the Translational Scientist

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Upfront

"May I Enjoy Life and Art"

... so states the Hippocratic Oath – and now research shows that art training can improve ophthalmologists' observational skills

September 2017

"Observation is a pivotal skill in medicine, especially in the field of ophthalmology, but medical education does not focus explicitly on teaching students how to observe," says Gil Binenbaum, attending surgeon in the Division of Ophthalmology at Children's Hospital of Philadelphia, Pennsylvania, USA. To explore how observation skills training might improve the medical – and more specifically the ophthalmological – observational skills of students, Binenbaum and a multi-center team performed a randomized controlled study. "We looked to the fine arts, a field that excels in its observation training," says Binenbaum.

Thirty-six first-year medical students were randomized into either art training or control groups. The 18 students in the training groups attended six art observation sessions at the Philadelphia Museum of Art over a three-month period; the control group was given free membership to the museum. Pre- and post-training, the students' observation skills were assessed by description testing (where they described works of art, retinal pathology images as well as external photographs of eye diseases) and emotion recognition testing.

Did the art training improve their observation skills? "Yes, it did!" says Binenbaum. The training group showed significantly improved observational skills compared with the control group ($p<0.001$). Furthermore, the training group scored significantly higher scores in all subsets of the description testing. "We believed that learning observational skills related to fine arts would allow students to hone general observational skills, but we were surprised by the extent to which they translated to medicine," says Binenbaum, adding that the results "highlight the art of medicine and encourage us to think outside the box when it comes to bridging across disciplines to improve medical training."

And does this team practice what they preach? Senior medical student and coordinator of the study, Jaclyn Gurwin, has herself participated in the art training in its first iteration and is now an ophthalmology resident. She commented: "It was incredible to see the training at work and how the medical students participated and expressed themselves in a way that I was not used to seeing in the medical school classroom. It seemed as though the students felt more of a freedom to share their own ideas and opinions, and they quickly built upon techniques they were learning."

Binenbaum hopes that their findings will help encourage medical schools and graduate medical education programs to recognize the importance of teaching observational techniques, and perhaps even incorporate this type of art observation training into their curricula. "As a result of our study, The Perelman School of Medicine at the University of Pennsylvania has already created two medical student elective courses in art observation training," says Binenbaum. Next, the team are planning to study the effect of art training on physician empathy, and have begun to pilot art observation training for post-graduate trainees. Binenbaum comments: "We hope that improved observational abilities from this training will translate to improved clinical effectiveness and empathy, and ultimately, make better physicians." It seems the medical schools of the future may place a little more emphasis on the finer points of the Hippocratic Oath.

Reference

1. *J Gurwin et al., "A randomized controlled study of art observation training to improve medical student ophthalmology skills", Ophthalmol, [Epub ahead of print], (2017). PMID: 28781219.*

A Breath of Fresh Air?

An epic Norwegian study confirms a new treatment direction for Parkinson's disease

September 2017

An epidemiological study of the entire population of Norway – some 4 million people – has found that the asthma medicine salbutamol may reduce the risk of developing Parkinson's disease (PD) by as much as 50 percent. Using data on 100 million prescriptions registered since 2004, the study investigated the effects of asthma and hypertension drugs on PD risk.

A classic symptom of PD is the accumulation of Lewy bodies – abnormal aggregates of alpha-synuclein. The study authors previously theorized that rather than try to clear the aggregate, or prevent the protein from forming Lewy bodies, they might be more successful in going further back in the process, and regulating the gene that codes for alpha-synuclein in the first place: SNCA. To do this, they screened 1,126 compounds – from drugs to supplements and vitamins – to see if they affected expression of alpha-synuclein. Three "hits" were found in beta-2 adrenergic receptor (β2AR) agonists that act as bronchodilators; the three drugs (metaproterenol, clenbuterol, and salbutamol) were then prioritized for further research. They also studied propranolol, a β2AR antagonist commonly used to treat hypertension.

In vivo and animal studies confirmed that the drugs affected the risk of PD, as did the aforementioned follow-up of the Norwegian population; interestingly, though salbutamol halved the risk of a person developing PD, pronanolol appeared to increase the risk (1). The team are hopeful that their findings will lead to new potential therapies, with the leader of the Norwegian prescription registry study, Trond Riise, commenting, "Our discoveries may be the start of a totally new possible treatment for this serious disease. We expect that clinical studies will follow these discoveries." (2)

References

1. *S Mittal et al., "β2-Adrenoreceptor is a regulator of the α-synuclein gene driving risk of Parkinson's disease", Science, 357, 891–898 (2017). PMID: 28860381.*
2. *University of Bergen, "Asthma medicine halves risk of Parkinson's", (2017). Available at: bit.ly/2eI6chI. Last accessed September 5, 2017.*

The Game Is Up

Thin layer chromatography and SERS track down Viagra in adulterated healthcare products

September 2017

What?

Drug counterfeiters, beware. A new method has allowed scientists from China to analyze for adulteration in widely available health supplements – detecting small amounts of Viagra, as well as other phosphodiesterase type 5 enzyme (PDE-5) inhibitors.

Why?

Adulterated medication and supplements can be extremely dangerous to human health. "Natural" aphrodisiacs are frequently adulterated with pharmaceutical drugs such as PDE-5 inhibitors. Drugs like Viagra can already cause side effects such as dizziness and a runny nose – not exactly conducive to an amorous encounter – but, more seriously, unmeasured or unapproved doses (which are impossible to judge in cases of adulteration) can cause cardiovascular problems and are dangerous for those with heart disease.

Current techniques?

Common methods used for this type of analysis include HPLC-DAD (diode array detection), nuclear magnetic resonance spectroscopy, LC-MS and GC-MS – each of which, while effective, require the skills of highly-trained technical staff and can be time- and resource-intensive. The team from Tianjin University of

Science and Technology, and Beijing Technology & Business University, both China, felt a more rapid solution was needed.

How?

The researchers spiked supplements with six PDE-5 inhibitors: sildenafil, hydroxyhomosildenafil, thioaildenafil, acetildenafil, vardenafil dihydrochloride salt and pseudo vardenafil before attempting detection using a combination of thin-layer chromatography (TLC), surface-enhanced Raman spectroscopy (SERS) and a BP neural network.

