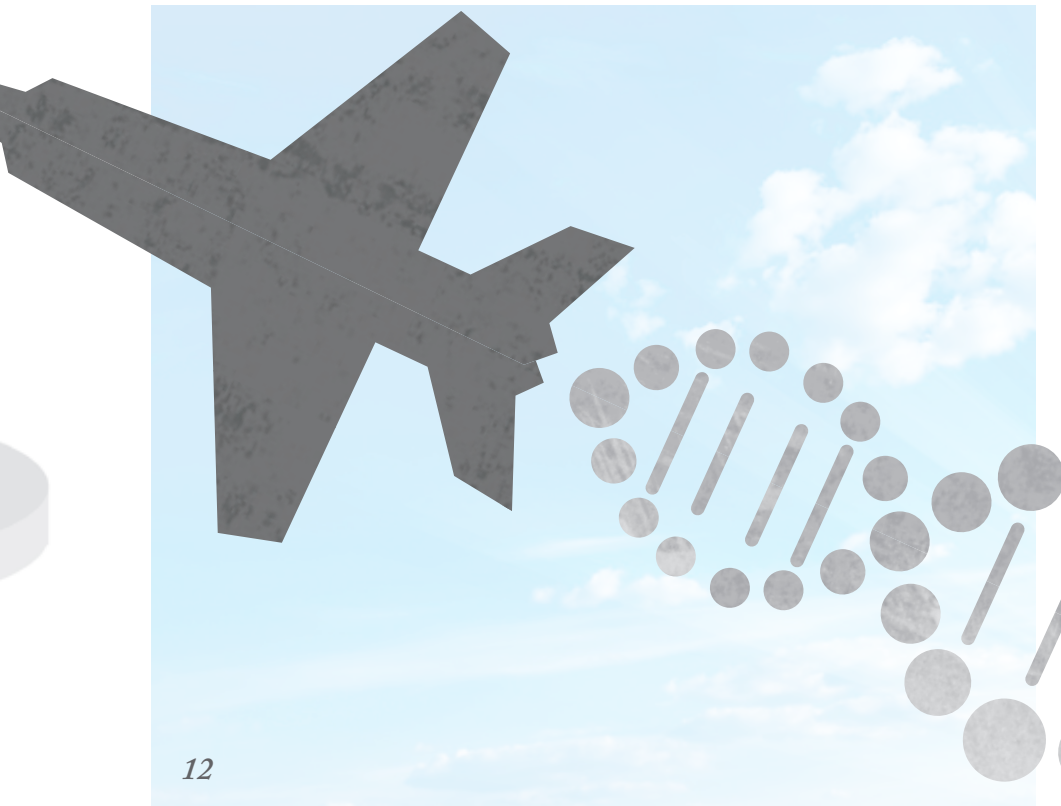
Chief Executive Officer - Andy Davies
andy.davies@texerepublishing.com

Chief Operating Officer - Tracey Peers
tracey.peers@texerepublishing.com

Change of address:
tracey.nicholls@texerepublishing.com
Tracey Nicholls, The Translational Scientist,
Texere Publishing Ltd, Haig House, Haig Road,
Knutsford, Cheshire, WA16 8DX, UK

General enquiries:
www.texerepublishing.com
info@texerepublishing.com
+44 (0) 1565 745200
sales@texerepublishing.com

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Sitting Down With

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Emphasizing multidisciplinary research that bridges the gap between basic research and clinical care.

TVST covers a broad spectrum of work, including but not limited to:

- Applications of stem cell technology for regenerative medicine
- Development of new animal models of human diseases
- Tissue bioengineering
- Chemical engineering to improve virus-based gene delivery
- Nanotechnology for drug delivery
- Design and synthesis of artificial extracellular matrices
- Development of a true microsurgical operating environment
- Refining data analysis algorithms to improve in vivo imaging technology
- Results of Phase 1 clinical trials
- Reverse translational (“bedside to bench”) research

Only Half the Battle

The true mission of translational science is to take new medicines and diagnostics from bench to bedside – and beyond

Editorial



In February 2016, the WHO declared the Zika virus a global health emergency. As scientists, our thoughts immediately turn to the development of rapid diagnostics, an understanding of the link to microcephaly, and the search for a vaccine. Sanofi, who recently saw approval of its Dengue vaccine in Brazil after a 20-year, \$1.5 billion development journey, believe its experience will speed efforts to prevent Zika, while several small biotechs and the NIAID are also testing candidate vaccines.

And yet, as we learnt in “The Missing Link” in last month’s issue (1), better diagnostics and medicines are just one part of the puzzle. It’s no coincidence that north-eastern Brazil – the epicenter of the outbreak – is the poorest and least developed part of the country. Families in houses with no running water must store their own; water tanks (and puddles of rainwater in uncollected rubbish) are perfect breeding grounds for Zika’s vector, *Aedes aegypti* – also known as the yellow fever mosquito. After being virtually wiped out in Brazil during the 1950s, *Aedes aegypti* has gradually recolonized the nation, bringing with it a range of diseases, including Dengue – and now Zika.

Certainly, a vaccine would be a great advance. But even if Zika is eradicated, without understanding and addressing the full range of factors contributing to the outbreak, we will be no better placed to combat the next emerging disease. Looking at the much bigger picture, Zika is not even the most serious threat to maternal and fetal health in Latin America – or elsewhere... Nevertheless, governments have called for women to “avoid becoming pregnant” during the outbreak; however, a complex blend of social, cultural and economic factors determine whether women have access to contraception or the desire/ability to prevent pregnancy.

It’s natural for scientists to seek scientific solutions, and for all human beings to want a straightforward narrative: “Scientists find the cure!” But as Michael Liebman points out in “Asking the Ultimate Question” (page 46), new and better technology is not always the answer. We must also ask bigger and bolder questions. How can we reduce the burden of neglected tropical diseases? How can we improve outcomes for mothers and babies around the world?

The Translational Scientist’s “In Perspective” section focuses on improving healthcare at the population level. The true path to translation lies not only in finding scientific solutions to the problems that threaten our health, but also addressing the barriers that prevent advances being applied where they are most needed.

Charlotte Barker
Editor
@translationsci

Reference

1. H Lambert, “The Missing Link”, *The Translational Scientist* 1, 46–48 (2016).

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

Chemotherapy All Wrapped Up

Could exosome encapsulation be the secret to delivering higher potency in chemotherapy?

A new study documents how exosomes can be used to encase paclitaxel – a chemotherapy drug – to deliver the drug to the cancer site with more than 50 times the potency of conventional delivery systems (1).

The team initially developed the exosome delivery system after experimenting with cell-mediated delivery of drugs in Parkinson's patients. "When we examined the mechanism of these effects, we realized that immune cells released exosomes loaded with these drugs, and delivered them to the brain," says lead researcher and Associate Professor of Pharmaceutical Sciences at the University of North Carolina, Elena Batrakova. Batrakova and her colleagues decided to isolate the exosomes from macrophages, and load them with drugs directly. As the body's own delivery vehicles, exosomes appear to bypass drug resistance mechanisms and the immune system.

Turning their attention to cancer, paclitaxel was an obvious choice since its potency and hydrophobic properties make it easy to load into the exosome capsules. Could exosomes deliver paclitaxel directly into drug-resistant lung cancer cells? After developing and comparing multiple approaches to loading the exosomes, they applied the exosomes to drug-resistant lung carcinoma cells in vitro and were delighted to find that exosome encapsulation increases the cytotoxicity of paclitaxel dramatically, results later confirmed in a mouse model. Such a delivery system could allow a much



lower dose of chemotherapy, and fewer harsh side effects.

However, as Batrakova explains, there's plenty more work to do: "One limitation is the amount of exosomes that can be collected from the patient. We are working on the storage and reproducibility conditions now so that we are able to produce a large amount of exosomes." Toxicology studies, formulation optimization, and proven effects in other animal models are also needed before clinical trials can be considered. *WA*

Reference

1. MS Kim et al., "Development of exosome-encapsulated paclitaxel to overcome MDR in cancer cells", *Nanomedicine* (2015). PMID: 26586551



Decoding Cancer Cross-Talk

Intercepting communication between cancer cells and endothelium uncovers clues to metastasis mechanisms

While many aspects of metastasis have been mapped out, the mechanisms by which cancer cells travel from primary to secondary sites have never been fully understood. A new study by researchers at Brigham and Women's Hospital helps fill the gap by tapping into the communication that allows cancer cells to induce healthy endothelium to become cancerous (1).

The researchers didn't initially set out to study metastasis, according to principle investigator Shiladitya Sengupta, but rather to study the behavior of metastatic breast cancer cells in the presence of endothelial

cells, which make up the blood vessels that sustain the tumor, "Normally breast cancer cells, when grown on a 3D tumor matrix, form spheroids called mammospheres. We had anticipated similar structures, with blood vessels growing towards the spheroids. However, when we introduced the cancer cells to the culture containing the blood vessels, the cancer cells didn't form spheroids but instead aligned on the vascular network," says Sengupta.

Surprised by their finding, the team used electron microscopy to investigate, and discovered nanoscale bridges linking the cancer cells to endothelium. Sengupta says, "This is a highly energy intensive process, and we rationalized that for the cancer cell to spend so much energy building the bridges, they might have a function. That's how we started studying the communication angle."

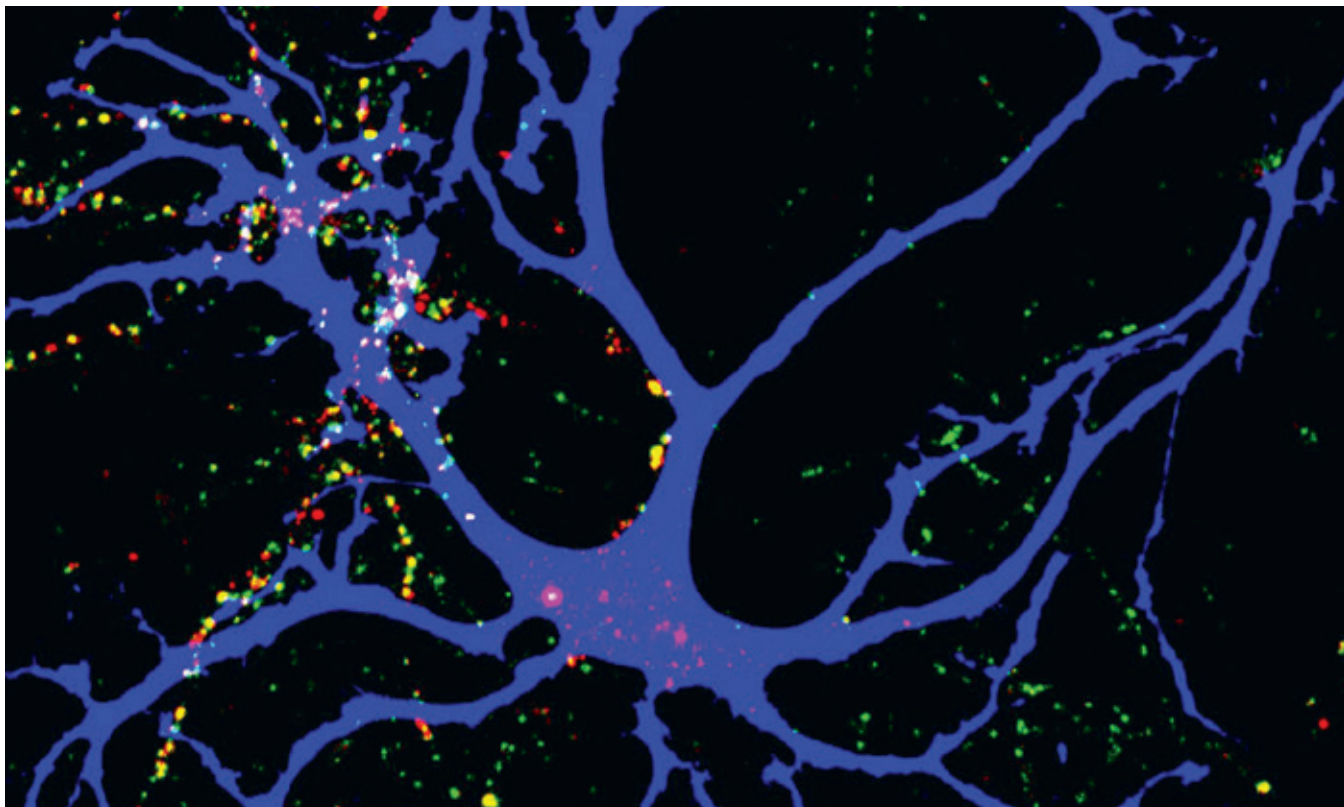
The team determined that the nanoscale bridges (composed of cytoskeletal elements) were actually used as a conduit for intercellular miRNA transfer to

transform healthy endothelial cells into a pathological state. Sengupta explains that the cancer cells inject miRNAs to essentially hijack the endothelial cells. The team went on to identify chemical compounds that could break down the connections between cancer and endothelial cells, and found that mice given these compounds had reduced metastatic disease.

"We have only just scratched the surface," says Sengupta. "We need to test if this behavior is ubiquitous. Are there any specific types of endothelial cells that attract such nanoscale bridges, or is it just a stochastic process? How is the information flow regulated? Much of the interesting science is still waiting to be explored." *WA*

Reference

1. S Sengupta et al., "Physical nanoscale conduit-mediated communication between tumour cells and the endothelium modulates endothelial phenotype", *Nat Commun* 6, 8671 (2015).



Missed Connections

A protein critical for synaptic plasticity in baby mice could hold clues to the origins of autism

The sheer number of changes that take place during early development can make it hard to pinpoint the cause of disorders such as autism. But a recent study sheds light on how the brain is wired to process sensory information during infancy and how disruption to that process could cause ongoing problems (1).

Cagla Eroglu, Assistant Professor of Cell Biology and Neurobiology at Duke University, and her team previously identified hevin as an astrocyte-secreted

protein. Their next task was to find out what role the protein plays. “We knew that hevin is localized to synapses, and also that in mice without hevin, synaptic junctions have defects both on the presynaptic (axonal) and postsynaptic (dendritic) compartments. These observations led us to hypothesize that hevin is capable of interacting with both sides of the synapse, and bridging the synaptic gap,” says Eroglu.

The researchers found that hevin did indeed interact with proteins on both sides of the synapse – specifically neurexin-1a (NRX1a) and neuroligin-1B (NL1). Their research also established how critical those interactions are in sensory development. Mice lacking hevin, NRX1a, or NL1 all have identical defects in synapses between thalamus and cortical neurons. During a critical window in development, activation

of this class of synapses by sensory experiences helps shape cortical circuits.

