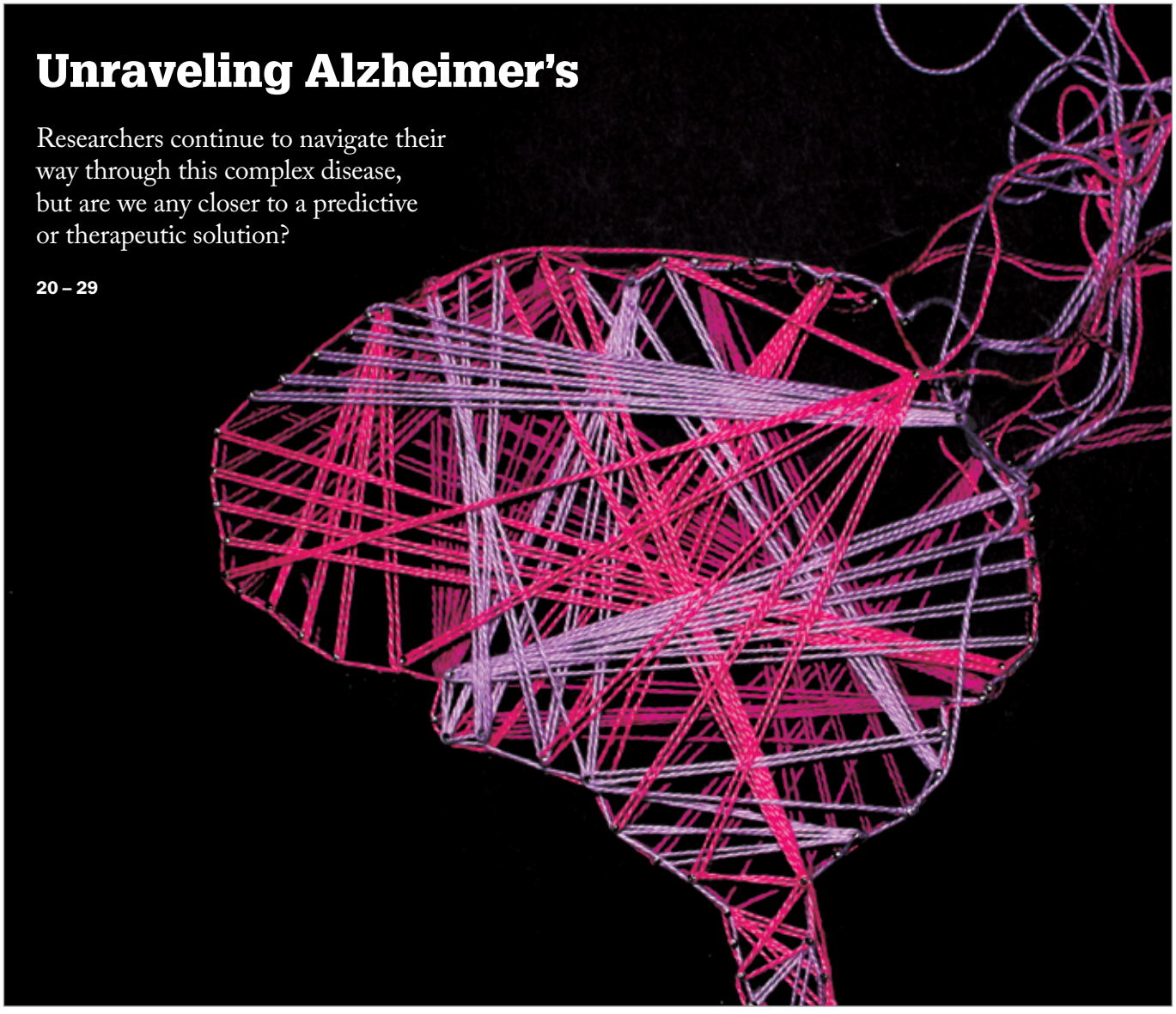
FEBRUARY 2016 # 16

the Pathologist

Unraveling Alzheimer's

Researchers continue to navigate their way through this complex disease, but are we any closer to a predictive or therapeutic solution?