Findings?

Using this technique, a limit of detection of less than 5mg/kg was obtained.

So what?

Its ability to cheaply and quickly achieve this level of sensitivity means TLC-SERS has scope in other areas vulnerable to adulteration, such as cosmetics, agriculture and food.

Reference

1. *N Sukenik et al., "Rapid detection of six phosphodiesterase type 5 enzyme inhibitorsin health care products using thin-layer chromatography and surface enhanced Raman spectroscopy combined with BP neural network", PLOS 12 (2017).*

Built for Speed

Urinary tract infections account for a large portion of antibiotic prescriptions – but could a new point-of-care test help doctors prescribe more selectively?

September 2017

The threat of antibiotic resistance needs no introduction, and yet antibiotics are still being incorrectly prescribed around the globe. New and improved approaches to diagnosing bacterial infection can help, and one team from the University of Uppsala, Sweden, have developed a rapid point-of-care (POC) test to determine the susceptibility of bacteria to antibiotics in urinary tract infections (UTIs). We spoke to Johan Elf, Professor of Physical Biology and Chair of Molecular Systems Biology, Uppsala University, to find out more…

How did you come to focus on a POC test for antibiotic susceptibility?

My lab was working on the basic science of cell-to-cell variation and we had developed very sensitive tools to measure growth rate at the single molecule level. When we started looking at-cell to-cell variation in antibiotic response to understand the origins of bacterial resistance, we realized that we could tell if the bacteria responded to the antibiotic in just a few minutes. The next step – envisioning a POC test for antibiotic susceptibility – was a small one.

And why focus on UTIs, in particular?

A hundred million women suffer from UTIs every year, and this accounts for a very large fraction of antibiotic use. At the same time, there is wide spread antibiotic

resistance. Doctors stop using the first line antibiotics when the local resistance is higher than 20 percent. But they could still be used in 80 percent of cases if we could only determine the antibiotic resistance profile, before prescribing the drug. It would allow us to both extend the lifetime of the existing antibiotics, and at the same time ensure we are always prescribing an effective antibiotic for that particular patient. It would also allow us to identify patients who don't have a bacterial infection at all.

How does the fast antibiotic susceptibility test (fASTest) work?

It's based on a microfluidic chip with structures small enough to allow us to selectively capture one bacteria in each of the 4,000 channels. Some of the channels are exposed to the test antibiotic, and we monitor the growth rate response by direct single cell imaging (see Figures 1 and 2). As we can detect the volume extension of individual cells and average over a few hundred cells, the average growth rates can be determined in just a few minutes (1). The principle is very similar to a standard plating assay, but miniaturized, which makes it much faster, as we do not need to wait for the bacteria to multiply.

What equipment and training will be necessary to administer the test?

For use in primary care, I expect that the test will have to be very simple and automatic. Ideally it should involve simply opening the lid of a shoebox-sized device, and placing a urine sample and a plastic consumable inside. A 10 minute wait and you'll have a result of a bacterial count, and within a maximum of another 20 minutes you should have an antibiotic susceptibility response for a few relevant antibiotics. As we couldn't achieve this next stage in a

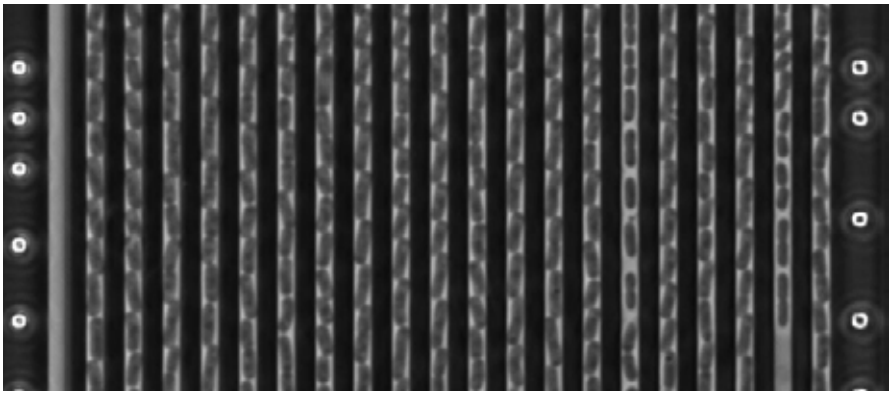


Figure 1. Klebsiella pneumoniae growing in the microfluidic chip imaged in phase contrast. The bacteria are 0.003mm long and divide every 30 min. Credit: Özden Baltekin