“We found that mice lacking hevin did not have the ability to remodel/reshape their cortical connections when their visual experience was altered. Remarkably, when we supplied hevin back to the cortical astrocytes, we could rescue plasticity. As we identified hevin receptors and their associated proteins, we started to realize all these proteins are encoded by genes that are linked to autism and other neurological disorders. This indicates that plasticity in the thalamocortical circuits may be critically impaired in autism,” says Eroglu. *WA*

Reference

1. SK Singh et al., “Astrocytes assemble thalamocortical synapses by bridging NRX1a and NL1 via hevin”, *Cell*, 164, 183-196 (2016). PMID: 26771491

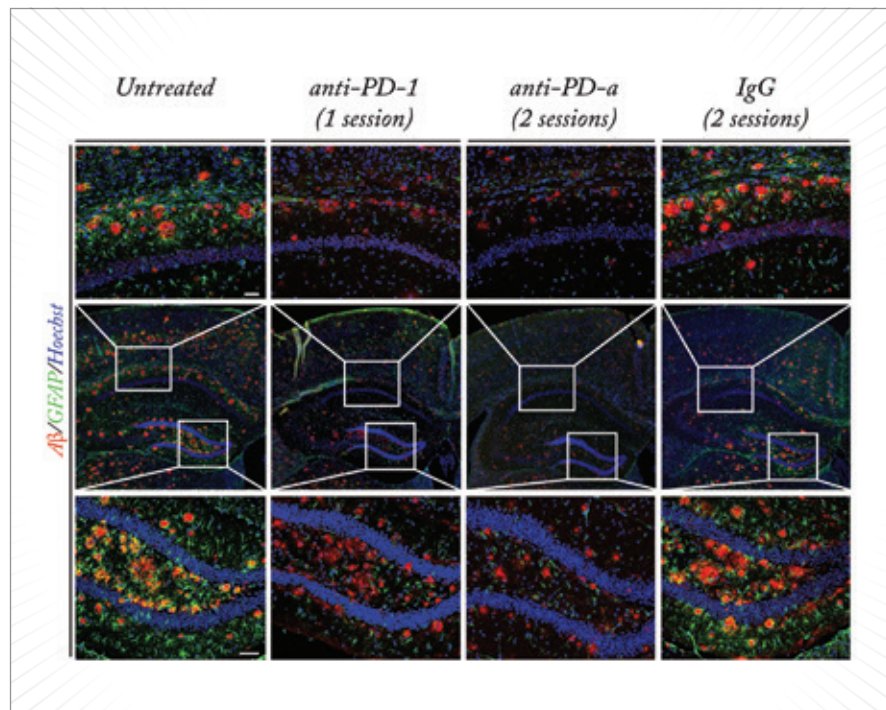
PD-1 on the Brain

Revolutionary cancer immunotherapies appear to alleviate Alzheimer's symptoms in mice

PD-1 inhibitors nivolumab and pembrolizumab were approved in 2014 as cancer immunotherapies, making headlines by inducing complete remission in some patients with hard-to-treat advanced metastatic cancers (read more on page 42). Now it seems the PD-1 pathway could have an important role in brain pathology too, after a new study showed that PD-1 blockade improved symptoms of Alzheimer's disease in mouse models (1).

Michal Schwartz, principle investigator and Professor of Neuroimmunology at Weizmann Institute of Science, explains the background to the study. “For the last 20 years, my research team has focused on understanding how the immune system participates in brain maintenance and repair in health and disease, and how to harness this power by peripheral immunomodulation. Prior to our early work, it was clear that brain pathologies are associated with local inflammation within the brain. Therefore, based on the old understanding of brain-immune system relationships, it was natural to try and suppress the immune system as a way of suppressing brain inflammation; however, these attempts largely failed,” says Schwartz. “But last year, we made a made a big step forward in our research when we understood that recruitment of the immune cells that locally clear accumulated toxic compounds from the brain requires activation of a unique ‘gateway’.”

In a previous study, the team demonstrated that in a mouse model of Alzheimer's disease this gateway is dysfunctional, but can be re-activated by



PD-1 blockade reduces A β deposition (red) and astrogliosis (green) in the brains of Alzheimer's disease mouse models. Scale bar, 50 μ m. Image courtesy of Baruch et al., (1).

reducing systemic immune suppression. These findings, which suggested that peripheral immune suppression interferes with the ability to fight Alzheimer's disease pathology, reminded the researchers of similar processes in cancer immunology. “Therefore, we decided to test whether immune checkpoint blockade – an immunotherapy which mobilizes the immune system to fight the tumor (and has revolutionized cancer treatment in recent years) – would be effective in the context of Alzheimer's disease,” says Schwartz.

Blockade of PD-1 pathway evokes an interferon- γ -dependent immune response and recruitment of monocyte-derived macrophages, leading to the clearance of cerebral amyloid- β plaques – the potential mechanism behind the improved cognitive performance observed in mouse models, according to the team. “Since the suggested therapy is mechanism-driven rather than targeting

symptoms of the disease, and the proposed therapies are (FDA-approved) antibodies targeting PD-1, we believe these findings could be translated in a relatively short time for clinical studies in Alzheimer's disease,” says Schwartz.

Alongside efforts to initiate human trials, Schwartz and her colleagues are focusing on pinning down the mechanism of action. “We are currently involved in further elucidating the mechanism by which PD-1 blockade is affecting pathology in different Alzheimer's disease mouse models. Additionally, we are collaborating with the biopharma industry to take this approach forward to the clinic,” says Schwartz. *WA*

Reference

1. Baruch et al., “PD-1 immune checkpoint blockade reduces pathology and improves memory in mouse models of Alzheimer's disease”, *Nat Med* 22, 135–137 (2016).



Catching the Thermodynamic Express

A new method could dramatically speed up analysis of nucleic acid thermal behavior

The current standard for analyzing thermodynamic behavior of DNA or RNA – melting curve analysis – can take months. A new method developed by US researchers promises to slash the time needed to hours – and boost accuracy to boot (1). We spoke with David Zhang, Head of the Nucleic Acid Bioengineering laboratory at Rice University’s BioScience Research Collaborative, to find out more.

Why did you focus on finding a faster way to do thermal analyses?

We needed better parameters to design primers and probes for pathogenic DNA and RNA sequences. The current methods give a standard error of around 3 kcal/mol, which requires further empirical optimization, taking up significant time and money.

At first, we thought that maybe someone in industry had already done the work, but after extended discussions with partners at Integrated DNA Technologies and at Cepheid, we

realized this is not the case. It’s been around 15 years since someone last did DNA characterizations, so we figured that unless we did the work, no one else would.

What has been the reaction?

The attitude of academic researchers we’ve spoken to is “cautious optimism.” In this work, we laid out a new method and used the method to obtain some new parameters, but to really improve DNA primer design, RNA folding, and so on, we’ll need a lot more parameters. It’s like a leaky ship... There are many holes that you need to patch to get it watertight – and patching up just one hole doesn’t bring much obvious improvement. Right now, we patched up one hole (DNA dangles) and we are working on a few others (DNA bulges and mismatches), but it’ll be a while until we’re comprehensive enough that there are major benefits to the research and biotechnology communities.

Tell us about your proposed “thermodynamic database”.

John Fang, one of the graduate students in the lab, is leading our efforts in this area, developing a software package we call “NABTools”. So far, we have built and launched several tools for DNA probe design and formulation, and we are working on a nucleic acid hybridization evaluator that allows users to visualize the structure and binding of primers to genomic DNA or RNA, with

a graphical user interaction that allows dynamic changes in the primer sequence or structure. NABTools Evaluator is close to completion, and we are excited to enable researchers and students to better design DNA reagents for the study of nucleic acids.

Why did you choose not to seek a patent?

As a lab, we seek to maximize our impact on the research community and on society in general. In some cases, such as our more applied technologies on rare allele PCR, seeking a patent increases the motivation for industry partners to build from our work. In other cases, such as the current work on nucleic acid thermodynamics, keeping the methods in the public domain will have a greater impact.

What’s next?

My lab is committed to the basic biophysical study of nucleic acids, because it really is the foundation of any applied work on DNA analytic and diagnostic assays. Just as shaky foundations will limit the height of a skyscraper, robust DNA biophysics knowledge is absolutely necessary as our society pursues precision medicine in which DNA molecular information is used to guide clinical treatment.

Reference

1. C Wang et al., “Native characterization of nucleic acid motif thermodynamics via non-covalent catalysis”, *Nat Commun*, 7 (2016).

One Man's Trash...

Researchers dive through genetic “junk” to discover a small protein with lots of heart

The DWORF (dwarf open reading frame) micropeptide was derived from what was previously thought to be non-coding ‘junk’ RNA. So why go searching in the genetic scrapheap? There were a number of clues suggesting that some transcripts annotated as long non-coding RNAs (lncRNAs) may actually have coding potential but had evaded detection algorithms, according to the study authors (1).

In order to identify true protein coding regions of candidate transcripts, the team used a published evolutionary conservation algorithm (2) to infer whether the sequences were likely to be protein coding. They identified numerous hypothetical small peptides with this method and decided to pursue the DWORF protein, partially as a validation of the method, and partially out of interest in the function of the protein since it is quite abundantly expressed in heart and skeletal muscle.

Their intuition was spot on. Though DWORF may span only 34 amino acids, it has a big impact in the heart. DWORF binds to the major calcium pump that drives calcium removal from the cytoplasm and modifies it to increase activity. The end result is that, in the presence of DWORF, muscle cells contract more strongly.

DWORF's ability to strengthen contractions, and its abundant expression in the heart, lead the researchers to speculate on its potential as a therapy. In various models of heart disease where muscle function is



impaired, they found reduced levels of DWORF. They believe that DWORF is a positive regulator of contraction, and postulate that manipulation of its abundance and activity could serve as a strategy to enhance contractility in cases of heart disease.

With translation in mind, the research team hopes to expand its knowledge of the potentially helpful micropeptide by analyzing how the expression and activity of DWORF is regulated, and how regulation changes in response to stress and disease. The ultimate aim? To

further understand the molecular basis of heart failure, potentially moving us closer to finding effective therapeutics for cardiovascular disease. *WA*

References

1. BR Nelson et al., “A peptide encoded by a transcript annotated as long noncoding RNA enhances SERCA activity in muscle”, *Science*, 351, 319–418 (2016).
2. MF Lin et al., “PhyloCSF: A comparative genomics method to distinguish protein coding and non-coding regions”, *Bioinformatics*, 27, i275–i282 (2016). PMID: 21685081.

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

Sugars in the Spotlight

It's time to recognize that there is a world beyond genes and proteins. Glycobiology should take its rightful place at the center of biomedical research.



By Cathy Merry, Stem Cell Glycobiology Group, Nottingham University, UK.

I've always been a little unconventional – perhaps that is what attracted me to glycobiology. There is no template for sugars; they don't conform to the central dogma of biology – DNA to RNA to protein – but instead are made on an ad hoc basis with a high degree of variability. Of course, that doesn't mean that glycans are produced at random, but they are controlled by forces that, until recently, we did not fully understand. Scientists working in gene and protein biology can easily amplify and sequence their samples, whereas analyzing glycans is no easy task.

Uniqueness is a common theme when glycobiologists talk about their subject. “Sugars are different, sugars are interesting, sugars are challenging,” is our refrain. It sounds rather evangelical but sugars really are special, and glycobiology attracts a special breed of scientist. While fellow undergraduate students groaned at carbohydrate chemistry lectures, we listened rapt. While conventional biochemists examined gene expression in minute detail, we were struggling to narrow

down our analyses to a single tissue. Traditionally, we have been the poor relation to proteins when it comes to funding and tools, and if it wasn't for our passion for the subject, most of us would have given up years ago. What drives us is the absolute conviction that sugars lie at the heart of some of the most pressing problems facing medical science today, from cancer to malaria to drug resistance.

That conviction is spreading in the wider scientific community; glycobiology is going mainstream. New tools and equipment are allowing sophisticated glycan analyses, which confirm the importance of sugars in all manner of physiological and pathogenic processes. For example, we always knew that glycans played a key role in development – if you alter certain glycosaminoglycans in mouse embryos, their development is so disordered that they die in utero. Now, novel analytical tools allow us to determine the type and amount of different glycans in different tissues throughout development, and pick up a huge amount of beautiful detail that you couldn't see before, including clues to disease mechanisms and developmental abnormalities.

“It sounds rather evangelical but sugars really are special, and glycobiology attracts a special breed of scientist.”

Life scientists used to working within the rigid confines of genes and proteins are finding themselves thrust into the very different world of glycans – and it scares many of them. I often review grants from people with strong specialties; for example, cancer biology. Imagine they have spent their career studying cancer metastasis, and suddenly find out that a crucial metastasizing factor is a sugar. If they have only ever taken a few classes of carbohydrate chemistry at undergraduate level, they are faced with a pretty big

gap in knowledge and skills. Often, they turn to inter-disciplinary collaborations – many of our best collaborations have come when talented scientists in other areas have approached us with this type of problem. It is great to see scientists in other areas taking an interest in glycobiology – and raising the profile of our field – but if glycans are ever to be as well understood as proteins, in the long term we need more dedicated glycobiologists. Glycobiology needs to be taught at undergraduate and PhD level, so that all biochemists have at

least a basic grounding. As analysis gets more accurate and glycans give up more of their secrets, I hope more people will choose to join this emerging field. In particular, glycobiology has always been a field that attracts female researchers – my own lab is overwhelmingly female. The strong women who carved out a niche and made great breakthroughs in the field, in the face of scientific, funding and professional barriers, are a continuing inspiration to me, and I hope to see that tradition of female-led research continue.

Can You Benefit from a Drug You Never Use?

Raising awareness of the hidden beneficiaries of medical innovation could help boost funding for drug development.



By Nicholas W. Papageorge, assistant professor in the Department of Economics at Johns Hopkins University, USA.

It is no secret that those with an illness will welcome new, groundbreaking and effective medication. Most people, however, are not sick. But many of us are able to at least anticipate the possibility that one day we might become ill, and will likely feel more positive about our future if we know that new medicines will be there if we need them.

In newly published research, my co-authors and I aimed to use a real-world example to illustrate the idea that healthy people value medications for diseases that they don't have – but might one day get (1). I'd like to explain our research here to encourage more discussion on this point.

In our work, we went back to a pivotal moment in pharmaceutical history; the invention of life-saving treatments for HIV – known collectively as highly active antiretroviral therapies (HAART) – which are credited with transforming HIV infection from a virtual death sentence into a chronic, but manageable condition.

Who benefitted from HAART? HIV-positive people are the natural beneficiaries; their survival rates soared, as did their quality of life. But in our research, we were more interested in HIV-negative individuals. We turned to a data set from the Multi-Center AIDS Cohort Study, following thousands of men who have sex with men, starting in 1984 – about half of whom were HIV-negative (2). The study asks questions about sexual behavior and conducts blood tests to see if anyone has become infected, and if so, whether or not their immune health is declining (a condition well known as AIDS).

“The appropriate allocation of research dollars should account for all potential beneficiaries of a new drug – and not just those who are already sick.”

We found that HIV-negative men started having riskier sex (for example, multiple partners and inconsistent use of condoms) after HAART came onto the market and we argue that this shift in behavior reflects changes to their views about the future. For HIV-negative men, the invention of HAART functioned somewhat like an insurance policy. By making HIV infection less terrible, HAART allowed

HIV-negative men to go about their life (including riskier sex) with less fear of infection.

Our study does not condone risky sex (nor does it condemn it). It simply builds on the idea that individuals want to live long lives but also enjoy themselves. Therefore, when it comes to making decisions about risky behavior – the types of things that are enjoyable today (for example, alcohol, drugs, junk food), but may have negative consequences down the line – individuals tend to weigh the risks against the benefits, and then land somewhere in the middle.

Some people see this example of HIV and risky sex as extreme, but even the most careful among us take risks every day – for example, when we drive a car or even leave the house. We could certainly avoid most car accidents if we never drove, but then we would also sacrifice other things we enjoy, such as going out

to restaurants or visiting friends, or even miss out on career opportunities.

I believe our findings have strong implications for how research dollars should be spent. Most people are not HIV-positive, but our study suggests that the “market” for HAART can be extended to include virtually anyone who is sexually active and at risk of infection. This is an important consideration; the appropriate allocation of research dollars should account for all potential beneficiaries of a new drug – and not just those who are already sick.

In terms of the perhaps not-so-obvious beneficiaries of pharmaceutical innovation, we also considered the value of a hypothetical, fully functional HIV vaccine. Clearly, HIV-negative men would be beneficiaries, as they would no longer have to worry about infection at all. However, HIV-positive men may also benefit from a vaccine because HIV-

negative men would likely react to a vaccine with increased sexual risk-taking. As a result, HIV-positive men may have an easier time finding sexual partners.

The vaccine scenario is similar to the HAART example. In both cases, there is a group of obvious beneficiaries of a medical innovation, and less obvious groups who will also benefit. The big question is: how can we better promote the wider benefits of medical development? With every medicine, I encourage developers to think more about the true market for their innovation.

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Fresh-(Faced) Funding

Making grant application processes tougher can have an unexpected and dramatic effect on the demographics of applicants. Let’s not force young investigators to fall at the first hurdle.