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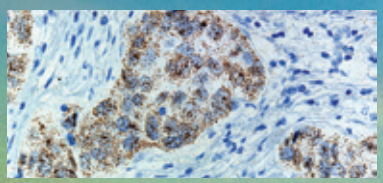

HER2 expression in human breast cancer FFPE tissue using RNAscope® 2.0 HD Reagent Kit-BROWN

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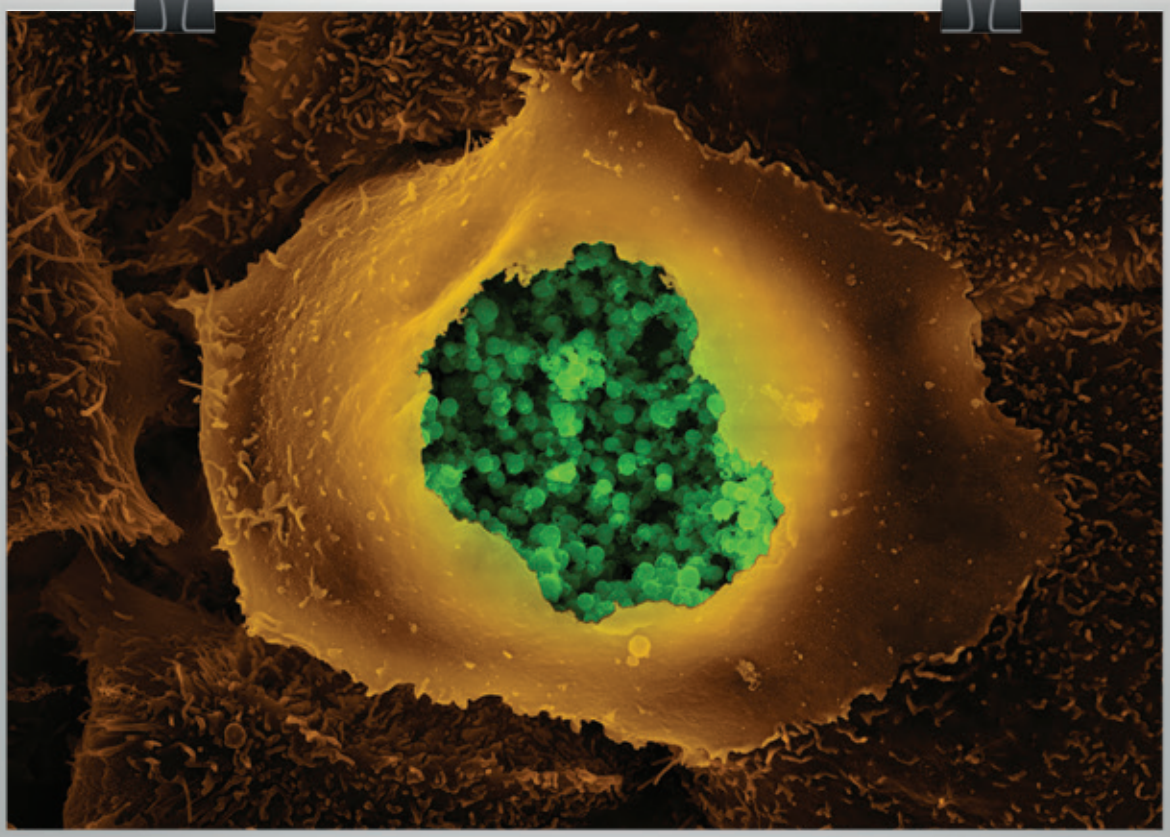
Image of the Month



This human epithelial cell (yellow) is infected with *Chlamydia trachomatis* (green). Volker Brinkmann from the Max Planck Institute for Infection Biology in Berlin, Germany, has opened the cell to expose its interior, where the bacteria have formed an inclusion. This image was prepared using a ZEISS FE-SEM scanning electron microscope.

Credit: ZEISS Microscopy.

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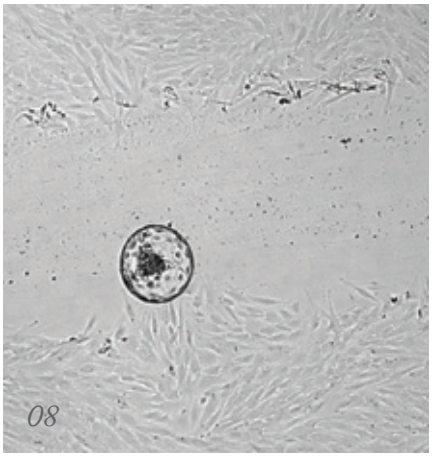

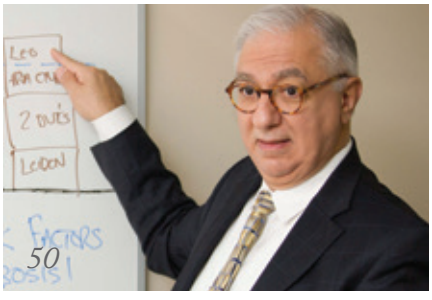

On The Cover

Woolen representation of the complexities of the brain.