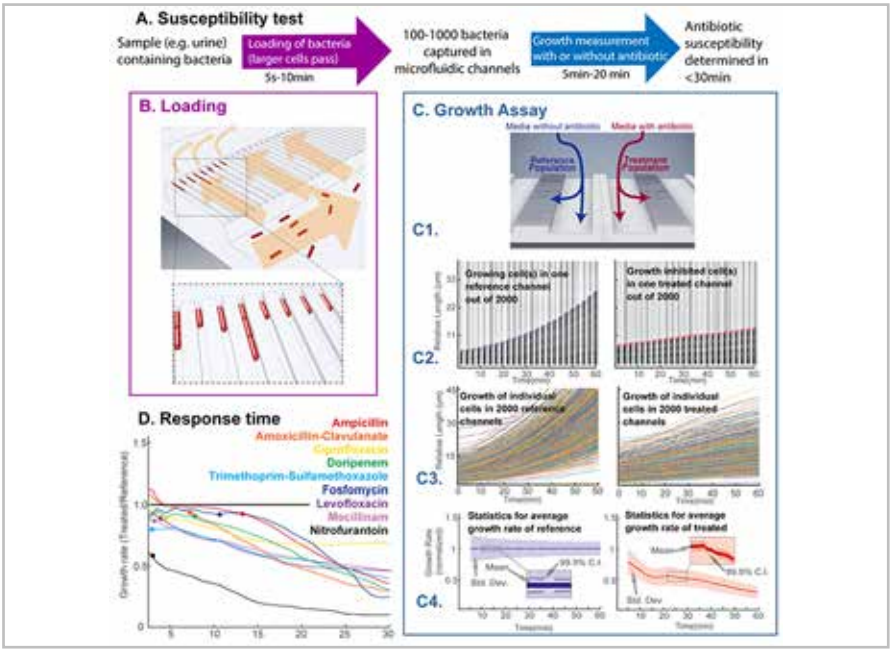


Figure 2. A. Overall workflow for the fASTest test B. Individual cells are sucked into the cell channels where they get stuck at the 300 nm constriction at the end (inset) C1. One row of 2000 cell channels are treated with an antibiotic and the other row is used as a reference. C2. Growth in one individual cell channel without antibiotic (left) and one with antibiotic (right) monitored over time (x-axis) as observed with phase contrast microscopy. C3. Length extension over time as determined for cells in 1600 individual cell channels without antibiotic (left) and with antibiotic (right). C4 Average growth rates for the bacteria in C3 together with 99.9% SEM and population standard deviation D. The average growth rate and 99.9% SEM for susceptible bacteria exposed to one of nine different antibiotics (colors), normalized to the growth rate in the non-treated reference channels. Only data from one typical references channel is displayed (gray). Dots indicate when the growth rate has dropped below untreated reference with 99.9% probability. Credit: Johan Elf

University setting, a company in Uppsala has taken over the development.

Could fASTest be adapted for use in other types of infection?

Sepsis obviously comes to mind because of the sensitivity and speed, as we only need a few hundred bacteria. But other diseases, such as meningitis or mastitis, could also be considered.

How important is POC testing in trying to curb resistance?

It definitely has its role to play. We need to stop using antibiotics when there is no bacterial infection, and we need to save broad spectrum antibiotics and new drugs for when they are truly needed. However, right now, there are no actual POC susceptibility tests and doctors have to base the first treatment on statistics alone. Using POC susceptibility testing, we can keep using old antibiotics in the cases they are effective, even if the average resistance is very high.

When will fASTest likely hit the clinic?

The method needs to be made user-friendly, and the consumable chip and reader device need to be produced at a large scale to become inexpensive. This task is now in the hands of the start-up company – Astrego Diagnostics. If they work with a bigger company for production and to reach the clinics, I would hope that it could be done in about three years.

What's next for your laboratory?

We will continue with our fundamental science projects related to intracellular biophysics and methods development for single molecule tracking in live cells. We will also do some work on the molecular mechanisms for cell-to-cell variation in antibiotic response, which underlies the development of resistance.

fASTest is a great example of the importance of basic research. If we had not pushed the measurement technology to answer our basic science questions, we would not have the microfluidics and image analysis tools we needed to create this test.

Reference

1. *Ö Baltekin et al., "Antibiotic susceptibility testing in less than 30 min using direct single-cell imaging", Proc Natl Acad Sci, 114, 9170–9175 (2017). PMID: 28790187.*

Spinal Tap

Metal speciation in cerebrospinal fluid may bring new understanding of neurodegenerative diseases

September 2017

Debilitating and often incurable, neurodegenerative diseases could affect over 12 million Americans by 2030 (1). Finding treatments – or, even better, cures – for these conditions is a high priority. But first, we need to understand them.

High levels of metal ions in the cerebrospinal fluid (CSF) are currently thought to play a key role in protein misfolding – a hallmark of neurogenerative disorders, so a multinational team of researchers developed a method for simultaneous redox speciation of iron (II/III), manganese (II/III), and copper (I/II). Based on strong cation exchange chromatography and inductively coupled plasma sector field mass spectrometry (ICP-sf-MS), the new method was optimized and tested using real CSF samples taken from amyotrophic lateral sclerosis (ALS) patients and neurologically healthy controls (2).

"The underlying hypothesis of our studies is that, unlike cycling body fluids (for example, blood or serum) or excretory media, the CSF is in direct contact with the brain parenchyma and brain extracellular fluid," says Nikolay Solovyev from St. Petersburg State University. "So, slight changes of trace element speciation caused by exposure or redox dis-homeostasis related to neurological pathology would be more clearly reflected in the CSF than in other matrices." Less cerebrally put: higher levels of the primary species of interest detected in CSF could act as 'red flags' for various neurodegenerative diseases (3).

Next, Solovyev and the team plan to complement their metallomics studies on ALS with non-specific metabolomics research to see how metal species interact with metabolites in the CSF with the ultimate aim of discovering candidate biomarkers.

Solovyev and the team want to apply analytical lessons learned in other disease areas, and will soon begin an investigation into copper speciation in Wilson's disease as part of a biomarker research project alongside new partners from Guildford, UK: "Here, we would like to improve the current approaches for ceruloplasmin determination using hyphenated techniques – and implement this into clinical chemistry. I would like to thank my colleagues from Germany, Italy and the UK for our collaborations."

References

1. *Harvard NeuroDiscovery Center, "The challenge of neurodegenerative diseases". Available at: bit.ly/2soDGmD. Accessed July 7, 2017.*
2. *N Solovyev et al., "Redox speciation of iron, manganese, and copper in cerebrospinal fluid by strong cation exchange chromatography–sector field inductively coupled plasma mass spectrometry", Anal Chim Acta, 973, 25–33 (2017).*
3. *B Michalke et al., "The importance of speciation analysis in neurodegeneration research", TrAC Trends Anal Chem, [Epub ahead of print] (2017).*

The Golden Touch

Researchers move a step closer to improving the effectiveness of cancer drugs by "manufacturing" therapeutic compounds in vivo using gold nanoparticle catalysts

September 2017

For decades, scientists have been trying to figure out ways of reducing the toxic side effects of chemotherapy drugs. But what if patients could receive inactive chemical precursors along with a catalyst to produce therapeutic compounds at the site of the tumor?

The trouble is finding the right catalyst. According to researchers from the University of Edinburgh in Scotland, gold nanoparticles are a good prospect: they work at or even below room temperature, are recyclable, and harmless to human beings. Their application in biological systems, however, is hampered by their affinity for thiols – sulphur analogues of alcohols. The near covalent bond formed between gold and sulphur leads to the spontaneous self-assembly of monolayers at the surface of the catalyst, masking its catalytic properties.

Asier Unciti-Broceta, Reader in Innovative Therapeutics at Edinburgh, and co-author of a recent study (1), have been able to protect gold nanoparticles from thiols within a polymeric device – a PEG-grafted low-crosslinked polystyrene matrix – allowing gold to work as a catalyst even in the presence of serum proteins (which are rich in thiol groups).