By Robin Barr, Director, Division of Extramural Activities, National Institute on Aging, USA

As the training officer at the National Institute on Aging of the NIH for 10 years, I helped trainees and junior investigators recognize their enthusiasm for research and creativity, before bringing them into our research programs. So when US Congressman Andy Harris (Rep-MD), a member of the House Committee on Appropriations and himself a former NIH grantee, expressed concern that the average NIH grantee in 2014 was well into their 50s, despite ongoing efforts from NIH to encourage younger investigators, it struck a chord with me. I was happy to join a committee to try to get to the bottom of why early-stage researchers were losing out on NIH funding and what we could do about it. Together with Rene Etcheberrigaray and Chuck Dumais from the Center for Scientific Review, I reviewed data from seven years of research project grants

“The average NIH grantee in 2014 was well into their 50s, despite ongoing efforts from NIH to encourage younger investigators.”

(R01) and made some interesting – and I hope useful – observations.

When we introduced our new and early-stage investigators policies back in 2007, there was a very positive response from the community. A review

“New investigators who feel that they cannot find funding may simply choose an alternative career.”

of the data from 2007 to 2009 suggests that young researchers significantly increased their applications for R01 awards. Separating out new and early-stage investigator applications in the review process and actively advancing new investigators seems to have led to a healthy increase in applications from junior researchers. But then something happened. Between 2010 and 2014 the number of junior investigators submitting applications topped by almost 40 percent. So what changed? In 2009, the NIH streamlined the grant review process, by cutting the number of amended applications investigators could submit from two to one. We all expected that applications would decrease as a result, but what we didn't foresee was that virtually all of the decrease would be made up of applications from the most junior investigators, who were most likely to need more than one round of amends. The change, while not targeting junior researchers, effectively wiped out all the gains made by the new and early-stage investigator policies.

The move to reduce the number of reapplications proved unpopular with the research community, and was scrapped in 2014, reinstating the chance for a second resubmission (albeit with a slightly different process). I was

able to look at the first two council rounds after we changed the policy and the shift was extraordinary. There was an all-round increase in applications, as you might expect, but it was the junior investigators who increased their applications the most. In fact, their submissions shot up over less than a year by more than 40 percent, reversing the decline. Over time, we hope to see the increase in submissions become an increase in awards.

Are we doing enough to bring down the average age of principal investigators? We don't know. But we're going to be monitoring the situation closely over the next few years, to see if there is a shift in the age of investigators receiving grants, and in particular, the age of first-time investigators. With the right policies in place, we hope new and early-stage investigators will be a growing segment of our grantees.

The crux of our findings was that junior investigators applying for grants were strongly impacted by any policies that made it harder or easier to get an award. Senior investigators on the other hand, with research careers well under way, submit applications regardless. I think we all agree that we must have a healthy pipeline of new researchers coming into the NIH stream, to maintain the vibrancy of research – and funding is a major factor for young scientists when deciding whether or not to pursue a research career. In simple terms, new investigators who feel that they cannot find funding may simply choose an alternative career, irrespective of their talent or creativity. The clear message for us – and other funding bodies – is that we must think very carefully before making the funding process harder; it will always hit our early-stage investigators hardest.

Read more from Robin at the Inside NIA Blog: www.nia.nih.gov/research/blog

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Chris HARRISON
(San Diego State University)



No Biomarker, No Trial?

The vast majority of experimental cancer drugs fail during the later stages of clinical development – after considerable time and expense has already been invested. The right biomarkers could help us weed out unsuitable candidates early on, and focus our efforts on therapies with real potential.

By James Ritchie, Sidath Katugampola and Paul Jones

The way we have developed cancer drugs to date is far from optimal and, in most instances, results in failure (1). The stark truth is that the majority of drug discovery and development activity yields little benefit to patients, while exposing them to potentially toxic drugs and wasting billions of dollars in the process. With the majority of experimental cancer drugs falling down at the costly mid-to-late stages of clinical development (Phase II or III clinical trials), we've reached a stage where something has to give. We need to bring critical decision points forward, ideally into the initial stages of clinical development (Phase I), before costs, timelines and patient numbers escalate. The shrewd application of biomarkers in early-phase clinical development can help us to make these critical go/no-go decisions in time.

Biomarkers come in many flavors

A biomarker is defined by the FDA as “a characteristic that is

objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.” Under the umbrella term of “biomarkers”, there are many different flavors. One of the most commonly adopted biomarker classification systems is that suggested by Bradley (2), which categorizes biomarkers (with some minor modifications) as follows:

- Pharmacodynamic
- Proof of mechanism (PoM)
- Proof of principle (PoP)
- Proof of concept (PoC)
- Predictive biomarkers (sometimes known as patient stratification, selection or enrichment biomarkers)
- Safety biomarkers

These biomarker categories are explained in more detail on page 20.



<i>Type of marker</i>	<i>What it tells us</i>	<i>Examples</i>
Proof of Mechanism (PoM)	<i>Does the drug engage the intended target?</i> Demonstrate that the drug binds to the intended target and/or produces the expected pharmacological effect; this does not necessarily have to be shown in the tumor – surrogate tissues are often used during Phase I dose escalation to obtain PK and PD data.	Dose-dependent target receptor occupancy in circulating PBMCs Reduction of downstream kinase phosphorylation in PBMCs or skin
Proof of Principle (PoP)	<i>Does the drug have a pharmacological impact on the disease?</i> Prove that the drug has a pharmacological impact on either the tumor cells or tumor microenvironment. As this aspect involves disease tissue the scope for repeated samples may be limited (at least for solid tumors), meaning assays may be restricted to expansion cohorts or even a subset of this cohort.	Reduction in Ki67 Cleaved caspase 3 Reduction in tumor angiogenesis Functional imaging
Proof of Concept (PoC)*	<i>Does it produce a clinically meaningful change on disease?</i> Demonstrate that the drug produces a change in an accepted clinical end point for patients with the disease. In the majority of cases PoC is beyond the remit of early-stage trials.	B-cell knockdown in chronic lymphoid leukemia Progression-free survival Response rate
Predictive Biomarkers (for patient selection)	<i>Do we know which patients are most likely to respond?</i> Are there markers that can be used to pre-select patients most likely to respond to the agent? With the exception of antigen-targeted agents it may be difficult to select patients purely on such markers in early-phase trials; however, recruitment can be biased towards patients with a particular marker – termed enrichment.	HER2+ disease for Herceptin ALK-EML4 fusion for ALK inhibitors BRCA mutation for PARP inhibitors
Safety Biomarkers	<i>Can we detect early signs of expected toxicity?</i> Are there markers that can be used to detect toxicity before symptoms appear? Phase I trials have extensive safety testing; however, there may be drug-specific risks that require extra monitoring. Examples include cardiac damage, effects on eyesight and immunomodulatory effects.	Troponin T ERG assessment

*PoC is generally included in the list of biomarkers although by definition the measures of PoC will generally be clinical end points rather than biomarker assays. PK – pharmacokinetics; PD – pharmacodynamic; PBMCs – peripheral blood mononuclear cell; ALK – anaplastic lymphoma kinase; EML4 – echinoderm microtubule-associated protein-like 4; PARP – poly (ADP-ribose) polymerase; ERG – electroretinogram. Adapted from (2).

Biomarkers to the rescue?

Biomarkers can provide us with data to make critical development decisions in early-phase cancer trials in at least two important areas.

Confirming the biological effect

The use of PoM and PoP pharmacodynamic biomarkers allows an early assessment of pharmacological activity of a new drug. Traditionally, dose-finding first-in-human oncology trials have relied on escalating the dose of the drug up to a maximum tolerated dose (MTD; the highest dose of a drug or treatment that does not cause unacceptable side effects), which is then declared the recommended dose for further development. For many (if not most) emerging oncology therapies, dosing to MTD is either impractical or nonsensical. The assumption that higher doses correlate with therapeutic effect, which is inherently linked to toxicity, may be appropriate for “traditional” cytotoxic drugs but not for many of today’s molecularly targeted agents.

In the absence of desirable “off-target” pharmacology, dosing beyond a relevant pharmacodynamic plateau is likely to offer little benefit but instead risks increasing toxicity or even producing confounding effects. Rather than blindly escalating the dose to the MTD, applying appropriate PoM pharmacodynamic biomarkers during the dose escalation stage of a clinical trial can provide an estimated optimum dose, without having to expose patients to unnecessary toxicities. PoP biomarkers can then be examined in patient expansion cohorts treated with the identified dose, in order to confirm the pharmacological impact on the tumor.

Selecting the right patients

Biomarkers that identify patients most likely to respond to a given therapy are known as predictive biomarkers. At the most basic level, molecularly targeted agents and therapeutic IgG antibodies will not work in tumors that lack the relevant target/antigen or are not reliant on that target for survival; for example, where simultaneous blockade of two pathways is required to cause cell death (3). Well-known examples include:

- Herceptin won’t work in tumors not expressing HER2;
- Inhibitors of PARP have a greater effect on tumors with existing deficiencies in DNA repair, such as BRCA1/2 mutations;
- The ALK inhibitors crizotinib and ceritinib have no efficacy in non-small-cell lung cancer patients lacking EML4-ALK translocations.

The use of predictive biomarkers has the potential to make clinical trials smaller, shorten development timelines and avoid exposing non-responsive patients to unnecessary toxicity. As a result, they are becoming a cornerstone in patient therapy. Even so, we must remember that, while attractive, markers of sensitivity or resistance may be challenging to establish in early trials and can be more complex than expected. For example, at least five subtypes of EML4 ALK are now known, each with different sensitivities to crizotinib, which can prove challenging when screening large numbers of patients for these rare markers.

“The majority of drug discovery and development activity yields little benefit to patients, while exposing them to potentially toxic drugs and wasting billions of dollars in the process.”

Best practice for biomarkers

We’ve established that biomarkers can bring forward critical go/no-go decisions, and make the drug development process more efficient by allowing candidates to “fail early and fail fast”. But there are a host of practical considerations to take into account before deciding when and how to make use of biomarkers in a given trial.

Biomarker development should begin as early as possible, ideally at the target validation stage, and continue throughout early drug discovery and beyond. It is particularly important to establish the key pharmacokinetic and pharmacodynamic relationships well ahead of a clinical trial. For example:

- Concentration required to produce a pharmacological effect in vitro;
- Duration of the biological effect during and after administration of the drug in vitro;
- Administered dose, schedule and plasma concentration needed to produce biological effects in vivo;
- Any toxicological changes that could be detected by adding safety biomarkers into preclinical studies and/or the clinical trial.

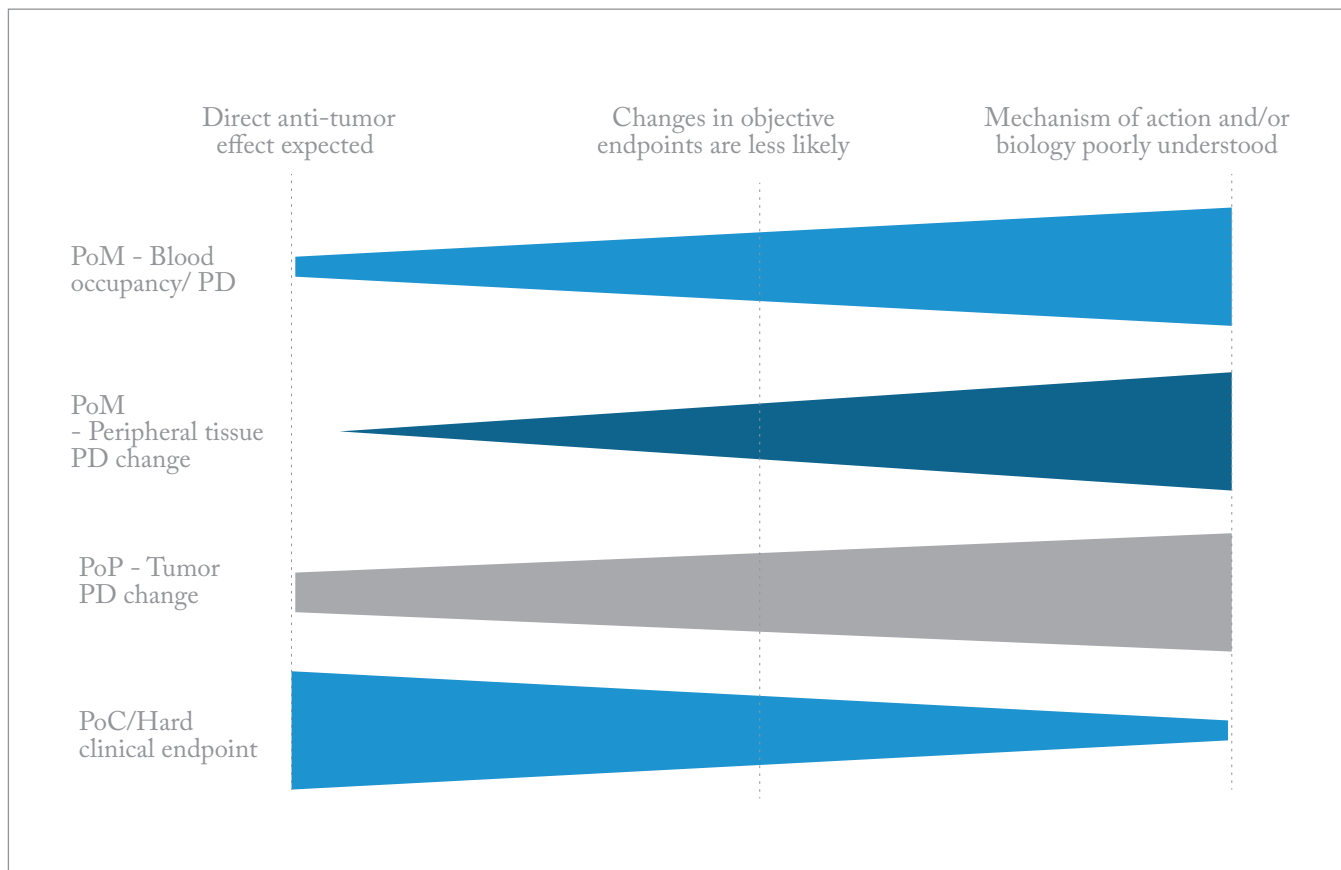


Figure 1. One size doesn't fit all. Shows the relative importance of different biomarker types, depending on the properties of the drug being tested.

Without an understanding of these elements, the clinical dose and schedule cannot be selected with any certainty.

During biomarker assay development, consideration must be given at all times to the four “S”s:

- Science – is there a scientifically relevant biomarker we can use?
- Suitability – can the assay reliably detect the pharmacodynamic change we expect to see clinically?
- Study design – do we know when to take a clinical sample and how many we will need to provide a statistically robust decision?
- Sample – can we actually deliver this assay in the real world?

Particularly for first-in-class trials, it can be difficult to define a scientifically relevant biomarker, as the underlying biology may not be adequately characterized. Although it might be possible to generate a potential biomarker empirically

in vitro or in vivo, without a thorough understanding of the underlying target biology, translation of the biomarker to the clinic will always be hazardous and the results difficult to interpret. It is also essential to gain a thorough understanding of assay performance prior to a clinical trial; close attention must be paid to analytical validation of assays. While assessing the sensitivity of an assay is relatively achievable, understanding what magnitude of change is biologically relevant in patients is much more challenging. Indeed, the clinical utility of a new biomarker may need to be investigated at the same time as the drug being tested, which makes setting critical study go/no-go decision criteria related to changes in pharmacodynamics very challenging, especially in the early stages of drug development.

During preclinical development, identifying when the peak pharmacodynamic effect is observed (both after a single dose and over repeated dosing) is essential, preferably in the context of the drug pharmacokinetics achieved. Establishing appropriate sampling times based on pharmacodynamic results

during a Phase I trial is impractical; however, it is feasible to use preclinically established pharmacokinetic/pharmacodynamic relationships to focus on appropriate time points in the clinic.

An oft-neglected aspect of biomarker development is sample integrity. Even the most established assay cannot produce reliable information from a degraded sample. Assays of live cells, phosphoproteins, mRNA and metabolites are generally the most challenging in this regard. Ensuring consistent sample handling between multiple sites, with subsequent analysis at a single site, may be necessary to make sure biomarker results are comparable between trial patients.