Upfront

In My View

Feature

the Pathologist

ISSUE 16 - FEBRUARY 2016

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Crossing a Line in the Sand

The UK has given the green light to use the CRISPR–Cas9 gene editing technique in human embryos. Can and should its use be controlled?

"Scientists get 'gene editing' go ahead". This headline – and other variations of it – made front-page news at the beginning of February when the UK approved the genetic modification of human embryos. Unsurprisingly, the announcement was greeted with cheers and disdain; and the media frenzy that followed carried much opinion and analysis of the consequences. A particular favorite attention-grabber of mine, "Just wait until they start introducing 1/2 human 1/2 animal". Far-fetched, I know, but the development did get me thinking… Where do you draw the line?

This landmark approval grants UK-based researcher Kathy Niakan permission to use the genome-editing technique CRISPR–Cas9 in healthy human embryos during the first few days after fertilization. Her primary reason? To understand IVF success rates in the hope of supporting the development of improved treatments for infertility. An undeniably admirable objective. But are the fears of those who believe that we're now one step closer to the "designer baby" justified? Well the stipulations of the approval are clear: the experiments must stop after seven days, after which time the embryos will be destroyed – so absolutely no implantation will occur.

The development has stirred debate on the use of gene editing to eliminate inherited disorders. In fact, scientists in China have already begun, announcing last year their correction of a gene that causes a blood disorder in human embryos (1). In their paper, however, the scientists admitted that their results reveal "serious obstacles to using the method in medical applications", and there's still huge uncertainty surrounding the long-term impact of such prenatal dabbling.

Jennifer Doudna from the University of California, in line to receive a Nobel Prize for the development of CRISPR-Cas9, vented her concerns in Nature: "Human-germline editing for the purposes of creating genome-modified humans should not proceed at this time, partly because of the unknown social consequences, but also because the technology and our knowledge of the human genome are simply not ready to do so safely," (2). While George Church, a geneticist at Harvard Medical School, argues that banning it "could put a damper on the best medical research and instead drive the practice underground to black markets and uncontrolled medical tourism." (3)

The debate will no doubt roll on. My own views on the matter are conflicted. Certainly a strong and emotional case in favor was made by a woman who attended the International Summit on Human Gene Editing in Washington last December. Having lost her child at six days old to a genetic ailment, she implored the research community, "If you have the skills and the knowledge to eliminate these diseases, then freakin' do it!"

Fedra Pavlou
Editor

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Upfront

Reporting on research, innovations, policies and personalities that are shaping pathology today.

Do you want to share some interesting research or an issue that will impact pathology?

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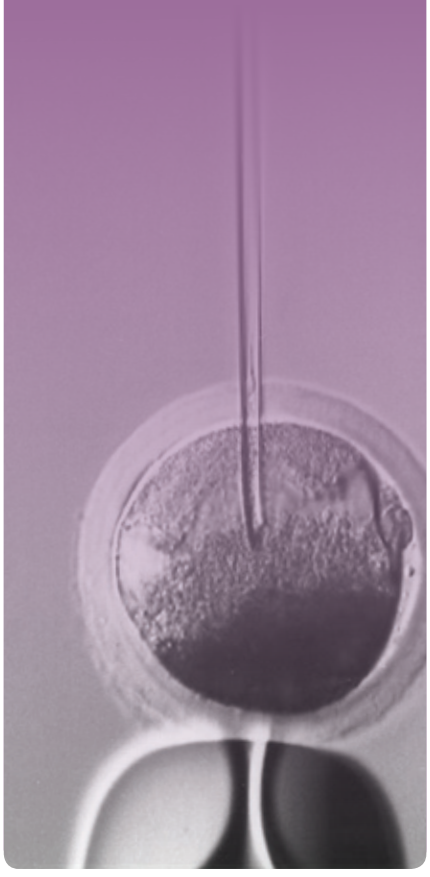
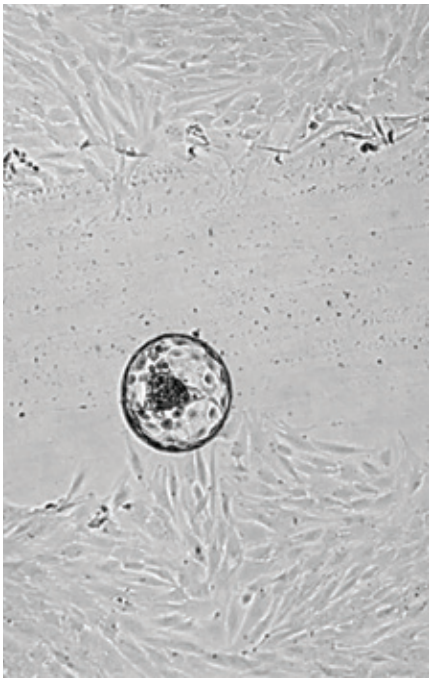