"We have demonstrated the potential of our therapeutic device by manufacturing chemotherapy drugs in the presence of cancer cells," says Unciti-Broceta. The nanoparticles have also been tested in a living system, with Unciti-Brocera and his co-authors demonstrating the locally-controlled release of a florescent dye in the brain of a zebrafish. "This opens up new avenues both in therapy and biomedicine, as we can now release drugs, probes or biomolecules in specific locations within the most complex and sensitive organ with spatiotemporal control," says Unciti-Broceta.

The researchers are now working with neurosurgeons and urological surgeons to use gold implants in cancer treatment. "We are currently investigating a two-component strategy consisting of surgical implantation of gold devices inside locally-advanced cancers; for example, brain tumors, and then giving inactive starting materials that will be converted into active anti-cancer drugs after reacting with the gold inside the tumor," he explains. "The chemotherapy drugs will be 'catalytically' generated just within the tumor, so the side effects of the chemotherapy in healthy organs will be minimal, and the treatment will last as long as the patient keeps taking the drug precursors."

Reference

1. *AM Perez-Lopez et al., "Gold-triggered uncaging chemistry in living systems", Angew Chem Int Ed Engl [Epub ahead of print] (2017). PMID: 28699691.*

Writing Off Cancer

When it comes to identifying cancerous tissue, is the "MasSpec Pen" mightier than the sword?

September 2017

Meet the MasSpec Pen, a handheld mass spectrometry device with the potential to speed up accurate and intraoperative diagnosis of cancer. The pen – which releases a single water droplet onto suspected cancer tissue before drawing it back up for chemical analysis – was able to predict cancer with high sensitivity (96.4 percent), specificity (96.2 percent), and an overall accuracy of 96.3 percent (1).

Finding and removing the edges of cancerous tissue by sight alone is a particular challenge for surgeons, and successful resection of all the cancerous tissue clearly has huge health implications for the patient. The resulting demand for precise, accurate and rapid detection has already inspired one similar device: the electrosurgical iKnife, which uses rapid evaporative ionization mass spectrometry (see tas.txp.to/0215/PRECISIONMEDICINE). Both approaches use mass spectrometry, but the MasSpec Pen has one major difference: unlike the iKnife, which burns the target tissue and uses the smoke for analysis, it doesn't destroy tissue as it analyzes it.

The MasSpec Pen was conceived by Livia Schiavinato Eberlin, Assistant Professor, Department of Chemistry, University of Texas at Austin – but for this small, yet seemingly mighty technology, it is only the beginning.

"We are going to further validate the technology in my lab with larger sample sets and expand to other cancer types – then we'll start testing in surgeries with our colleagues in the Texas Medical Center to compare our results with current results from clinical practice," says Eberlin. "Then we should expand to larger clinical trials to properly evaluate if the technology can improve surgical treatment and patient care." Eberlin and team hope to be able to trial it during operations within the next 12 months.

Eberlin says it is very rewarding to work on a project with such high potential impact. "Since working with R. Graham Cooks during my PhD, the last 10 years of my career have been dedicated to

translational and clinical research, and I am excited about the recent development of the MasSpec Pen," she says. "I am very passionate about the field, and specifically about developing mass spectrometry technology that can make a real difference in clinical practice. My amazing research team and I have been working extremely hard on this project. It is amazing to see what they have accomplished so quickly!"

Reference

1. *J Zhang et al., "Nondestructive tissue analysis for ex vivo and in vivo cancer diagnosis using a handheld mass spectrometry system", Sci Transl Med, 9, eaan3968 (2017).*

TRI a New Kind of Spectrometer

An inexpensive, smartphone-based device could offer a wide range of point-of-care tests

September 2017

What

Everyone's vision of the "laboratory of the future" is different – but most agree that it should be reliable, versatile and efficient. And if those attributes don't come with huge costs or space requirements – even better. Enter the US$550 spectral transmission-reflectance-intensity (TRI)-Analyzer (1), which can perform an array of tests on the spot by harnessing clever optics and the power of a smartphone. But is it the future?

"Several years ago, we completed an early demonstration of a smartphone as a spectrometer. The spectrometer itself was handheld, but to interface with any sort of meaningful biological sample, it needed to be attached to some benchtop optics. The next step was to produce a truly handheld device with everything inside, including the light source and sample interface." To that end, the research team condensed three general optical techniques – transmission, reflection and intensity, each of which uses a different optical path – into a compact package to minimize size and cost. Best of all, the system wasn't designed for a specific test. "So many recent advances in the point-of-care testing realm focus on miniaturizing a test for a single condition. The TRI-Analyzer is a handheld instrument capable of measuring thousands of commercial tests."

How

The TRI-Analyzer was developed from the ground up. "We wanted to design a device that maximized spectral resolution (and therefore sensitivity) and versatility," explains Long. He and his colleagues began with optical simulations to develop the ideal light path, and then substituted in commercial optical components. First, they designed the custom fiber-optic assembly and the 3D-printed cradle in which the optics are mounted; then, they built a prototype and tested each of the three modalities with basic samples, such as food coloring. "We also wanted to run some proof-of-concept experiments using biological samples from a context where a portable device would be beneficial," says Long. To that end, the team assessed the TRI-Analyzer's performance with an ELISA assay to detect an indicator of pre-term birth (fetal fibronectin protein) and a fluorescent assay to measure phenylalanine, an indicator for phenylketonuria.

Who

The regulation of new medical technologies is stringent, so it will be some time before the TRI-Analyzer is approved for routine clinical use. In the meantime, though, veterinary pathologists take note: "The best patient right now would be a cow or horse. Just like people, they catch diseases and are highly mobile. 'Clinic access' is often challenging, and getting results back to patients after laboratory analysis can be difficult when they're out in the pasture. Having a device that could perform a test on-site would obviously be beneficial."

Personally, though, Long says he is incredibly interested in global health applications. "I'd love to see the TRI-Analyzer used by clinicians in rural or remote places where there might be clinics, but not clinical laboratories. Perhaps a doctor who travels to a dozen clinics on a regular basis could take the TRI-Analyzer with them as a portable lab system instead of collecting clinical samples, sending them off to a lab, and then trying to reconnect with a patient a couple of days later."