The problem with predictions

While in many instances suitable predictive markers may not be available or required for a first-in-human trial, such markers may be appropriate or even vital for further development of the agent. There are several preclinical and clinical approaches that can be taken to identify such markers, but they generally fall into three groups:

- Rational selection – choosing potential predictive biomarkers based on known biology and then testing the hypothesis in appropriate cell lines and preclinical in vivo cancer models.
- Screening-based selection – looking at the efficacy of the agent across a wide panel of cell lines, such as the Wellcome Trust Sanger “Genomics of Drug Sensitivity in Cancer” project (<http://www.cancerrxgene.org/>), and selecting potential predictive biomarkers based on the genotype of sensitive cells lines. This can be based on single or multiple genes; although in this latter case testing the hypothesis experimentally can be very challenging.
- Retrospective response analysis – collecting biopsy material from patients during a trial and retrospectively genotyping to differentiate a predictive signature based on responders and non-responders. This is very challenging in early-stage clinical trials with small patient numbers but tumor material can at least be collected for future analysis.

In cases where a single characterized tumor marker is required (for example, monoclonal antibodies and small molecules targeting specific mutations), patient selection is relatively straightforward. However, for many agents a single, simple marker for efficacy is not available and in these cases it may not be possible or even advisable to select patients in early-phase trials.

Where a predictive biomarker is available, in the vast majority of cases it will necessitate the analysis of tumor tissue, which

in itself can be a challenge. While existing historic biopsy material is relatively easy to obtain, each patient’s disease evolves over time and with treatment, so historic tissue may not reflect the current status of the tumor. Historic tissue also tends to be formalin fixed, which can limit the utility of antibodies in patient screening. In contrast, a contemporary biopsy taken as part of the clinical trial will be far more relevant to the patient’s disease and can be processed as needed. The downside is that the requirement for biopsies as part of trial inclusion may severely limit recruitment. This is especially true where a relatively small proportion of the patient population is expected to be positive for the biomarker and most biopsies will therefore prove futile.

The decision on the use of biopsies for patient selection should be made on a case-by-case basis according to the biology of the target, the design of the clinical trial, and the intended patient population. In addition, successful patient selection or enrichment can depend on the assay type, reagents used, preparation of tissue and performance of the assay. Therefore, defining a positive result for inclusion across a range of patients, assays and trials can be very challenging. Decisions on setting “positivity” limits to entry criteria based on patient selection markers should be made on a case-by-case basis with reference to both the known biology of the target and, if possible, additional data on the patient population to be investigated (from biobanks, for example). A balance needs to be struck between setting limits too high to achieve recruitment or too low to get any biologically meaningful data. Finally, for any trial intending to use predictive biomarkers, it is vital to understand the size of the patient population – and the logistical challenges associated with screening early on – to allow a rational discussion on the clinical viability of the project.

One size doesn’t fit all

Unfortunately, it is impossible to have a single prescriptive biomarker strategy for all possible oncology drug classes and targets; there is just too much diversity to allow this. Even so, provided the four “S”s described on page 22 are adhered to, a biomarker approach can be developed for any cancer trial.

We must remember that, fundamentally, clinical trials are experiments conducted to answer a scientific question about a particular therapy and/or the underlying tumor biology. Given this, the biomarkers chosen for an early-phase clinical trial will be highly dependent on the nature of the scientific and clinical aims of the trial, type of agent, etc. There are many ways in which this might be considered but, at the Cancer Research UK Centre for Drug Development, we generally use four broad categories as a reference framework:

1. Clinical trials in which a direct, quantifiable anti-tumor effect is expected

Such trials might include cytotoxic agents, synthetic lethal combinations, antibodies with anti-tumor antibody-dependent cellular cytotoxicity or antibodies armed with cytotoxic or other therapeutic payloads. The emphasis in these trials is on PoP and objective clinical markers of PoC such as response rate or progression-free survival. The use of predictive biomarkers may or may not be required, depending on the mechanism of action of the agent. Cell-based immunotherapies, such as chimeric antigen receptor technologies, may also fall into this category given that they have direct anti-tumor effects and have been shown to be effective in treating advanced, hard-to-treat cancers.

2. Clinical trials in which direct changes in objective clinical endpoints are less likely

Here, agents may target tumor cell migration, metastasis or angiogenesis. Agents in this class will generally have PoP biomarkers that are well characterized, making the detection of any biological effect of the agent comparatively straightforward. Patient selection for such targeted agents is likely to become very important. In the absence of a clinical response, peripheral PoM biomarkers to guide dose selection are likely to be necessary, as well as PoP in tumor to gain evidence for further development.

3. Clinical trials in which the mechanism of action and/or downstream biological effect on the tumor are poorly understood

Examples here might include agents targeting tumor-associated immune cell phenotypes or tumor metabolism. Unlike the category above, the purpose of such a trial is likely to be focused as much on exploring the mechanism of action of the agent, as advancing its development. In extreme cases the aims of the trial may be entirely related to hypothesis testing and the agent used purely as a tool with no inherent “developability”. In these cases both PoM and PoP biomarkers become necessary to fulfil the aims of the trial but suitable assays are unlikely to have been characterized clinically or even exist preclinically. Predictive biomarkers are unlikely to be possible. In these cases a very comprehensive preclinical assessment of potential clinical

biomarkers is required before a decision can be made on whether to translate a project to the clinic. These studies are likely to be a clear-cut case of “no biomarker, no trial”.

4. Therapeutic cancer vaccine trials

These tend to fall outside the above approaches as simple pharmacokinetic/pharmacodynamic relationships do not strictly apply and the definitions of PoM and PoP are more concerned with the development of an anti-target immune response than a direct anti-cancer response per se. PoC clinical efficacy readouts such as progression-free or overall survival might be considered for such trials depending on the patient population being studied. Demonstrating clinical benefit in an advanced cancer population is unlikely, but may be expected in a newly diagnosed population with low tumor burden.

Biomarkers for all?

The aim of early phase clinical trials should be to establish if an agent warrants further clinical development or to robustly test a scientific hypothesis in the clinic. If a biomarker is needed to meet the aims of a clinical trial, performing the trial without an appropriate biomarker is futile. Overall, the situation is more complex than “No Biomarker, No Trial” and requires a firm understanding of what a clinical trial is aiming to achieve and how exactly biomarkers can support this aim. Nevertheless, rationale application of biomarkers in early clinical development can stop an agent from becoming another victim of mid- to late-stage cancer drug failure. Perhaps it would be more accurate to say “No Biomarker Strategy, No Trial”.

“Perhaps it would be more accurate to say ‘No Biomarker Strategy, No Trial’.”

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James Ritchie is a drug development scientist, Sidath Katugampola is a biomarker development specialist and Paul Jones is a preclinical sciences manager at Cancer Research UK Centre for Drug Development, London, UK.

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Finding HIV's Achilles' Heel

The human immunodeficiency virus has evaded the best efforts of vaccine developers for over three decades. And our own HIV vaccine has taken 15 years to make it into clinical trials. Here, I share why I believe we're finally in with a chance.

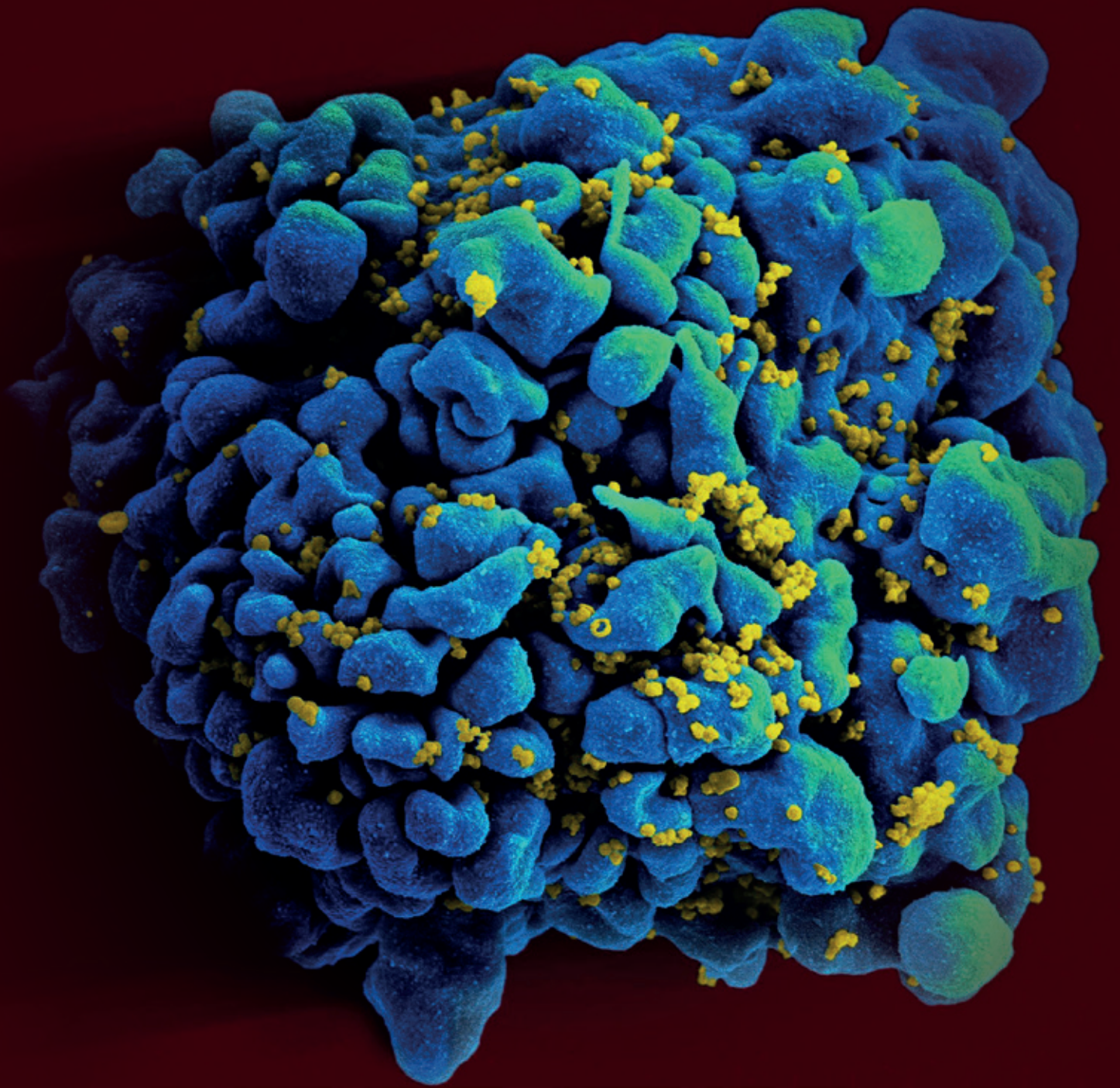
By Timothy Fouts

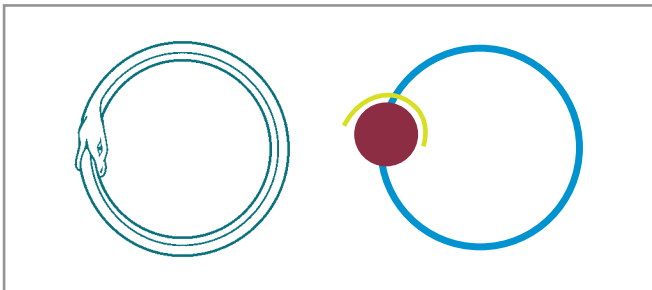
I have been interested in HIV ever since my grad student days, having lost friends to the virus before effective drugs were available to keep the virus in check. Over 30 years, amazing progress has been made to life expectancy thanks to antiretroviral therapies, but an effective vaccine still eludes us. The NIAID has funded over 100 clinical trials of over 50 HIV vaccine candidates since 1987, but none have made it to licensure.

There are three main reasons that creating a vaccine for HIV has so far proved impossible. First, the virus changes very rapidly. In fact, the virus in your system a month after infection is very different from the one that you were initially infected with – it's like hitting a moving target. Second, the virus has a number of other clever ways to evade the immune system (see "Evasive Maneuvers"

on page 30). Third, HIV infects the very cells that your body uses to combat an infectious agent, namely CD4-positive T cells.

Several candidates have shown promise. In one of the highest-profile clinical trials for HIV to date, RV 144, promising early results generated cautious optimism with many investigators. Unfortunately, the response was not durable, providing only a 30 percent decrease in infection rates by the end of the study. The vaccine tested in RV 144 was based on a prime–boost strategy – with four injections of a vaccine using attenuated canary pox virus as a carrier to present HIV envelope proteins to the immune system plus two injections of a subunit vaccine, targeting a different HIV envelope protein (see page 29 for more on "Vaccine Varieties).



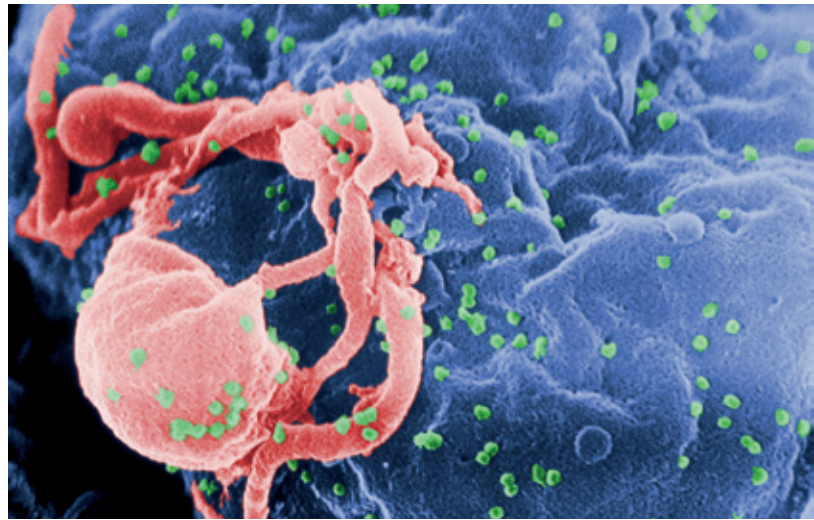


The FLSC vaccine is made up of sections of CD4 receptor (yellow) and its viral ligand GP120 (red), joined by a flexible linker (blue) that allows the two to bind, not unlike Jörmungandr, the snake from Norse mythology that circles the globe and eats its own tail.

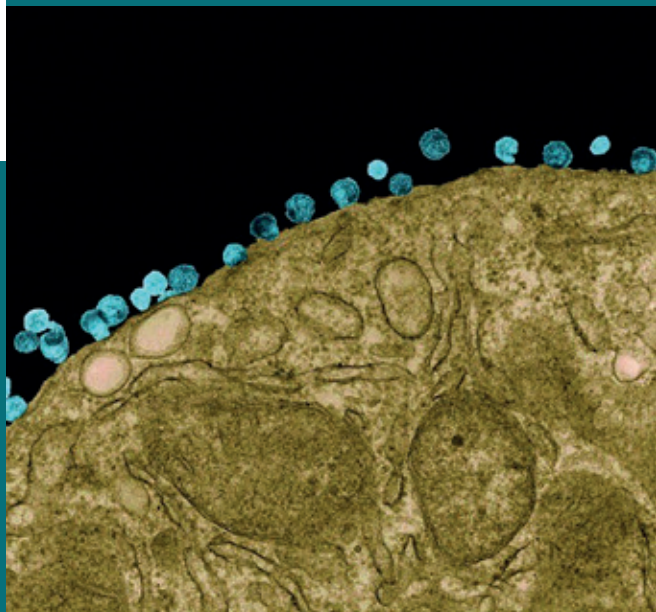
A chink in the armor?

The Profectus vaccine stems from an observation made by my colleague at the Institute of Human Virology – Tony DeVico – who noted that you can stop the virus from getting into the cell if you target sites that are normally hidden on the virus but become exposed when it engages its receptor. The first receptor the virus encounters is CD4, a cell-surface protein. The virus binds to CD4 using a “spike” made up of the viral envelope protein. Once bound, the viral protein changes shape to expose parts that engage a second receptor – this time a chemokine receptor (usually CCR5 or CXCR4). Only by binding to both receptors can the virus gain entry to the cell.