Fertilization Failure Forecast

There's a genetic basis behind recurrent IVF failure – and a better understanding of it may help counsel couples on their options

For couples with difficulty conceiving, *in vitro* fertilization (IVF) is often their only hope for a biologically related child. But while IVF has helped many couples, as many as one in 10 undergo recurrent implantation failure (RIF) despite repeated transfer of high-quality embryos. It's an expensive and understandably frustrating experience, and one that, at the moment, clinicians can only combat with empiric therapy. It's clear that there's a need to better understand the etiology of RIF, and thanks to a longstanding interest in endometrial factors, fertility experts at the University of Southampton have been exploring the genetics behind the problem.

Nick Macklon and his colleagues hypothesized that a disruption of endometrial gene expression may underlie RIF in some patients. After sequencing biopsies from 43 women with RIF and 72 who had undergone successful IVF, they identified and validated a 303-gene profile that appeared be associated with RIF (1). The majority of the changes in expression were downregulations, and gene ontology studies pointed to defects in cell proliferation, motility and ciliary action. This knowledge gives a better understanding of the genetic basis for RIF, and may one day lead to predictive testing for IVF success. "Endometrial genetic testing is beginning to enter clinical IVF," says Macklon, "but thus far is limited to helping clinicians to identify



Credit: C Weimar et al., PLoS One 2012.

the best time to place an embryo into the uterus. If we can develop a clinical test based on our findings, it could become part of the routine clinical investigations for infertility, and also guide couples before embarking on IVF."

Macklon's team are currently planning a prospective study in couples just beginning their IVF journey to determine the potential for a screening test based on endometrial genetics. At the same time, they're seeking to understand the pathways that govern the endometrium's role in implantation. His recommendations for doctors involved in IVF? "I encourage my colleagues to participate in studies of novel therapies with a sound biological rationale, rather than offering expensive but unproven, and possibly harmful empirical therapies." *MS*

Reference

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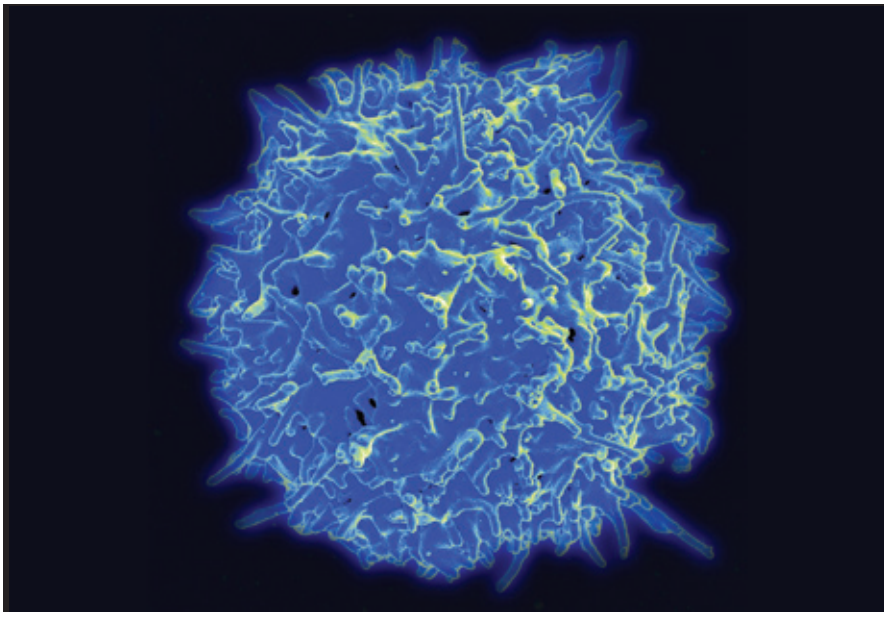
Memory Modifications

Epigenetic changes to the chromatin of memory T cells allow them to respond rapidly to re-infection



Credit: NIAID.