Why

Long and his colleagues hope that the TRI-Analyzer will help free many diagnostic tests from the centralized laboratory. Their ultimate goal? A tool that researchers and clinicians can use to quickly translate both existing and novel biomedical tests from the benchtop to the bedside. Better yet, they anticipate that the decreased logistics of sample collection, shipping, tracking, and follow-up will save time for physicians and laboratory professionals alike.

"I'd hope that we move away from having a separate gadget for each test we want to perform and toward a future where a single device can serve as a more universal portable laboratory capable of measuring many different types of tests," concludes Long. "I hope our work helps nudge the field in that direction."

Reference

1. *KD Long et al., "Multimode smartphone biosensing: the transmission, reflection, and intensity spectral (TRI)-analyzer", Lab Chip, [Epub ahead of print] (2017). PMID: 28752875.*

Escaping the Rat Race

Over-reliance on rodent models could be leading drug discovery in the wrong direction

By Stefan Amisten, translational research scientist, Diabetes Research Group, King's College London, UK

September 2017

When I was working at Oxford, a professor I knew said something that really stayed with me: "Mouse diabetes is not a big clinical problem."

There are only three real frontiers left to explore – deep space, the deep ocean, and the mysteries of biomedical science. And that made biomedical science an obvious career choice for me; it's where individuals and small groups can come together and create new things or discover new knowledge – all without a huge budget or the support of a large organization. Biomedical science truly is the final frontier. But I believe there is a huge knowledge gap in our field. Why? Two words: rodent models.

I focus on drug targets for diabetes and cardiovascular disease; and, in my experience, too much research focuses on what has come before. The same drug targets and receptors are given all the attention, and we're failing to look elsewhere. It also struck me that the vast majority of research – especially in diabetes – is conducted using mice and rats. But how well do rodent models really reflect their human counterparts? Looking further, I found that in-depth research into the similarities between mice and humans in this context is severely lacking – even though millions of pounds are spent studying mice and rats with diabetes.

There seems to be an assumption that the mouse is more or less the same as the human. But if you look at things from an evolutionary perspective, mouse and man had their last common ancestor around 10 million years before the dinosaurs died out! So besides the very obvious differences like our increased size and lack of tail, there are huge differences in our pharmacology – but no one seems to be working to comprehensively map these differences.

My colleagues and I set out to address the gap; after all, we all want to treat human disease, not simply learn more about mice. Our initial study found that some quite important and well-known receptors differ a great deal in terms of expression in mouse and man (1). I find this both worrying, because a lot of conclusions are being drawn using mouse models, and comforting, because it means there is much untapped potential in human tissues that we've missed by only looking at rodents.

Clearly, the availability of human tissue is an issue – and it's important to remember that behind every human tissue donation there is an individual tragedy. So, from a practical perspective, it isn't possible to sidestep the use of animal models for most research. But there are things researchers can do to address the issue, and this can be applied to any disease or tissue: look at human tissue first, then move to an animal model if necessary, and then once you have finished your mouse studies, go back to human tissue to validate your findings. There's no point spending three years coming up with a fantastic drug target in rodent models if that particular target is not present in humans. Don't waste precious time and resources studying animal-only phenomena.

I plan to continue my work in this area; my colleagues and I are currently working to publish a follow-up study looking at the peptides and proteins that interact with cell surface receptors, and how they differ in mice and humans. Based on our findings so far, it seems that some of the textbooks (which were created with the help of mouse data) will need to be rewritten, as there are many big differences that have been overlooked. I would urge all researchers using mice as part of their work to consider how well their findings will translate – if you jump to conclusions based on your findings in mice, you run the risk of making discoveries that will only benefit mice!

After all - "Mouse diabetes is not a big clinical problem."

Reference

1. *S Amisten et al., "A comparative analysis of human and mouse islet G-protein coupled receptor expression", Sci Rep, 7, 46600 (2017). PMID: 28422162.*

Treating Cancer

When you crunch the numbers on drug development, are the costs of cancer drugs justified?

September 2017

The road to creating a new cancer drug is long and winding, and paved with expensive R&D. But just how much money and time does it really take? The cost of anticancer therapies is continuing to rise, and the huge expense of bringing a new drug to market is often cited as the reason. Two US researchers decided to take a closer look at the claim, by analyzing the data on ten publicly traded drug companies. They chose companies with only one drug currently approved by the FDA, but also took into account the money the companies had spent on other drugs that ultimately didn't gain approval.

The authors found that the costs the companies incurred were significantly lower than some previous estimates (1) – and that companies quickly make more in profit than they initially spend

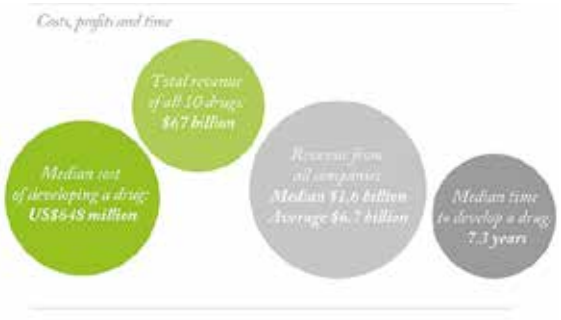
on R&D. The authors acknowledge that their method has limitations, but believe that the biggest challenge is shared by all studies attempting to analyze the cost of drug development: a lack of transparency. They conclude that "future work regarding the cost of cancer drugs may be facilitated by more, not less, transparency in the biopharmaceutical industry."

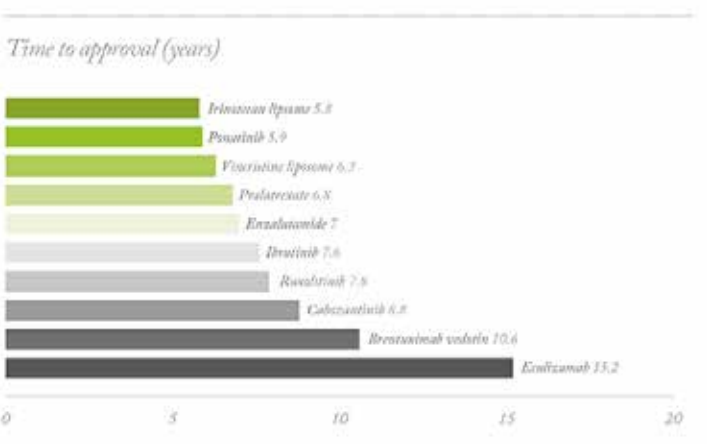
Here, we break down some of the key findings.