To evade the immune system, the sequence of the HIV spike changes constantly, but the receptor-binding domains must remain constant to allow cell entry. The obvious target is the CD4 receptor-binding site, so why not target that? The answer



can be found in the 3D structure of the envelope protein. The CD4 binding site is a deep cleft and obscured by loop structures and glycan groups, making it hard for the immune system to “see” and access the binding site. But when viral envelope protein binds to CD4 and undergoes its dramatic conformational change, other conserved areas are exposed and could be targets for the immune system. We realized that if we created an immunogen that looked like this altered spike protein, we could induce an immune response that would target the virus while it is in its transition state, before it enters the cell. The protein we produced consisted of a piece of CD4 and a piece of the envelope protein GP120, stitched together



with a floppy linker that allowed the two to engage in such a way as to make GP120 change its shape. We call this transition state vaccine the full length single chain (FLSC).

Profectus is born

The Institute of Human Virology was founded by Robert Gallo with two important goals: doing great basic research and translating this research into new therapies that can be developed into clinical products. Several years after I joined the Institute, I was asked if I would be interested in joining the spin-off company which would spearhead that translational mission – Profectus Biosciences. We had enough money from investors to launch the company – but not enough to move the vaccine into the clinic. I was doing the groundwork towards clinical translation, so I accepted the invite immediately. For me it was a no-brainer. I was not interested in becoming an academic and having to deal with all the rules that academics have to put up with. Since joining a small company, I have certainly spent a surprising amount of my time writing grant applications to help move our products into clinical trials. But there is a different mentality and way of working in a company that you really can't transplant into an academic setting.

In the wilderness

We first described our protein construct back in 2000, so it's taken us 15 years to bring it to Phase I clinical trial. Why the delay? It's the usual story – not enough money. Even getting funding to do some preliminary studies in animals was a

Vaccine Varieties

Attenuated virus

A weakened version of a pathogenic virus, designed to infect cells and cause an immune response, but not harm the host. Examples: Measles, Influenza.

Subunit

A portion or protein of the pathogenic virus. Typically co-formulated with an adjuvant to excite the immune system. Example: Hepatitis B

DNA vaccine

A DNA plasmid encoding proteins from the pathogen is injected, to be taken up and expressed by the body's own cells. Example: Only one DNA vaccine has been approved for human use (Japanese encephalitis).

Viral vector

A harmless virus is genetically engineered to express proteins from the target pathogen to provoke an immune response. Example: Profectus' replication-competent vesiculovirus (VSV) vector. None approved for human use.

challenge. We had promising results in small animals. Next came studies in primates, the gold-standard model for HIV, to build up a solid database of evidence to prove that a vaccine was worth pursuing. In the meantime, we were looking for money to develop a product we could take into clinical trials. This took us quite some time. It is frustrating because with the right funding we could have brought the vaccine to the clinic years ago – the vaccine really hasn't changed that much from the very first experiments we published. We've made a lot of variations on it for experimental purposes, but the principal construct has remained the same.

The breakthrough in funding finally came when the Bill & Melinda Gates Foundation, along with the Henry Jackson Foundation, stepped in to put a significant sum behind the vaccine. Concurrently, Profectus was able to get a Small Business Innovative Research grant (SBIR) from the National Institute

of Allergy and Infectious Diseases. A classic case of “feast or famine” – we were in the financial desert for a long time and then all of a sudden the stars aligned and we’ve been able to move forward rapidly to the point of entering clinical trials.

Into the clinic

We have now started enrolling the Phase I trial, and the first participants, healthy 22–45 year olds, have received the vaccine. At this stage, we’re solely interested in safety; in other words, does the vaccine make anyone sick? Of course, once we establish safety, the million dollar question is “Will it work?” I’m confident that we will evoke an immune response but also well aware that we may need to fine-tune the precise formulation. In our primate studies, while a straightforward subunit vaccine generated a response, it wasn’t the 100 percent lifetime immunity that you see with some vaccines. To get the best protection, we also found that we had to induce the correct “immune balance”. This comes back to the fact that HIV targets CD4-positive T cells. If the vaccine induces the formation of too many of these CD4-positive T cells, it actually works in the virus’ favor. So the goal is to induce a predominantly antibody-based response, with just the right amount of T cells – it’s a fine line. It may be that the subunit vaccine, as currently formulated, is enough. But to be certain, we’re also testing a variety of different prime–boost regimens to expand the repertoire of immune responses being elicited by the vaccine; for instance, combinations of DNA and subunit vaccines as well as combinations with viral vectors such as the canarypox that was used in the RV 144 study. Once the subunit vaccine completes Phase I, we will initiate these additional trials to help us understand the best way to elicit the immune balance we need. We can learn a lot from animal studies, but ultimately you need human trials to be sure you have the balance right. My clinician friends regularly remind me that people are not monkeys – but, given our previous work in primates, I have every confidence that the vaccine will be safe and that it will generate the right immune response.

Timothy Fouts is Senior Director of Virology at Profectus Biosciences, Baltimore, MD, USA.

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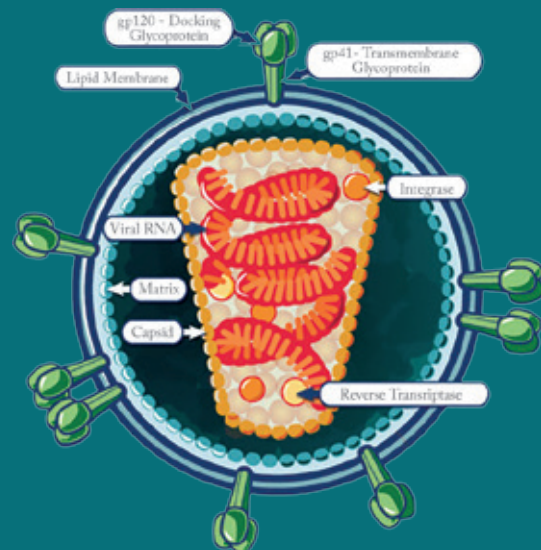
Evasive Maneuvers

HIV has a multitude of strategies to avoid detection and evade elimination by host defenses.

Shape shifting A recent study reported that the mutation rate of HIV-1 in vivo is the highest of any biological entity, and significantly higher than previously reported (1). Mutations are created both by viral reverse transcriptase and by host cytidine deaminases. High genetic diversity benefits the virus by allowing it to dodge immune responses but a large number of mutations can cause inactivation, so the virus walks a fine line.

Invisibility cloak Reverse transcription of viral RNA into double-stranded DNA normally triggers innate pattern recognition receptors. The capsid protein that surrounds the HIV-1 genome prevents infected macrophages from recognizing and destroying the virus, by binding to specific host factors. By using drugs to suppress the host factors involved, researchers have been able to “uncloak” the virus and prevent replication – one avenue for future antiretroviral therapies (2).

Going to ground While modern antiretroviral therapy allows patients to live largely normal lives, the disease is never truly wiped out. Instead, the virus hides out in cells and tissues protected from the immune system – ready to stage a re-invasion if treatment is interrupted. It was thought that the virus remained dormant during this tactical retreat, but recent research suggests that the virus continues to replicate and evolve within the lymphoid tissue, even in patients with undetectable levels of viral RNA in blood samples (3).



A single virion.



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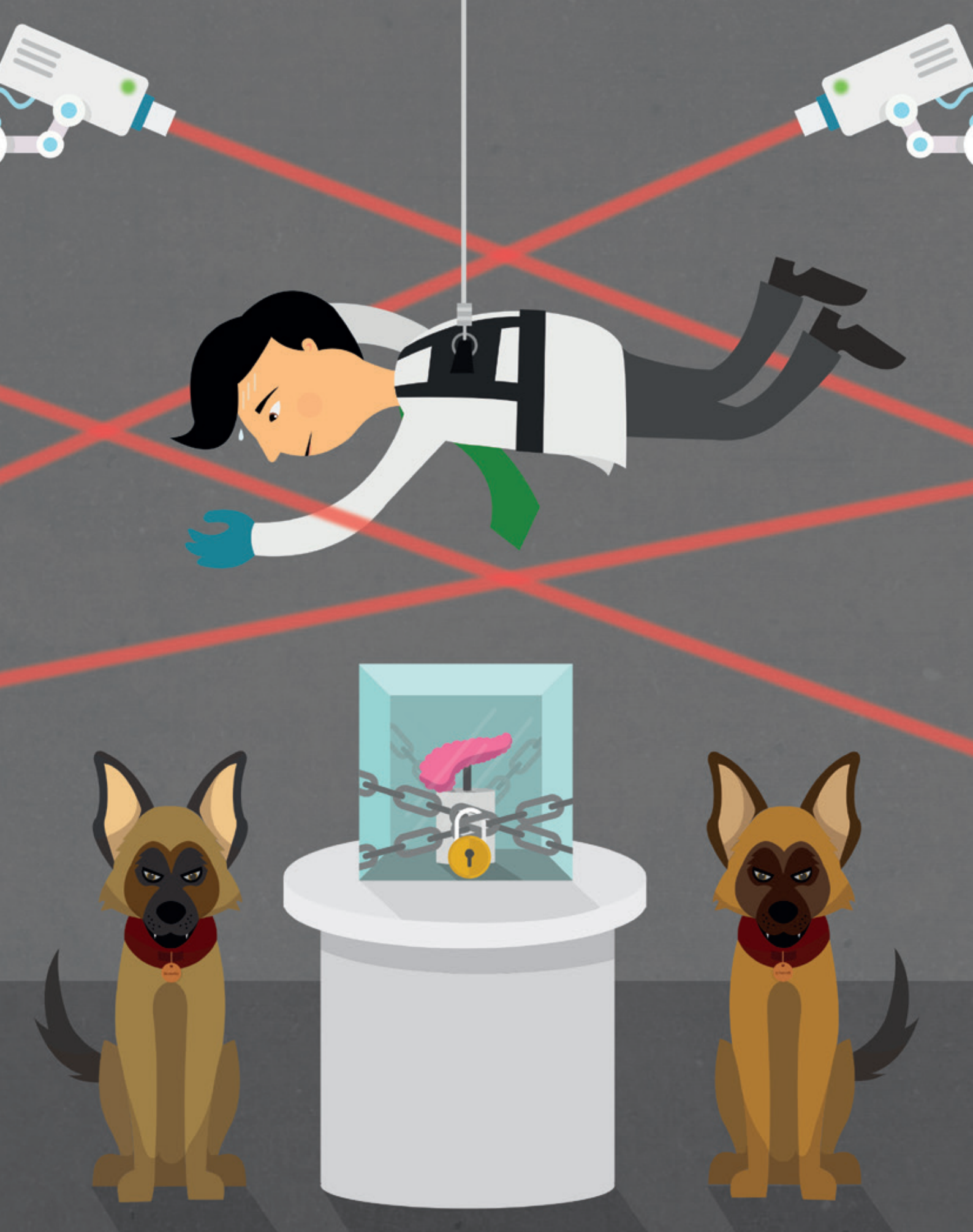
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Mission: Impossible

Pancreatic cancer is tough to understand, diagnose and treat. Undeterred researchers are on a quest to crack the case.

By Michael Schubert

Although only the 12th most common cancer worldwide (1), pancreatic cancer has gained increasing attention over the last few years. High-profile figures like Steve Jobs and Randy Pausch have put the disease under the spotlight, but despite the increase in research interest, progress remains slow. Why? A combination of factors: the potential causes of the disease are not well understood, screening techniques are imperfect, chemotherapy and radiation treatments have limited success, and the mortality rate is high – only about six percent of patients survive for five years after diagnosis (2), and that number drops to one percent after 10 years (3). But these are not just dismal statistics – they are a call to arms for researchers, and lately, that call has been answered, with new ideas for diagnosis, prognosis and treatment seeming to arrive every day. Here, we speak with some of the scientists at the

forefront of this research to learn more about what's being done. Will we soon see those survival statistics improve? It's still early days for the new wave of pancreatic cancer breakthroughs, but one thing's for sure – the promise is most certainly there.

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(Chemo)resistance Is Futile

The epithelial-to-mesenchymal transition in pancreatic cancer may play a role in its resistance to treatment – and inhibiting it may improve treatment efficacy

While late diagnosis remains a key reason for the dismal outcome of pancreatic ductal adenocarcinoma (PDAC), the fact that it is difficult to treat using non-surgical methods also presents a big problem. PDAC tumors are often resistant to chemotherapy; only two agents are currently approved to treat advanced disease. The first, gemcitabine, increases median survival by just over one month (from 4.41 to 5.65 months) compared with the previously used drug, 5-fluorouracil (1). Adding the second, erlotinib, has an even smaller effect – increasing survival by only one-third of a month (2). Despite the many Phase III trials conducted to improve the efficacy of chemotherapy – using everything from traditional chemotherapy to experimental targeted approaches – PDAC remains stubbornly resistant to treatment (3).

Why? Because very few patients ever experience a good response to chemotherapy, we know that PDAC's resistance to treatment is primary (innate), rather than secondary (acquired) as in most other cancers – but what we haven't known is what gives rise to this resistance. One research team from the University of Texas MD Anderson Cancer Center pointed the finger at the epithelial-to-mesenchymal transition (EMT). The EMT program plays a role in metastasis, but the researchers noticed that when cancer cells begin to migrate, they stop proliferating. Lead author Raghuram Kalluri explained that “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” (4).

To examine the role of EMT in PDAC, Kalluri and his colleagues generated mouse models of PDAC that featured a deletion of either Snail or Twist – two transcription factors responsible for the EMT program. Deleting either of these proteins had no effect on tumor pathology, invasion or metastasis, but did increase cancer cell proliferation and gemcitabine sensitivity (5). “We found that the EMT program suppressed drug transporter and concentrative proteins, which inadvertently protected these cancer cells from anti-proliferative drugs, such as gemcitabine,” said Kalluri – so suppressing the influence of that program resulted in a stronger response to chemotherapy, including reduced tumor burden and significantly better survival. What does this mean for patients? No research has been conducted yet in humans, but the promising results in mice indicate that EMT suppression may be an intriguing target worthy of further investigation.

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Breaching Cancer's Defenses

A new immunotherapy approach shows that engineered T cells are able to penetrate into pancreatic tumors and directly attack the cancer

By Ingunn Stromnes

Pancreatic ductal adenocarcinoma (PDAC) is unique among cancers for its survival mechanisms, which include the ability to survive with limited blood supply and low oxygen, and to protect itself from the immune system. The lack of angiogenesis means that it's difficult for chemotherapy to reach the cancer cells; the hypoxic tumor environment means that radiation therapy is of limited use; and the ability of the cancer to induce inflammation and condition immune cells in its favor means that it's able to avoid the body's natural defenses. As a result, despite advances both in treatment options and in our understanding of the disease, we remain

unable to effectively penetrate PDAC's fortress – the majority of patients present with locally advanced or metastatic disease that is inoperable, meaning that they have only months to live, and no known therapy provides lasting benefit.

The immune advantage

In previous research, we were able to deplete a particular subset of immune suppressor cells in PDAC and unmask the ability of the adaptive immune response to target the cancer (1). Continuing on from this work, we decided to investigate a way of overcoming the immunological barriers set up by PDAC, knowing that developing an effective immune therapy to treat this disease was likely to change the therapeutic landscape, and that the principles we learned would likely translate to other types of solid tumors. T cell therapy is not entirely new – it's currently under investigation in a variety of leukemias and lymphomas. But treating solid tumors with T cells is harder, because it's not always possible for the cells to penetrate the tumor tissue. So we knew that if we were able to develop a method that allowed T cells to attack PDAC effectively, we might be able to broaden our horizons to include other tumors as well.

Immunotherapy is quite attractive because it specifically targets the malignant cells, leaving healthy tissue unharmed. T lymphocytes, the type of cell we engineer to target and kill cancer cells, have the ability to form memory – so their antitumor activity can be long-lived. Lastly, immunotherapy lets us take advantage of millennia of evolution. T lymphocytes naturally traffic throughout all of the body's tissues. It's conceivable that no site is off limits, including distant metastases, dormant tumor cells and desmoplastic tumors. This is particularly important in PDAC tumors as they have the ability to form a dense shell around themselves, compressing blood vessels and preventing chemotherapy access.