Scanning electron micrograph of a healthy human T cell.

As we slowly emerge from the cold and flu season, pockets filled with tissues and bottles of hand sanitizer, the thought of a way to defeat these elusive viruses is never more attractive. Perhaps that's why a group of researchers at the University of Birmingham, UK, have explored the "immunological memory" that allows our immune systems to respond quickly to re-stimulation by a pathogen. As a result of this exploration, the team has discovered a new class of gene regulatory elements that imprint on the chromosomes of T cells, keeping the chromatin in its "active" conformation and ensuring that the cells can respond quickly to threats they've seen before (1).

It has been known for many years that recently activated T cells respond much faster to re-stimulation than naïve or immature T cells. We also know that these recently activated T cells acquire a different pattern of epigenetic modifications in the active regions of chromatin at gene regulatory elements. "When we put these two facts together," says Peter Cockerill, who led the team that made the discovery, "we realized that it was the epigenetic priming of cells that allowed them to respond much faster, and we saw that this had the potential to at least partly account for the increased immune response after vaccination or infection."

Immune memory involves both an expansion of the T and B cells that recognize a specific pathogen, and those cells' enhanced ability to respond to the same pathogen again. What the Birmingham researchers found is that one cycle of T cell receptor activation is sufficient to reprogram the genome by introducing "hotspots" that make specific chromosome regions more accessible. Upon closer inspection, Cockerill explains, it turned out that these regions encompassed the inducible transcriptional enhancers that regulate immune response genes – so the priming mechanism is a simple way to make hundreds of genes respond much faster.

The discovery may have implications not just for fighting infections, but also in autoimmune diseases. "You would expect to find autoimmune and inflammatory disorders associated with a higher proportion of 'memory-like' T cells," says Cockerill. Such diseases are also associated with excessive cytokine production. "We could aim to target the pathways that keep these regions open, which may involve these pro-inflammatory cytokines and the kinase signalling pathways linked to them. This is similar to what is already being done with anti-TNF therapy in rheumatoid arthritis, where the cytokine network is targeted at one key point."

Cockerill and his colleagues have uncovered part of the explanation for how immunity is acquired, and why naïve and memory T cells behave differently. "We identified the first stage of this process which is the establishment of epigenetic priming in direct response to T cell activation." But where will their research go next? "We know that immunity lasts for decades, and circulating memory T cells have the same modification patterns as recently activated T cells", he says. "The search is now on for the mechanisms that allow epigenetic priming to become re-established after each cell division, and to be maintained for years when these cells stop dividing." *MS*

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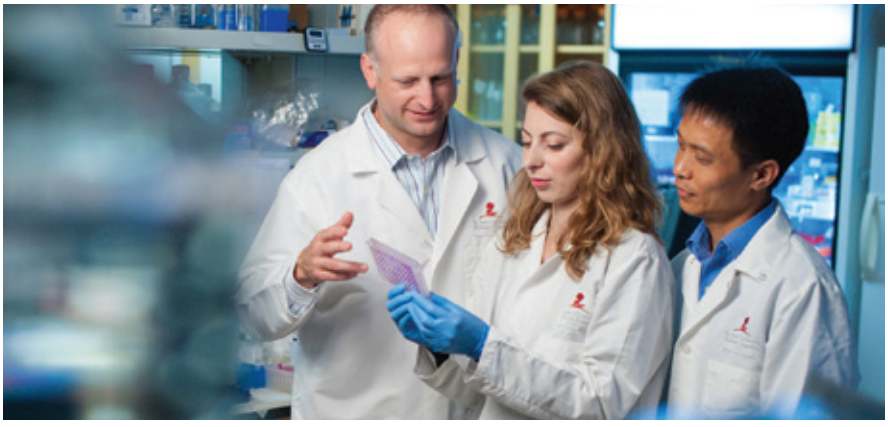
Pandemic Protein

The stability of a virus' hemagglutinin protein affects its ability to invade host cells, be transmitted, and potentially cause pandemics



The team at St. Jude Children's Research Hospital led by Charles Russell.