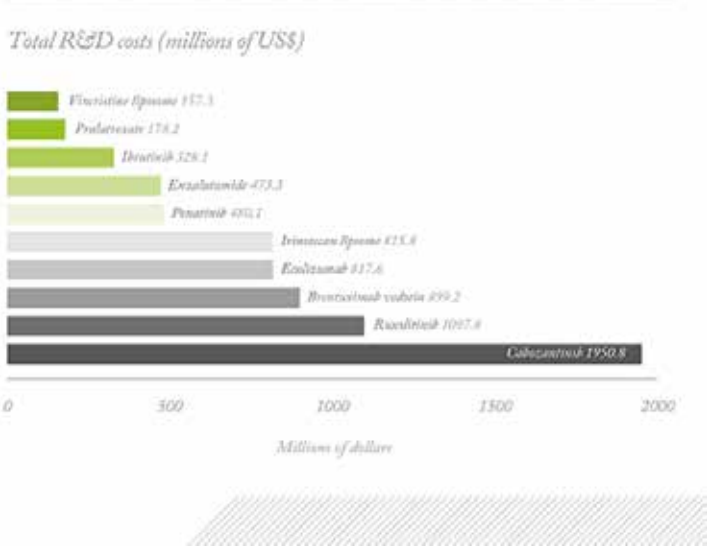
Reference

1. *V Prasad, S Mailankody, "Research and development spending to bring a single cancer drug to market and revenues after approval", JAMA Intern Med, [Epub ahead of print} (2017). PMID: 28892524.*

Company	Approved Drug
Alexion Pharmaceuticals	Eculizumab
Allos Therapeutics	Pralatrexate
Seattle Genetics	Brentuximab vedotin
Incyte Corporation	Ruxolitinib
Medivation	Enzalutamide
Talon Therapeutics	Vincristine liposome
Exelixis	Cabozantinib
Ariad Pharaceuticals	Ponatinib
Pharmacyclics	Ibrutinib
Merrimack Pharmaceuticals	Irinotecan liposome







Features

Recognizing Friend from Foe

The immune system scrambles into action when a foreign entity is detected, but not all foreign entities mean harm. New solutions are needed to teach the immune system to recognize biological drugs as partners rather than plunderers.

By Werner Cautreels

September 2017

The human immune system is an incredible defense mechanism that has the ability to interrogate and respond to any harmful entity (or 'antigen') that it is exposed to. When we are exposed to viruses, our dendritic cells sample the particles, process them, and then mobilize the immune system into action, resulting in the production of antibodies against the virus. The same mechanism has been exploited for vaccination, of course.

But the immune system also has a darker side – antibodies can form in response to anything deemed as 'foreign,' including biological medicines that are intended to improve – or to save – the patient's life. A well-known example is coagulation factor VIII – a clotting protein required by patients with hemophilia A. In a surprisingly large percentage of patients (over 30 percent), the immune system treats factor VIII as if it were a harmful entity and starts to make anti-drug antibodies (ADAs). This often results in a loss of efficacy and may also cause severe hypersensitivity reactions, including anaphylaxis.

Arrested development and allergic responses

When I started my career, most therapeutics were small chemical molecules, but today the focus has shifted to biologics. The immune system does not react to small molecules, but it can often react to biologic drugs, such as proteins, monoclonal antibodies and enzymes. A surprisingly large number of biologics already on the market induce the production of ADAs in many patients. Not only can ADAs reduce drug efficacy and modify pharmacokinetics and pharmacodynamics, they can also cause allergic responses. Over 100 approved biologics already list immune responses on their labels. As one example, a majority of patients taking Humira make ADAs (1). It often takes several months to a year for antibodies to build up and become a problem, but it is a key reason why patients on anti- TNF alpha inhibitors are often forced to switch medications.

The real problem arises when there is no alternative treatment. For instance, for patients with Pompe disease, there is only one approved enzyme: alglucosidase alfa. If patients develop ADAs to alglucosidase alfa – and the vast majority of patients do – the loss of alglucosidase alfa efficacy can prove to be fatal. ADAs also prevent a number of drugs from even reaching the market.

Antibody action

We need an approach to deal with ADAs that goes beyond 'wait and see.' At present, some physicians are avoiding certain approved medications because of the drug's immunogenic profile or are unaware that a patient has developed ADAs because they are not routinely monitored. Other physicians are experimenting with immunosuppressive cocktails to overwhelm the immune system to keep the ADAs at bay and allow the medication to work. However, the need to broadly immunosuppress patients comes with clear drawbacks and risks.

Teaching Old Drugs New Tricks

Many promising treatments do not reach the market because of immunogenicity. As one example, Ira Pastan, a senior investigator with the US National Cancer Institute (NCI), discovered mesothelin, a protein that is overexpressed in mesothelioma, pancreatic cancer and other solid tumors. After identifying the target, Pastan started to work on recombinant immunotoxins consisting of an antibody fragment fused to a bacterial toxin payload intended to kill mesothelin-expressing tumor cells. NCI subsequently developed a product candidate, studied it in clinical trials – and found that almost all patients developed antibodies against the immunotoxin, rendering the drug useless.

NCI then opened a small new Phase 1 trial in which a small number of terminal patients with a rare form of cancer known as mesothelioma were dosed with the immunotoxin and a potent cocktail of immunosuppressant drugs. The results were compelling. While the vast majority of patients still formed ADAs and were forced off therapy, one patient was able to receive four treatment cycles and another was able to receive six treatment cycles. Both of these patients saw marked tumor regression, and one of these patients remains alive today more than five years after his treatment (5).

Roche licensed the technology and reengineered the immunotoxin with NCI to make it less immunogenic by removing certain epitopes, creating a product candidate known as LMB-100. Roche initiated a new clinical trial with LMB-100, but found that the compound was still highly immunogenic. Roche then returned the product and technology to NCI. In 2016, NCI and Selecta generated compelling preclinical data showing how SVP can prevent the formation of ADAs to LMB-100, which led Selecta to in-license the product candidate in 2017. Selecta and NCI are currently planning a Phase 1b clinical trial for this new combination product candidate, known as SEL-403.