An engineered attack

Current treatments have minimal, if any, clinical benefit. Chemotherapy is not specific, very toxic, and typically has only transient or palliative benefit. It's also unable to penetrate bulky pancreatic tumors due to high interstitial pressure and compressed blood vessels. And of the small population

of pancreatic cancer patients who are able to undergo surgery, only 20 percent will survive for five years – so even surgery isn't curative in most patients. It's clear that we need a better way to attack these tumors.

Our immunotherapy method involves isolating a population of T lymphocytes and engineering them to express a particular affinity-enhanced T cell receptor. This receptor specifically recognizes an epitope of a protein overexpressed by tumor cells. We chose to target the protein mesothelin, which is highly expressed in most PDACs, as well as in several other cancers. After our T cells are ready, we expand them in culture and transfer them back into patients – who, in this preliminary study (2), were mice.

Eight days after infusing our T cells into the mice, we observed increased tumor cell apoptosis, showing that the cells were doing their job. But by day 28, that effect had been lost, thanks to the inhospitable environment of the PDAC tumors. We provided the mice with a second infusion of the cells to see whether or not the tumors remained susceptible, and saw the same effects again. Eventually, we randomized mice to receive either our T cells or a control T cell infusion every two weeks – and saw that, while control mice showed consistently progressing disease, those receiving our treatment showed objective responses, including increased tumor cell apoptosis, decreased metastatic disease and malignant ascites, and almost double the median survival time (54 days in control vs. 96 days in treated mice).

Taking T cells to trial

In these preclinical studies, the engineered T cells preferentially accumulated in the tumor and metastases, killed cancer cells, persisted indefinitely, and prolonged survival. They showed another advantage as well – they specifically targeted the cancer without toxicity to the mice. Some of the proteins that we target are also expressed at low levels in some normal tissues, which means that there is potential for some toxicity, but after extensive evaluation in our preclinical models, we detected none. So not only are these T cells able to penetrate the biophysical barriers that chemotherapy can't, they offer the chance for an improved safety profile as well.

But this is a living cell therapy, which means it's more cumbersome to generate and requires access to an experienced good manufacturing practice (GMP) facility. And at the moment, the suppressive tumor



microenvironment shuts down T cells over time – meaning that patients must receive regular infusions, increasing the burden on both patient and facility. We are currently working on how best to refine our approach so that we can sustain T cell expansion and function within the harsh tumor environment.

In the meantime, our first priority is to translate these results to patients as quickly as possible. We now have the equivalent T cell receptors for engineering human cells and hope to open a trial in the near future. My hope is that our approach will eventually significantly prolong survival in patients with advanced pancreatic cancer. The fact that it's more technically challenging to deliver is less of a concern – if we have an effective solution, it will change how

patients are treated and ultimately bypass the need for toxic chemotherapies altogether.

Ingunn Stromnes is a researcher at the Fred Hutchinson Cancer Research Center, University of Washington, Seattle, USA.

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An Epigenetic Epiphany

When genetics yielded unsatisfactory answers about pancreatic cancer's persistent survival, researchers looked beyond the genome – and found telling epigenetic changes

Many researchers have investigated the genetics of pancreatic cancer, hoping to find answers to the disease's mysteries. Some studies have struck gold with oncogenic events like KRAS mutations (1), which occur in almost all cases of pancreatic ductal adenocarcinoma (PDAC) – but then discovered that treatments targeting those mutations are hampered by dose-limited toxicity or disease resistance. More recently, next-generation sequencing has revealed mutations in several genes that code for chromatin regulators (2,3), suggesting that epigenetic factors might be responsible for some properties of PDAC tumors – perhaps even their persistent survival.

Based on their knowledge of the properties of proteins in the BET (bromodomain and extraterminal) family, researchers from the Technical University of Munich and Stanford University decided to use them to investigate the possibility of an epigenetics-based therapy for PDAC. BET proteins use their bromodomains to recognize acetylated lysines on histones; the proteins involved in DNA packaging in the cell. Histone acetylation is associated with increased transcription and a more open, accessible chromatin structure – including in oncogenes like MYC, thereby increasing the survival and proliferation of abnormal cells that would otherwise undergo apoptosis.

To generate their treatment, the researchers examined the expression of BET proteins in PDAC tumors and found three proteins – BRD2, BRD3 and BRD4 – in preneoplastic and

neoplastic lesions. They then used a mouse model to test a small molecule known as JQ1, which inhibits the function of those proteins (4). By inhibiting the BET proteins, the researchers were able to decrease both MYC activity and inflammatory signaling, suppressing PDAC development. But JQ1 alone wasn't effective enough – the mice still ultimately succumbed to their disease. So the researchers investigated agents that could be used alongside the small molecule to improve treatment and discovered that the addition of the small molecule SAHA – which inhibits histone deacetylation – had a synergistic effect.

This is an especially promising start because JQ1 (as TEN-010) is already in clinical trials and SAHA (as vorinostat) has been approved by the FDA for use in cutaneous T cell lymphoma. Because the researchers don't need to start from scratch, their treatment may reach the clinic more quickly than a brand new combination. With that in mind, they've already begun investigating potential biomarkers – like the gene p57, which may be a key mediator of the drugs' function and a predictor of treatment success.

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Building a Better Mousetrap

It's often difficult to target pancreatic cancer cells while sparing healthy tissue – but a new therapy concept not only makes this possible, but also enhances the potential effectiveness of adjuvant treatments

“Minimally invasive” would not typically be a term that you would associate with pancreatic cancer treatment, in fact quite the opposite, but one team in Ireland believe they've made a breakthrough.

It's a two-part process. First, tiny, oxygen-filled microbubbles with an inactive chemical agent attached are delivered to the tumor tissues by injection. Second, the sensitized tumor is exposed to low-intensity ultrasound waves, breaking up the bubbles and activating the attached drug. This serves more than one purpose – not only is the drug delivered directly to the tumor without damaging healthy tissue along the way, but the oxygen itself also assists with treatment, improving the function of therapies like radiation that require oxygen to work.

“Because we can control exactly where the sound waves go, we can selectively target the tumor and spare healthy tissue.”

Ulster University's Norbrook Chair of Pharmaceutical Science, John Callan, explained, “Because we can control exactly where the sound waves go, we can selectively target the tumor and spare healthy tissue, making this a highly targeted therapy with reduced side effects,” (1).

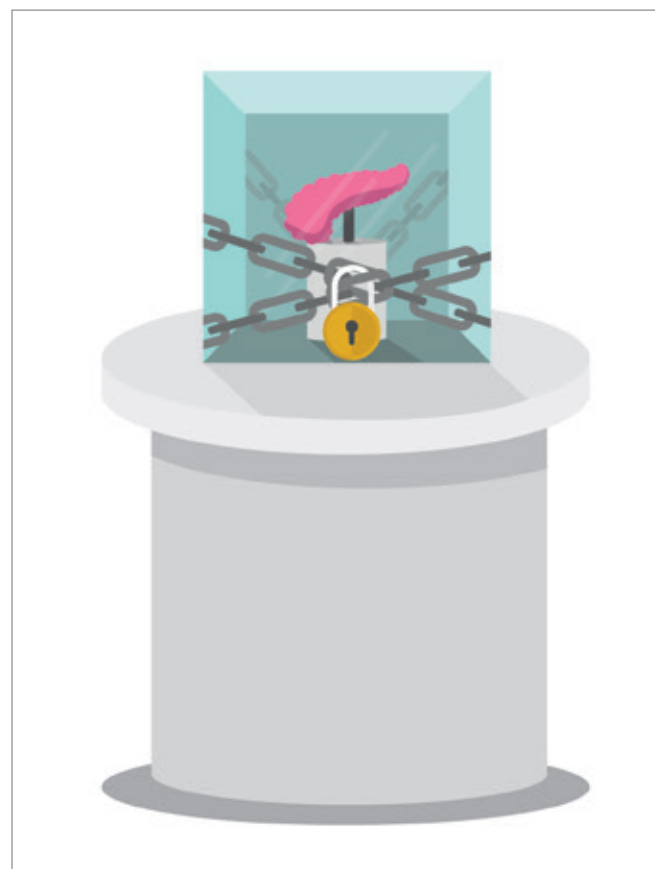
The researchers have named this technique “sonodynamic therapy” (SDT) and are excited by its potential, in particular given that their initial testing has shown a five-fold reduction in tumor size on pancreatic ductal adenocarcinoma (PDAC). SDT is not the first of its kind – similar techniques, like photodynamic therapy, exist – but it has advantages over other established treatments because ultrasound waves are capable of much deeper tissue penetration than light (2). It's a uniquely

beneficial approach for pancreatic cancer because of the disease's characteristic low blood supply and large tumor size at diagnosis; increasing the tumor's oxygen content can make radiotherapy and some chemotherapies more effective, while successful shrinking of the tumor can make surgery an option for more patients.

Ultimately, the researchers hope to make pancreatic cancer a treatable disease, even in patients who have more advanced, or less accessible, tumors.

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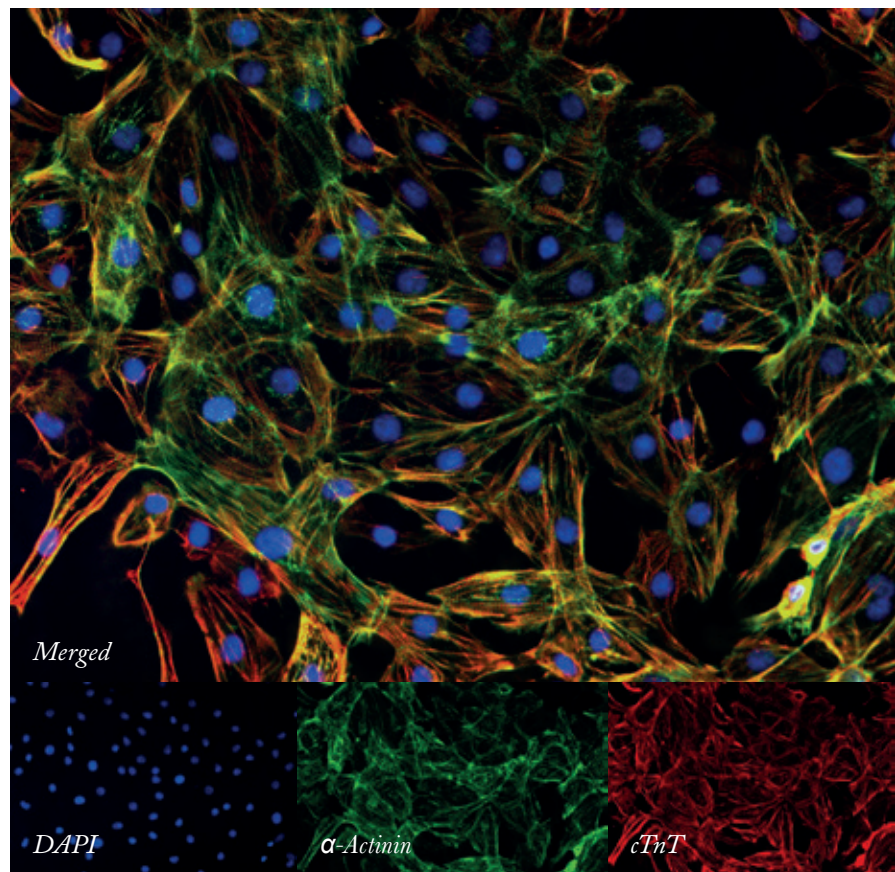
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State-of-the-Heart Tissue Models

A decade after Shinya Yamanaka brought us induced pluripotent stem cells, our ability to generate an endless supply of human heart cells is being put to good use in research and development.

By John Ahrens and Joseph C. Wu

It is estimated that only eight percent of new drugs will successfully transition from clinical trials to market launch. Even when drugs do gain regulatory approval, some are still pulled from the market due to unforeseen complications, with cardiovascular complications often cited as the most common reason for these withdrawals (1, 2). Given our current inability to effectively model how drugs affect the heart, it is not surprising that the decreasing rate of drug development has hit cardiovascular therapies especially hard. Between 2000 and 2009, the number of new cardiovascular drugs approved by the FDA decreased by 33 percent compared with the preceding decade (3). This is particularly concerning given that, according to the American Heart Association, cardiovascular disease is already responsible for one in every four deaths in the United States and by 2030 will kill 24 million people per year worldwide (4). Despite the urgent need for better cardiovascular drugs, the lack of cardiac models capable of accurately reflecting human physiology has led the FDA to place stringent demands



The quality and purity of cardiomyocytes differentiated from induced pluripotent stem cells can be assessed using immunofluorescence staining for cardiac-specific markers.

on cardiovascular trials, thereby further increasing their size, duration, and cost. Thus, there is a strong need for cardiac models with more accurate prognostic capabilities to enhance general drug toxicity screening and novel drug development.

Current models

While it is possible to isolate adult heart cells from patients after heart surgery, the difficulty of obtaining such tissue is further compounded by the fact that human cardiomyocytes rarely divide and do not survive more than a week when grown on a petri dish, severely limiting their application for larger-scale studies. In lieu of primary heart cells, screens for cardiotoxicity and investigations of novel drugs rely on different in vitro and in vivo models. Unfortunately, current models are either overly simplistic or not indicative of human heart function. Two of the most common in vitro models are transgenic Chinese hamster ovary (CHO) cells and human embryonic kidney (HEK293) cells that overexpress human cardiac ion channels (5). While easy to culture and quick to divide, these heterologous cells cannot accurately emulate the complex multichannel relationships that direct the function of a real human beating heart cell. In vivo mouse models are also common and represent a more dynamic environment to study cardiovascular systems. However, mice have vastly different physiology compared to humans. For instance, on average a mouse heart contracts roughly seven times faster than a human heart (6). These deficiencies ultimately mean that many drugs fail in human trials when cardiac safety cannot be adequately established using conventional models; and conversely, some drugs with a significant risk of inducing serious cardiac complications – including seizures and sudden cardiac death – may slip through the safety net (7).

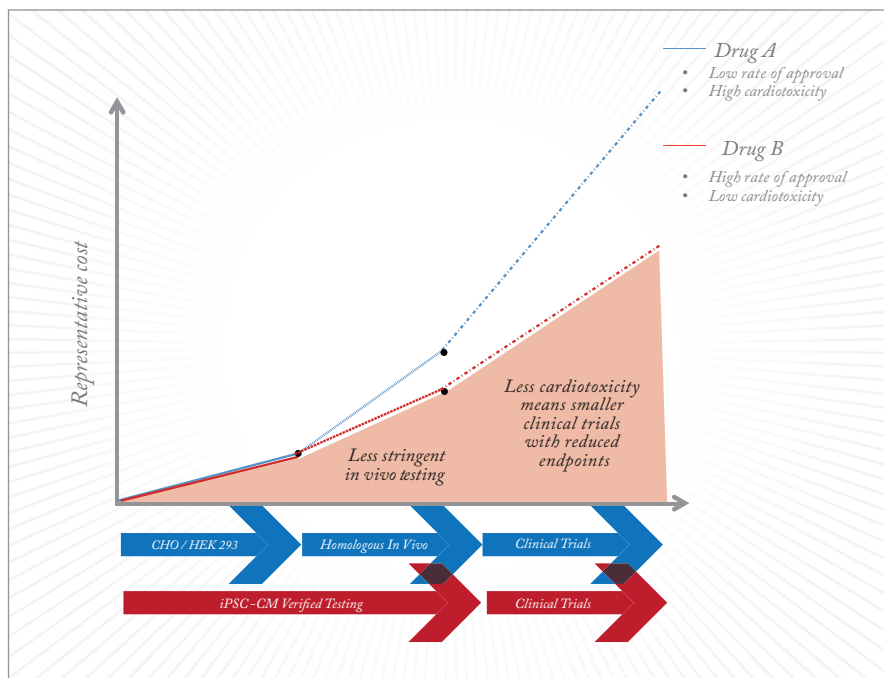


Figure 1. The current drug discovery process is limited by the cardiac models used, which increase costs and restrict development.