Picture a pandemic and you may think of smallpox, tuberculosis, cholera and the Black Death. Closer to home, the 2009 influenza pandemic is still fresh in people's memories – and the H1N1 virus that caused it is still under study. Recently, scientists at St. Jude Children's Research Hospital discovered that in the human viruses, the hemagglutinin protein responsible for fusing the virus to its target cell became more stable in an acidic environment than it was in earlier swine viruses (1) – exactly the property needed for airborne human-to-human transmission. They also demonstrated that when the protein was mutated to increase its activation pH, it lost the ability to spread via airborne particles. We spoke with Charles Russell, who led the project, to find out more.

What led you to examine the stability of hemagglutinin as a function of pH?

The influenza virus hemagglutinin protein has long served as a model for how a protein can cause membrane fusion. The high-resolution structure of hemagglutinin was determined over 30 years ago. Since then, researchers have studied how low pH in the endocytic entry pathway activates it to undergo structural changes that cause membrane fusion. So we know how the molecule changes its shape, but we had questions about how these structural changes affect the ability of an influenza virus to first infect one host (like birds or pigs) and then another (like humans).

What changes occur in hemagglutinin when moving to a more acidic environment?

Think of the influenza virus as a ball with spikes sticking out. The protein is attached to the virus' membrane by a stalk; sticking out farther is a globular head domain with a pocket that points at and binds to receptors on a host cell. After binding, the cell "swallows" the virus into an endosome. Over time, the virus pumps protons into the endosome. When the pH has dropped enough, a complete hemagglutinin shape change is triggered.

First, the receptor binding domain heads pop off. Then the fusion peptide (part of the stalk), shoots out like a harpoon into the membrane of the endosome. After that, the protein bends back on itself so that it looks like a hairpin, fastening the virus to the host cell. You can think of hemagglutinin like a mousetrap. When the trap is set (the protein is folded and activated by cleavage), it waits until the mouse bites the cheese (the pH is low enough). After it is triggered, the spring in the trap snaps the wire down to catch the mouse (just like the hemagglutinin protein shoots its fusion peptide into the target endosomal membrane, then snaps back to pull both membranes together).

What implications might this have for disease prediction and prevention?

We now have a better idea of what animal viruses pose a threat of human transmission and pandemic. So we will be better prepared to control infections of risky viruses, and we will have a better idea which viruses to target with vaccines to prevent possible pandemics. It takes months to prepare an influenza vaccine, so we need to have a head start. There are also experimental drugs that bind to the hemagglutinin stalk. We know that, in some cases, resistance to these drugs occurs by decreasing hemagglutinin stability (raising the activation pH). But now, we also know that the penalty for such resistance is lower transmissibility – so we should continue to pursue antiviral drugs that stop hemagglutinin membrane fusion.

What are the next steps for your work?

We want to know whether the other influenza viruses that have caused human pandemics have also had altered hemagglutinin activation pH, and what the tolerable range of hemagglutinin activation pH is in influenza virus reservoirs, intermediate hosts, and humans. Finally, we want to know how altering the hemagglutinin acid stability with a mutation can help us make live influenza virus vaccines that are grown more efficiently and better stimulate protective immunity.

Reference

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Cracking the Case of Brittle Bones

New research has discovered the culprit behind brittle bones in Hajdu-Cheney syndrome: an excess of osteoclasts, the cells responsible for bone resorption

The inspiration:

Patients with Hajdu-Cheney syndrome have malformed skeletons and fragile bones. Nearly five years ago, two publications in Nature Genetics demonstrated that these patients have specific mutations in the *NOTCH2* gene (1,2). At the time, Ernesto Canalis' laboratory at the University of Connecticut had been working on Notch signaling for about 10 years. They believed that the only way to study the rare bone resorption disease was to recreate those mutations in a mouse model.

The experiments:

In the 2011 papers, individuals with Hajdu-Cheney syndrome underwent exome-wide sequencing. Mutations were found in exon 34 of *NOTCH2* upstream of the domain required for protein degradation, creating a stable, truncated *NOTCH2* protein (1,2). "We have discovered that the Hajdu mutant mouse has increased bone resorption because of an increase in bone-resorbing cells," says Canalis (see Figure 1). "It is conceivable that the use of specific anti-resorptive agents might ameliorate the bone disease." But the mouse model lacks the craniofacial and neurological abnormalities of human disease, so the researchers are currently aging the animals to see whether these manifestations appear (3).