At Selecta, we are aiming to improve the efficacy and safety of biologic medications by resolving the ADA issue. One of our cofounders, Ulrich von Andrian (the Mallinckrodt Professor of Immunopathology at Harvard Medical School) is one of the world's leading immunologists, and much of his work has been focused on the role of dendritic immune cells. The dendritic cell acts as the teacher and sentinel of the immune system. They sample viruses and nanoparticles in general and, if they sense danger, they activate the immune system to respond by inducing the activation of virus-specific T cells and B cells, which leads to the production of specific antibodies to fight the danger. Von Adrian demonstrated that you can also achieve the opposite result by taking dendritic cells out of an animal and teach them to induce immune tolerance to an antigen. He then reinjected those dendritic cells into another animal, which prevented the animal from making antibodies against the specific antigen.

We believe that it is also possible to combat ADAs in vivo by using synthetic vaccine particles (SVPs). We have designed these nanoparticles with the goal of permitting them to "talk" to the immune system – telling it when to fight and, just as importantly, when not to fight. We hope to use SVPs to program the immune system to elicit tolerance to a specific antigen, without impacting the rest of the immune system. Rather than taking the dendritic cells out of the patient and dosing it with a biologic and an immunomodulator in a petri dish to prevent ADAs, we enable the critical process – specifically SVP-Rapamycin dosed in combination with a biologic – to take place within the patient to induce longer term immune tolerance.

The design of SVP-Rapamycin took a significant time as we were looking to overcome serious scientific challenges and had to meet many important criteria. For instance, we wanted them to work when dosed both subcutaneously or intravenously. We wanted to ensure that these nanoparticles resembled viruses so that they would be taken up selectively by the dendritic cells. We designed the nanoparticles to remain intact once they were injected and to only release their payload once they were taken up by the dendritic cells. In addition, of course, we had to develop a means to produce the particles in a way that made business sense and could facilitate our scale-up. We have already translated our SVPs from in vitro, to mice and to non-human primates – and this research has been published (2). But, of course, we needed to make the most important translational step of all – demonstrating that our approach would work in humans.

The right indication

In order to pursue our first commercial path for SVP-Rapamycin, we needed a suitable biologic candidate to showcase the potential of SVPs, and we had the following criteria:

It had to be a product that we owned; we could have chosen to license out our technology, but we wanted to own the product for the first applications so

that we would have full control of the development path and timeline.

At the same time, we needed this to be a real commercial opportunity to address real unmet patient needs.

We also wanted a product that would enable us to demonstrate a benefit very rapidly – both from an efficacy and from an ADA-mitigation aspect.

In some cases, immunogenicity is built up immediately; flu shots are designed so that you only need one shot to have an immune reaction, and some biologic drugs provoke an equally strong response. With many other drugs, ADAs build up more slowly over the course of many months.

We also wanted to find a medication that had clear biomarkers of efficacy as opposed to a longer-term clinical outcome.

Lastly, we wanted to work with adult patients for our first indication. With hemophilia and other genetic diseases, the focus is often on treating young patients. However, as SVP is a new technology, starting with children would have erected high hurdles from regulatory agencies, parents and ethics committees.

Our screen led us to the chronic severe gout market. Gout is a very prevalent disease – there are around eight million patients in the US alone. It is caused by metabolites from proteins; specifically uric acid, which normally circulates in the blood at healthy levels below 6 mg/dL. Gout patients have an imbalance between how much uric acid is formed and how much is excreted through the kidney. If the concentration goes above 6.8 mg/dL, uric acid is no longer soluble, leading to the formation of crystals that can cause inflammation in joints and tissues. To get rid of the imbalance, you may need an enzyme called a uricase that targets uric acid. However, as the human body doesn't make uricase, it is viewed as foreign by the immune system, and ADAs form in the vast majority of patients (3).

We licensed one such enzyme, pegsiticase, and then combined it with our technology. By co-administering the enzyme drug with our SVP technology, we have generated data that show that we can prevent the formation of ADAs in human patients (4). I like to describe SVP-Rapamycin as a "negative vaccination." With a vaccination, you are sending a danger signal to the immune system to induce the formation of antibodies to fight an antigen. With SVP-Rapamycin, we seek to teach the immune system that the biologic is not dangerous and that ADAs should not be formed. We have already generated clinical data in support of the idea that SVP-Rapamycin that is administered with pegsiticase mitigates the formation of ADAs to pegsiticase. We are now in the middle of a phase II study and expect to share additional information about this trial in late 2017. We have already started looking at the design of our phase III program, which we plan to begin in 2018.

Treat and retreat

Gene therapy could be a particularly promising area for SVP. Going back to hemophilia; what if we could teach a patient's liver cells to make the missing coagulation factor? Gene therapy would involve delivering genetic information encoding the coagulation factor into the liver cells, but to do that you need a vehicle, such as a viral vector. Of course, as these vectors are "viral," they are always immunogenic when you dose them systemically. Initially, the viral vector should induce liver cells to start making the missing protein. But, over time, expression may wane due to cell turnover in the liver. Currently, it is not possible to re-administer gene therapy because the immune system will have made ADAs after the first injection. This is a particularly challenging issue for pediatric patients, as cell turnover in the liver will be high as the children grow. As a result, systemic gene therapy dosing has been mostly limited to adult patients thus far. In preclinical studies; however, we have shown that by combining viral vectors with our SVP technology, ADAs can be prevented, making it possible to re-administer gene therapy.

As the problem of ADAs becomes more understood, I expect to see greater regulatory oversight – and perhaps agencies in the US and other developed markets will begin to require companies to not only study immunogenicity during clinical trials, but also after a drug has been approved and is in regular use on the market. We urgently need to address this issue as the next generation of biologic therapies are developed. Particularly in the case of gene therapies, retreatment will be incredibly important for a number of inborn diseases for which no treatments exist today. If we want to progress medicine to the next level, we need to tackle ADAs. And I believe that the most effective way to do this is through antigen-specific immune tolerance.