A new model for research

The emergence of human induced pluripotent stem cells (hiPSCs) presents the opportunity to redesign the current drug development paradigm – and to avoid some of its problems. hiPSCs were first generated in 2007 by the overexpression of four transcription factors that revert adult differentiated cells to an embryonic stem cell-like state (8). Similar to embryonic stem cells, hiPSCs have the capacity to differentiate into any cell type, and ever-more efficient derivation protocols now provide ready access to a limitless supply of human heart cells (hiPSC-CMs) (9). In addition, hiPSC-CMs retain the genetic mutations of individual donors, a fact that allows researchers to investigate the potential for individualized therapies (7, 10).

Two types of cardiac diseases commonly studied using hiPSC-CMs are cardiomyopathies, which involve the deterioration of heart muscle

contraction, and channelopathies, which involve the repolarization of ion channels. So far, hiPSC-CMs have been used to model several different

“The emergence of human induced pluripotent stem cells presents the opportunity to redesign the current drug development paradigm.”

“Continued development of these automated, scaled systems will further cut costs, streamline analysis, and provide an unbiased platform to screen a wide range of drugs simultaneously.”

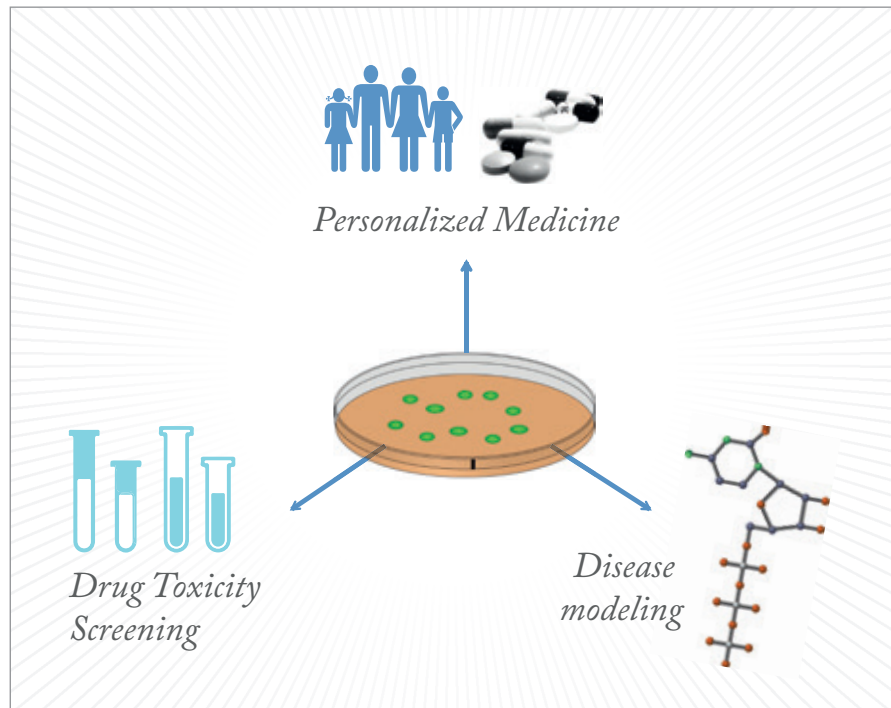


Figure 2. Applications for cardiomyocytes from induced pluripotent stem cells.

cardiomyopathies, such as dilated, hypertrophic, and arrhythmogenic right ventricular cardiomyopathy (11-13). The most commonly studied channelopathies are those that induce long QT syndrome, a prolonged action potential duration that can cause arrhythmias, seizures, and sudden death (10, 14, 15). Preliminary channelopathy studies not only found that disease phenotypes were expressed in hiPSC-CMs, including prolonged action potential and reduced repolarization velocity, but also demonstrated their physiological response to beta blockers, a common class of cardiovascular drugs that slows the heart rate. Further, by correlating how the efficacy of different beta blockers can vary based on patient genetics, studies have demonstrated the potential of hiPSC-CMs for personalized medicine.

However, a major limitation for the use of hiPSC-CMs for drug

discovery and toxicity screening is the relative immaturity of the cells compared with cardiomyocytes in adult primary heart tissue. There are many differences in genetic and phenotypic properties, including cell size, sarcomere organization, calcium handling dynamics, cardiac-specific genetic expression, and others (16). To bridge this maturation gap and improve the predictive ability of hiPSC-CMs, engineered constructs have recently been developed to culture cells in a more natural setting that recreates the physical and topographical cues of the heart. As hiPSC-CMs mature, the relevant structural and functional abnormalities caused by mutations are more easily identified. One identification metric is the alignment and uniformity of cellular sarcomeres. Similar to the coils within a spring, the sarcomeres within cardiomyocytes are the mechanisms of contraction. However,

the hiPSC-CMs cultured in traditional monolayers in plastic petri dishes are often so disorganized that there is little room to distinguish between healthy and diseased cells. To address this issue, researchers at Harvard used micropatterned substrates to culture hiPSC-CMs with a mitochondrial cardiomyopathy, which increased the average sarcomere alignment, improving the discrimination of healthy and diseased cells (17).

Other engineered constructs like collagen-based engineered heart tissue allow us to directly analyze contraction, instead of relying on indirect indicators of contractile force such as calcium handling and sarcomere alignment. Engineered heart tissue is cultured on composite pillars that are stiff enough to apply passive stress but also flexible enough to measure contractile forces by the length of their bending, mimicking the tension within the natural heart. A recent study

used engineered heart tissues to model dilated cardiomyopathy, identifying a mutation in a sarcomere protein that causes a decrease in contractile strength (18). Crucially, this phenotype was not otherwise evident in single-cell assays, highlighting the importance of tissue-scale disease models. In addition, multiple studies have illustrated that effective doses of beta blockers are closer to physiologic levels when administered to 3D cardiac constructs compared to traditional 2D cell culture systems (19). Taken together, these studies showcase how applied tissue engineering is improving cardiac models, supporting the use of hiPSC-CMs as potential clinical diagnostic tools that can reflect in vivo human physiology.

A new model for drug screening
While improved maturation can help increase the accuracy of cardiac disease models, progress is also being made in scaling these constructs for use in drug development – specifically, to enable high-throughput screening for cardiotoxicity. To this end, the field is tailoring in vitro platforms toward more automated processes. For instance, the use of microelectrode arrays allows us to investigate electrophysiology as a functional readout for drug efficacy without killing the cells. Recently, a microelectrode array was used to screen for drug-induced arrhythmias in hiPSC-CMs and to accurately identify cardiotoxic drugs (20). In addition, microfluidic cardiac chips reduce culture costs while enabling the physiological administration of drugs in a continuously circulating flow. These microfluidic chips are often paired with bioprinting, which enables researchers to consistently create spatially organized tissues with microscale resolution. Continued development of these automated, scaled systems will further cut costs, streamline analysis, and provide an unbiased

platform to screen a wide range of drugs simultaneously.

hiPSC technology offers clear potential to improve the drug development process by increasing the meaningful identification of novel targets while streamlining the process of cardiotoxicity screening. The development of more mature, scalable constructs will likely promote the commercial application of hiPSC-CMs to a wider range of diseases. In the future, these hiPSC-CMs may help make drug development less costly and therefore lower the barrier of entry for new cardiovascular therapies.

Joseph C. Wu is Director of the Stanford Cardiovascular Institute, and John Abrens is a Research Assistant at Stanford University, USA.

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Translated

*Celebrating success
Translation in action
Bench-to-bedside*

Unlocking the Potential of Checkpoint Inhibition

Like many drugs today, pembrolizumab (Keytruda) was the result of a group effort. Here, we catch up with two scientists who shaped the destiny of the biotherapeutic at very different stages in its development.

The Human Touch

David Matthews is associate director of biotherapeutics at MRC Technology. We spoke with Matthews to find out how MRC Technology helped the drug's inventors take the first steps towards human trials.

Why is pembrolizumab important? Firstly, it is active in hitherto very difficult-to-treat cancers, in some cases inducing complete remission, which is amazing. Secondly, it was the first successful PD-1 pathway inhibitor and one of the first checkpoint inhibitors. It's a game-changing approach, and has opened our eyes to the possibility of targeting more cancers using similar mechanisms. The pharmaceutical industry is now putting a lot of investment and effort into understanding the pathways involved and hopefully coming up with more checkpoint inhibitors that can target more cancers.

How does PD-1 blockade lead to tumor regression?

There is a whole slew of molecules that are used to keep the immune system in check, to prevent inappropriate immune activation, which can lead

to autoimmune disease. PD-1 is one such molecule. Found on T cells, when engaged it dampens the immune response to prevent immune cells attacking the body's own tissues. Certain types of tumor cells take advantage of this mechanism by expressing a PD-1 ligand (PD-L1). When PD-L1 on a cancer cell binds to PD-1 receptor on a T cell, it prevents the immune system recognizing the tumor as abnormal. Blocking PD-1 binding with the pembrolizumab antibody means that T cells regain the ability to recognize the tumor. Put simply, pembrolizumab unmasks the tumor, so the body's own immune defenses can recognize and destroy it. It's not a panacea – not all cancers express PD L1 – but the hope is that other similar checkpoint inhibitors can be identified and blocked.

What is the overarching aim of MRC Technology?

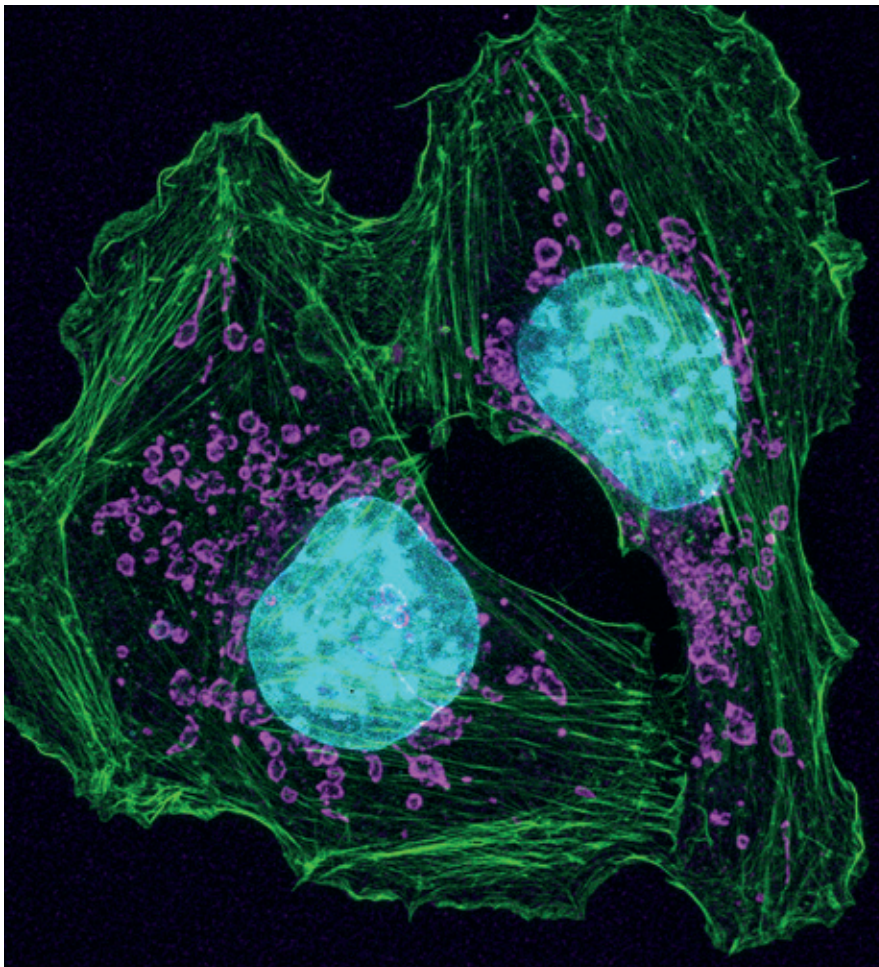
We're a nonprofit organization whose mission is to bridge the gap between academia and industry in developing new therapeutics. We team up with academics and biotechs to take care of the non-research side of drug development; for example, therapeutic antibody development, assay design and

"It's a game-changing approach, and has opened our eyes to the possibility of targeting more cancers using similar mechanisms."

target validation. It isn't the type of work that is very publishable, but it is a vital step in turning an idea into a candidate therapeutic. Essentially, together with a university or research institute we de-risk projects to make them more desirable for pharma to take on and develop into the clinic. MRC Technology develops small molecule and antibody drugs for a wide variety of diseases, but my team is focused on biotherapeutics.

What was your role in developing pembrolizumab?

We helped the original inventors at



Organon create a humanized version of the antibody. Other people had worked on PD-1 antibodies before Organon, but unfortunately they never took them forward, otherwise drugs like pembrolizumab could have been in the clinic a decade ago. Organon had the confidence and determination to take it to the clinic but, as a small company, it lacked the skills and experience to humanize the antibody in-house, so they asked us to take on the job back in 2007. We have been carrying out antibody humanization since the early 1990s so we're one of the most experienced groups out there - this is the fourth therapeutic antibody we have humanized to be approved for treating patients.

What were the challenges?

In this case the mouse and human genes were not a good fit – a fairly common occurrence but it meant we had to do some additional engineering. You can read published protocols on how to humanize antibodies, but they won't tell you how to fix those types of problems. That's where our know-how comes in. Assuming the antibody is potent enough, the next questions we ask focus on whether the antibody looks like a drug. Is it soluble? Does it aggregate? Is it stable at room temperature? We do a panel of biophysical assessments to make sure it has the properties needed for a viable drug and, if it makes the grade, it's ready to go onto the next stage: preclinical development and manufacturing.

At a Glance

International non-proprietary name (INN): Pembrolizumab

Brand name: Keytruda

Previous name: MK-3475, lambrolizumab

Developed by: Organon (acquired by Schering-Plough in 2007)

Marketed by: Merck & Co/MSD (merged with Schering-Plough in 2009)

Drug class: PD-1 inhibitor

Approval status: Approved in USA for advanced melanoma patients already treated with ipilimumab in September 2014 with an expanded indication granted in December 2015 for patients with unresectable or metastatic melanoma, and in October 2015 for treatment of patients with metastatic PD-L1-positive non-small cell lung cancer. In Europe, it has been recommended for the treatment of unresectable or metastatic melanoma since May 2015.

How did you feel when you heard pembrolizumab had been approved? Seeing pembrolizumab on the market and having a truly life-changing impact for some patients made us feel very proud. Our part in the drug development process is a long way before the treatment reaches patients, but creating a drug that might help someone is why we do what we do, on a personal and organizational level. How many people can say that have been directly involved in creating something that saves lives?

Into the Clinic

Eric Rubin, vice president of oncology clinical research at Merck & Co, tells us how the company guided the drug through a trailblazing regulatory approvals process – and shares what’s next for cancer immunotherapies.

When did you get involved in the development of pembrolizumab?

The drug came to us when we merged with Schering-Plough in 2009. I was part of the group tasked with examining the merged pipeline to decide how to prioritize our efforts. One of the compounds we selected at that time was the anti-PD-1 antibody MK3475, now known as pembrolizumab. That was around the beginning of what I would call a renaissance of immunotherapy in oncology, and data from trials of anti-CTLA-4 antibodies suggested that checkpoint inhibitors were promising targets, although there was a fair bit of toxicity associated with that particular target. We decided to start with a relatively small Phase I study of MK3475 about four years ago and it’s been an amazing journey since then. That study has now become a great example of rapid drug development. From the initial small trial, it grew to become a 1200-patient study, which is obviously very unusual for a first-in-human trial. The drug is now approved in patients with advanced melanoma and lung cancer, along with a companion diagnostic. For me, it has been a great example of collaboration between various stakeholders involved in drug development.

Not the typical regulatory path...