The implications:

"We have learned a great deal about mechanisms of bone turnover and bone loss, and confirmed that *NOTCH2* plays a critical role in these processes. The model will allow us to pursue other disorders associated with mutations in the Notch signaling pathway." What are Canalis and his laboratory doing now? "We are studying the function of B cells in Hajdu mutant mice, as *NOTCH2* is known to play a key role in these cells. We are also creating additional mutants affecting other members of the Notch signaling pathway in an effort to relate them to other genetic disorders associated with Notch signaling."

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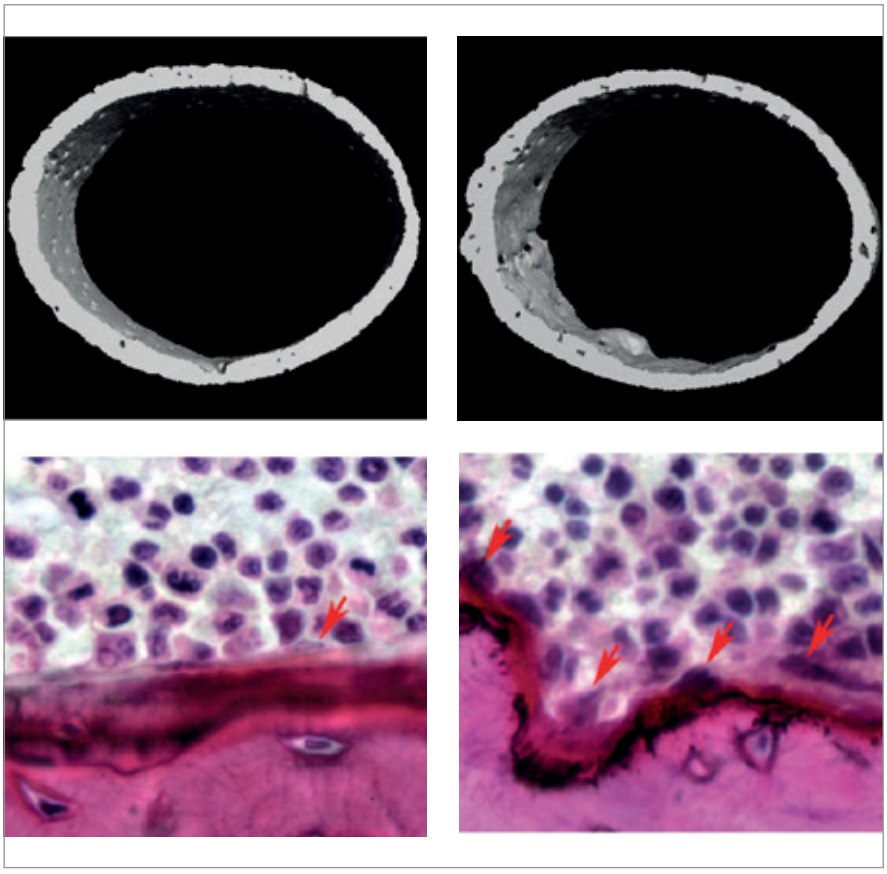

Credit: Stefano Zanotti/Canalis Lab, UConn Health.

Figure 1. A cross-section of the femur of a mouse with a *NOTCH2* gene variant. The arrows indicate an excess of osteoclasts resorbing bone.

Diversity's Double-Edged Sword

H. pylori's attachment protein allows it to adhere to multiple blood group antigens – but also contains a disulfide-clasped loop that may be its downfall

Helicobacter pylori is a well-known culprit in cases of gastritis, peptic ulcer and gastric cancer. The bacterium is able to survive the acidic stomach environment by attaching itself tightly to the epithelial cells of the gastric mucosa – a key mechanism in the establishment of chronic infection and mucosal inflammation. In the 90s, Borén identified the ABO blood group antigens as binding sites for the *H. pylori* BabA adhesin in the gastric cell lining (1,2). Vaccine development has so far shown limited progress, and although *H. pylori* is one of the most prevalent infection in humans, antibiotic therapy is restricted because of the risk of resistance and therefore only recommended for use against peptic ulcers. With no way of identifying the patients at greatest risk of stomach cancer, and only poor ways of fighting the disease once it develops, there's an urgent need to develop new therapies against *H. pylori* infection. Fortunately, a team of researchers in Belgium and Sweden have uncovered what they deem the bacterium's Achilles heel: a weakness in the binding protein BabA (3).