Werner Cautreels is Chairman, President and CEO of Selecta Biosciences, Inc.

References

1. *NK Bender et al., "Immunogenicity, efficacy and adverse events of adalimumab in RA patients", Rheumatol Int, 27, 269–274 (2007). PMID: 17006705.*
2. *RA Maldonado et al., "Improving the efficacy and safety of biologic drugs with tolerogenic nanoparticles", Nat Nanotechnol, 11, 890–899 (2016). PMID: 27479756.*
3. *FDA, "Krystexxa (pegloticase) injection label", (2012). Available at: bit.ly/2y5ArHc. Last accessed September 26, 2017.*
4. *Selecta Biosciences, "Selecta Biosciences reports data from ongoing phase 2 trial of lead candidate, SEL-212, in development for chronic severe gout", (2017). Available at: bit.ly/2fO11d8. Last accessed September 26, 2017.*
5. *I Pastan, R Hassan, "Discovery of mesothelin and exploiting it as a target for immunotherapy", Cancer Res, 74, 2907–2912 (2014). PMID: 24824231.*

Kallikrein Dream

Sitting Down With... Eleftherios P. Diamandis, Hold 'em for Life Chair in Prostate Cancer Biomarkers, Head of Clinical Biochemistry, Mount Sinai Hospital and University Health Network; Professor & Head, Division of Clinical Biochemistry, Department of Laboratory Medicine & Pathobiology, University of Toronto.

September 2017

How did you first become interested in cancer biomarkers?

My involvement goes back 35 years. In the 1980s, there was a lot of interest in new biomarkers, and there was a flurry of activity with the discovery of prostate specific antigen (PSA), and a number of other markers, like CA 125 for ovarian cancer, CA 15-3 for breast cancer, and C 19-9, which is used for pancreatic and colon cancer. As a young biochemist, I was fascinated by all these new discoveries, and naturally wanted to get involved.

When PSA was discovered, the general consensus was that the family of PSA genes included three members. But in the early 1990s, there were new reports describing genes homologous to PSA, in the same genomic region. So we developed a hypothesis that there may be other undiscovered members of this family. We initiated a genomic effort to find them, and were surprised to find a whole family, not of three genes, but of 15 different genes on exactly the same genomic locus on chromosome 19. We subsequently cloned, characterized, and named the enzymes that they code – serine proteases that belong to the kallikrein family. And I'm still studying them to this day.

What makes the kallikreins so fascinating?

Many researchers are looking into their biological function. The family appear to be very nice biomarkers not only for prostate cancer, but also ovarian, lung and other cancers. And we're only now beginning to understand what these genes – and the proteins they produce – are doing. For example, we're now convinced that the kallikreins participate in diverse biological functions, such as semen liquefaction, in which the major player is PSA. They also play a major role in the cascade of events involved in skin desquamation and regeneration, and we've found them in cervical fluid, and in sweat. Kallikrein 6 is highly expressed in the brain, and we suspect it may play a role in the development of neurodegenerative diseases such as Alzheimer's. It's becoming clearer and clearer that these proteins have diverse functions in various parts of the body.

In the last five years, there has also been tremendous interest in developing therapeutics based on kallikrein enzyme inhibition. We have shown that in certain diseases, the activity of these enzymes is increasing. Examples include Netherton syndrome (a rare skin disease) and also more common conditions like atopic dermatitis and psoriasis – we have shown that they are all likely to involve increased proteolytic activity of kallikreins. So developing inhibitors would be a logical therapeutic intervention.

Just this one family of enzymes appears to lead your research in many different directions – how do you choose what to pursue?

The diversity of our projects reflects the diversity of the expression of these enzymes! Of course, we can't make rapid progress in all of these areas – we need to look for the low hanging fruit. We believe that our work on developing therapeutics for skin diseases is currently the most promising area. And we're not the only ones – there are many groups working to develop specific inhibitors for these enzymes, and even therapeutic giants like Novartis are making major investments in this area.

What do you look for in a potential cancer biomarker?

Most of the markers we currently have in the clinic are used for monitoring previously identified cancer patients, to see if their therapy is working. Unfortunately, this means the impact of existing markers is relatively small. We need to look to population screening, and find something that we can test for in asymptomatic individuals. The impact this could have on clinical care is huge. If we can detect cancer early and implement effective therapies much earlier, this could make a big difference to patient outcomes.

Which of your current projects are the most exciting?

We are working to develop assays to measure a small number of tissue-specific proteins. It's an area that hasn't really been looked into before, and we're hoping to identify their clinical value, as we suspect that they have hidden potential.

We've also just published a paper in which we put forward the idea of creating a database of personalized cancer biomarkers that are useful in different patients. We have named them rare markers – markers that may be highly useful, but only in a few patients. We think this could be

another exciting new avenue. For the last 30 years we have tried to find one biomarker that will work for all patients. But molecular studies show that different types of cancer are not specific diseases – breast or ovarian cancer is actually a group of related diseases, with different molecular features and signatures. And that means we have to accept that finding one biomarker to work for all of these patients is not very realistic. We believe that the way forward is identifying rare biomarkers and developing repositories for people to report them – eventually we could create a rich enough database to look up a biomarker for any patient.

Do you have any advice for newcomers to the field?

I'm actually in the middle of preparing a new lecture on mentorship, and I do think it's important to share your experiences with younger people. An important tip is to be honest with your science. There is a lot of press lately on fabrication of results – this is totally unacceptable. It doesn't build careers; it destroys them. So I always tell my students: never consider fabrication, don't go there. Be honest with yourself and with your work.

I would also say: work hard, develop multidisciplinary approaches, and read widely to expand your knowledge. But don't forget to have interests and passions outside of science. I don't want to create robots with tremendous output, but to develop human beings who enjoy life. Finally, be persistent – don't be discouraged by failures. If you get 99 failures and one success from 100 attempts, embrace it! Learn and move forward.

If you weren't a scientist, what would you be?

I've had a great deal of fun exploring new knowledge – and it's a privilege to work with very talented young people. My number one alternative would be a musician; however, though I love listening to music, I have no talent for making my own... Without science, I'd probably have chosen something athletic – perhaps a tennis player. But given how wonderful and rewarding my career has been so far, I don't think I'd change it!

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