No. The typical purpose of a Phase I trial is assessing safety and dose finding. Many of them will have some efficacy component and our original protocol had a small number of patients with

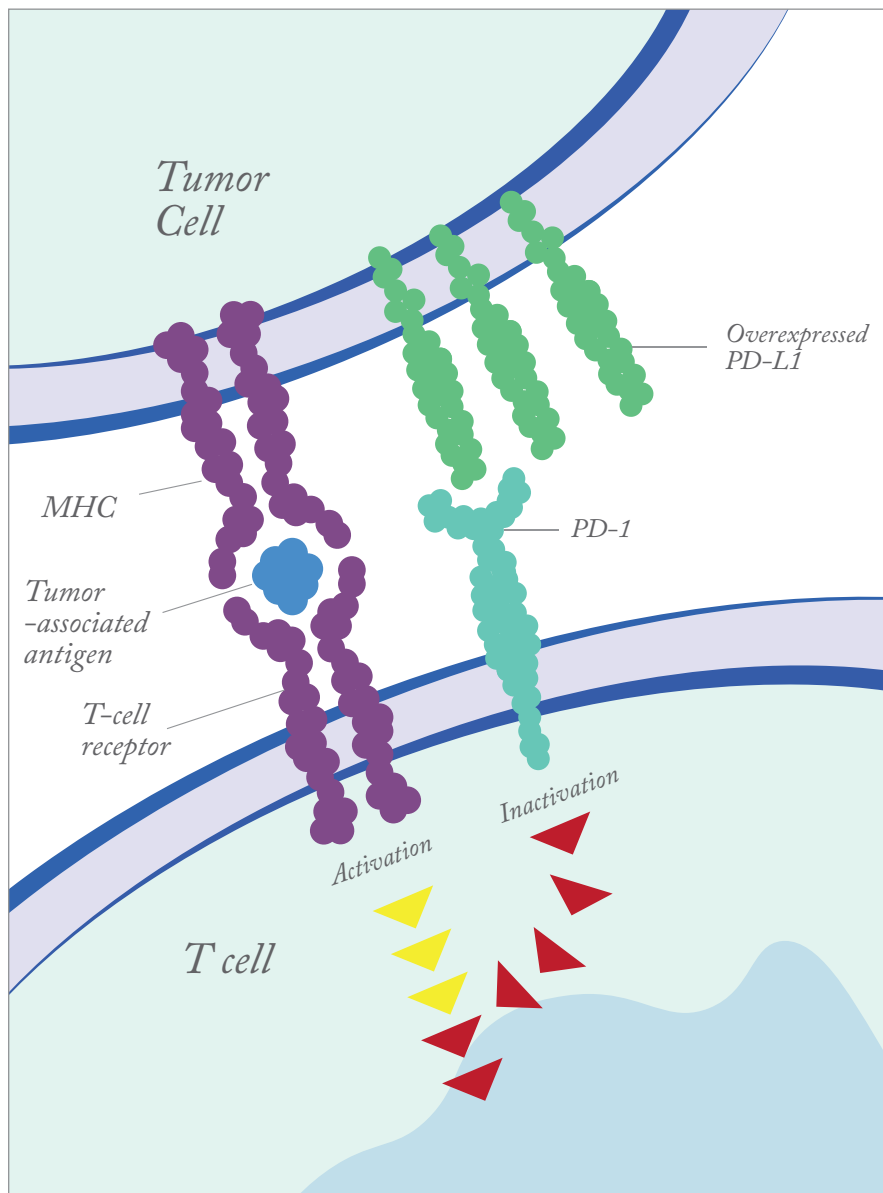


Figure 1. Tumor cells can dampen immune responses by overexpressing PD-L1.

melanoma to try to get a sense of the efficacy. In the traditional approach, you would then go to a Phase II trial and ultimately a randomized Phase III trial. But in this case the dramatic results we saw right from the very beginning led us to seek an accelerated path. Luckily for us, around that time new legislation was enacted in the US to allow the FDA to designate breakthrough status

to groundbreaking new therapies. Pembrolizumab was the first oncology drug to get breakthrough designation, which gave us the flexibility to grow the Phase I study and obtain enough information to demonstrate a positive risk-benefit ratio.

Was it tough being the first to take that path?

“For me, it has been a great example of collaboration between various stakeholders involved in drug development.”

Being first is always challenging and we all had to learn as we went along. But actually, I think there are challenges for drugs awarded breakthrough designation now too. When Congress authorized this designation they did not authorize an increase in FDA staffing to deal with the additional workload. When there were only a handful of drugs in the pathway, it was relatively easy to access FDA officials. Now, there are dozens of drugs with breakthrough designation, so it is much harder for the FDA to live up to that expectation. There are a couple of bills that are working their way through the US government to try to increase resources at the FDA to maintain the efficiency of the accelerated approval process. In addition, I think the FDA is keen to make it clear that this route is intended only for true breakthrough drugs. It's important that people understand that traditional randomized trials will still be required for the vast majority of new therapies. This was a nice demonstration of how it can be done, but it isn't the expected path for every drug.

What's next?

I've been lucky enough to meet people who were facing a guaranteed

death sentence before being treated with pembrolizumab and are now approaching three years with no detectable cancer. It is wonderful to hear their stories, but it's a sad fact that most patients won't have such a good outcome – their cancer will eventually progress despite getting pembrolizumab. We're working very hard to find ways to extend the benefits of the drug.

How do you hope to achieve that?

A very active area in cancer medicine right now is combination therapies. There are so many potential combinations with pembrolizumab, it's hard to decide which ones to study first. We have nearly 80 clinical trials ongoing or planned with combinations of pembrolizumab and a variety of other drugs, including: other immune modulators, such as antagonist/agonist checkpoint inhibitors and vaccines; standard therapies, such as chemotherapy and radiation; and targeted therapies, such as kinase inhibitors. All look promising for some patient groups based on translational and preclinical work, and we're excited about the possibilities.

Of course, you need to know which drug (or combination of drugs) to give to which patients. We already have a companion diagnostic on the market to help select lung cancer patients most likely to respond to pembrolizumab. Developed in collaboration with diagnostics company Dako, the test looks for the presence of PD-L1, one of the ligands for PD-1. Lung cancer patients with tumors expressing high levels of this protein have a response rate to pembrolizumab of 40 to 50 percent, compared with below 10 percent in patients with low expression. We'll be conducting further studies of both PD-L1 and PD-L2 to find other ways to identify patients most likely to respond.

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Asking the Ultimate Question

In today's highly competitive academic world, are we losing sight of the real goal of biomedical research in our rush to secure funding and embrace the latest technologies?

By Michael N. Liebman

Over the last 30 years, I've watched biomedical research rapidly embrace new technologies aimed at developing better drugs and improving patient care and outcome. This evolution extends from molecular modeling to bioinformatics, translational medicine, and now the conversion of personalized medicine into precision medicine and its enhancement with big data. Although these approaches typically develop from academic research, they have all migrated to commercial activities (and investment opportunities), while promising to improve healthcare.

In many cases, approaches have evolved from breakthrough science to commoditization and integration into standard research practice. For example, molecular modeling progressed from computational/quantum evaluation of chemical properties to visualization/graphics and molecular dynamics. Now, no drug is developed that does not use some form of this analysis. Bioinformatics evolved from protein structure–function analysis to sequence analysis of proteins, nucleic acids and genomes. Molecular biologists now routinely apply complex

algorithms developed in advanced research in disparate areas.

Today, precision medicine is replacing personalized medicine. I believe this reflects a focus on selection among existing medicines, rather than the development of drugs that only work for an individual. The more limited definition focuses on genomic data while the broader view includes clinical history, lifestyle, and environment.

Big data integrates results from many different approaches along with clinical data. The term indicates “more data than can be adequately managed with available algorithms, storage and visualization technologies.” But these boundaries continually evolve so today's “big” data, is tomorrow's “typical” data. Extensive data mining efforts are applied to identify new correlative relationships. The associated technologies that support these progressions range from high-performance computing and “the cloud”, to array technologies and next-generation sequencing.

So, we're seeing a boom time of great advances. However, good basic research still does not routinely lead to actual clinical utility. The difficulty typically lies not in the “handoff”, but rather an

inability to recognize the difference between “unmet clinical need” and “unstated, unmet clinical need.”

“Unmet clinical need” implies underserved diseases, such as Alzheimer's disease or ALS, where clinical needs are not adequately met for lack of the right tools or approach.

Unstated, unmet clinical need describes a gap in knowledge or adequacy of existing processes and procedures in clinical practice; for example, diagnosis and disease stratification, or understanding the complexity across the patient, physician, provider, payer, pharma, regulator, family/caretaker and community interface. An example of this is the difficulty in treating heart failure patients with “preserved ejection fraction”. The diagnosis itself involves dealing with a complex syndrome and subjective evaluation of the patient. Even the determination of a specific threshold for “preserved ejection fraction” remains difficult to support based on observational data. As a result, seemingly definitive criteria for diagnosis can yield an extremely heterogeneous population for evaluation of new therapeutics and result in limited success.

- i) Disease stratification. Most diagnoses represent syndromes or complex disorders that need resolution into clinical subtypes. The Institute of Medicine estimates that 10 percent of patients are misdiagnosed, but this significantly underestimates the impact of not using disease stratification based on clinical presentation to improve patient care and outcome.
- ii) Co-morbidities and polypharmacy. Virtually all patients come to a physician with a history of previous disease, current disease or additional undiagnosed disease. Patients are often taking multiple prescription medications and over-the-counter remedies that will impact diagnosis and response to treatment.
- iii) Clinical trials do not enroll real-world patients. Clinical trials rarely deal with the complexities of either the disease (for example, stratification) or the patient (for example, co-morbidities).
- iv) Comparative effectiveness. If the physician doesn't prescribe the drug according to guidelines or the patient does not take the drug as prescribed, any drug can be rendered ineffective, so simple comparison of efficacy between drugs in a clinical trial is not adequate to predict effectiveness in real-world medicine.
- v) Disease is a process. In disease, biological processes may change over time, which can be monitored using clinical observations to define the "dimensions" of the disease. The direction of this vector defines the disease subtype. How far along the vector a patient
- is defines their "stage", and how quickly they progress along the vector defines their velocity. In chronic diseases such as diabetes, the patient's underlying biology is also in a state of change and this can impact the presentation of disease. These can (and should) be addressed mathematically to enhance potential diagnosis and treatment.
- vi) Biomarkers are not diagnostics. Biomarkers are measurable indicators of the status of underlying biological processes. Diagnostics are indicators of the presence of disease or stage of disease progression. These are not necessarily the same. Although diagnostics are used to indicate disease state or stage, they are not typically based on understanding the disease etiology, but instead are accessible markers for measurement.
- vii) Clinical guidelines. Guidelines are typically developed using a consensus method or involving evidence-based methods; for example, randomized clinical trials. In a consensus guideline, potential variability in the confidence associated with each step is not presented in a transparent manner that would enhance clinical decision-making. In evidence-based guidelines, the use of varying inclusion/exclusion criteria and lack of comparison to real-world patients, as noted above, limits generalized use. In each instance, greater transparency could enhance the utility of guidelines in common practice.
- viii) Electronic health records. Current efforts focus on achieving interoperability while maintaining

privacy. Unfortunately, little effort focuses on what data should be included in the electronic health record to make it useful. Learning from the experiences of countries where nationalized healthcare systems already have universal electronic health records could greatly benefit compliance and utilization for new efforts in this area.

The reality is that most physicians, when faced with a patient across the desk, cannot take the time to wait for solutions to these issues, and typically may not even acknowledge them on a daily basis. But in the application of design thinking, these issues become the focal point for research and action. Big data can provide the mechanisms to identify and collect the data critical to address these problems. Translational research/medicine can focus on developing solutions or partial solutions, and precision medicine can provide the mechanism for delivering the results to the patient. But while all of these techniques will help us find answers, it is asking the right questions that will deliver real benefits to patients.

Michael N. Liebman is Managing Director, IPQ Analytics, LLC (USA) and Adjunct Professor of Pharmacology and Physiology, Drexel College of Medicine, USA.



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CENTRAL ORGANIZATION

Prof. Luigi Mondello
Chromaleont S.r.l.
Phone (+39)-334-3612788 - Fax (+39)-090-9080155
e-mail: iscc@chromaleont.it
website: www.chromaleont.it/iscc

LOCAL ORGANIZATION

Elisa Giglio
Margherita Barilà
Chromaleont S.r.l.
Phone (+39)-334-3612788
Fax (+39)-090-9080155
e-mail: iscc@chromaleont.it

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A close-up portrait of Julian Solway MD, a middle-aged man with grey hair and glasses, wearing a dark suit, white shirt, and patterned tie. He is smiling slightly. The background is dark with several yellow gear icons of varying sizes and orientations.

Engineering Better Health

Sitting Down With... Julian Solway MD,
Walter L. Palmer Distinguished Service
Professor - Medicine and Pediatrics,
Director - Institute for Translational Medicine,
Dean for Translational Medicine, University of
Chicago, USA.

What made you decide to go into medicine?

Originally, I went to MIT to study electrical engineering and was fascinated by the then-new field of biomedical engineering. It became obvious to me that training as a physician would allow me a more insightful perspective for research and development, and of course studying medicine was attractive in its own right.

I entered medical school in the Harvard–MIT joint program in Health Sciences and Technology. There was a very strong focus on engineering and physical science principles in medicine, with many engineers on the faculty. It was a wonderful multi-disciplinary approach to learning about disease.

Once I started to see patients, I discovered another great passion – clinical medicine. I enjoy meeting, getting to know, and helping people. So rather than pursuing a PhD in electrical engineering, I became a medical resident, and then a fellow at the Brigham and Women’s Hospital, where my clinical focus was on pulmonary and critical care medicine. However, I wanted to move closer to my family – including my dear twin sister – in Chicago. A wonderful opportunity came up at the University of Chicago in 1985, and I have been here ever since. It’s a fantastic environment and a wonderful place to make one’s career.

Do you still see patients?

I do – although I only see inpatients these days; I wish I had time to see outpatients too!

What’s the main focus of your research work?

My laboratory uses a wide range of tools to explore the biology of airway muscle in asthma. The airway is encircled by smooth muscle. During an acute asthma attack, that muscle tightens like a boa

constrictor, and we’re trying to find ways to prevent that from happening. We have studied airway smooth muscle gene expression, cell biology, and contractile function, and how all of this influences tissue behavior within the airway wall. It’s not all in vitro work – we’re involved in genetics studies (in collaboration with superb geneticists, like Carole Ober) and in clinical trials and mechanistic bronchoscopy studies. We also participate in comparative effectiveness studies through the Chicago Area Patient Centered Outcomes Research Network (CAPriCORN). I’m a previous co-chair and a present steering committee member of the NIH/National Center for Advancing Translational Science’s Clinical and Translational Science Award (CTSA) consortium, so I’ve had an opportunity to observe and perhaps influence the wider direction of translational and clinical research.

What does translational science mean to you?

Since former NIH director Elias Zerhouni initiated the forerunner of what would become the CTSA program, I became aware of the term becoming more widely used. Of course, people have long been working to advance knowledge to improve health, which is the broader definition of translation. It’s important to remember that the direction of knowledge doesn’t only flow from basic to applied research, it’s also gaining insights from the clinic and the community, then testing them in a research environment – a two-way process.

Was the University of Chicago Institute for Translational Medicine (ITM) initiated in response to the CTSA program?

I would say that we formalized ourselves in response to the CTSA program. Like

other institutions that have applied for and received CTSA grants, we already had many interests and activities that fell within the domain of translational research. What the CTSA grant allowed us to do was to dramatically improve these programs.

What are the goals of the Institute?

David Meltzer, section chief of hospital medicine here at the University of Chicago, coined a wonderful phrase, which I have adopted as a great summary of what we do: we assemble, integrate, and create. We assemble the resources we already have in terms of ongoing activities, support, interest and expertise. We integrate them so that they work better together, to reduce redundancies and improve efficiency. And where we find we have a gap in our capabilities, we create new resources to fill the gap.

The ITM has become an organizing center and a cheerleader of sorts for translational medicine, pointing out the wonderful opportunities that come from bringing different fields of expertise together. Part of the brilliance of Zerhouni’s original concept for the CTSA program was that it would bring together knowledge on a variety of areas, including regulation, informatics, community engagement, clinical research centers, population scientists, preclinical studies, ethics, and education.

In short, we support great ideas and provide the resources to make them a reality.

What’s the proudest moment of your career?

I believe it has yet to happen. Naturally, I’m grateful for, and happy with, the successes I’ve had in my research, but discovering a new way to improve the lives of asthmatics would make me most proud. After all, that’s the ultimate goal for any translational scientist.



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