Thomas Borén, who led the Swedish contingent, explains that BabA binds to the ABO blood group glycans presented on the gastrointestinal epithelial cells (4): "In the stomach, *H. pylori* manage to adapt extensively to changes in the stomach such as during chronic inflammation." He and his team have determined that BabA is extensively polymorphic due to adaptation

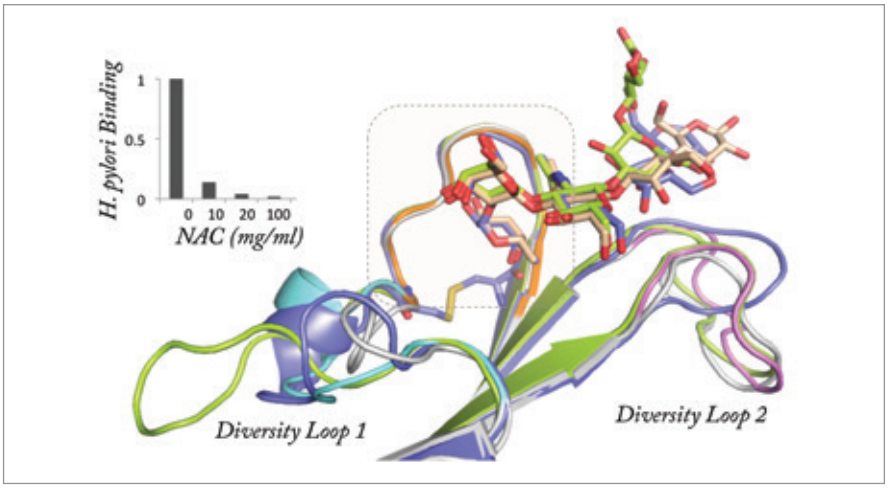

Figure 1. Close up view of the BabA protein's glycan binding site in complex with Lewis B, its main blood group antigen receptor in the stomach epithelium. Shown is a superimposition of the BabA proteins from four clinical isolates, highlighting the diversity inside the binding pocket. Redox-active pharmaceuticals such as N-Acetylcysteine (NAC) disrupt the conserved cys-clasped loop in the binding pocket (boxed area), and lead to the loss of *H. pylori* adherence to the stomach epithelium (see bar graph).

(4). By crystallization of the protein, Han Remaut's team in Brussels could show that the functional polymorphism is owing to two diversity loops inside the protein's glycan binding site, whose amino acid sequences can be changed to alter binding properties, such as blood group preference. But at the same time, the teams discovered that the binding "holdfast" in the BabA protein is formed by a disulfide-clasped loop that can be inactivated by reduction – providing hopes that the source of the bacterium's strength may also be the source of its defeat.

"The possibilities we now have uncovered to specifically interfere with *H. pylori* adherence and reduce the intensity of the chronic inflammation processes are exceedingly exciting," says Remaut, "especially since this does not involve the use of broad-range antibiotics that are known to have undesired collateral impact on the healthy microbiota." Instead, the two teams propose to treat infections using redox-active drugs that inactivate the disulfide-clasped loop, causing difficulties only to *H. pylori* (see Figure 1). "This could open the door to a new type of antimicrobial drugs," says Remaut. "The structural information also provides promising possibilities for how to develop novel vaccines against adherent *H. pylori* infections, says Borén. The two teams are currently studying the way *H. pylori* adapts to changes in the stomach during disease progression, and they anticipate that better understanding of the *H. pylori* attachment processes will also help epidemiological studies. *MS*

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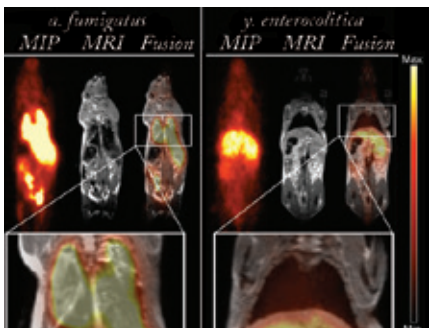
Breaking the Mold

Diagnostic imaging may detect fungal lung infections faster and more accurately than current methods

Pulmonary aspergillosis, a disease caused by the *Aspergillus fumigatus* fungus, is dangerous in patients with compromised immune systems. It's also notoriously difficult to diagnose; blood tests take time and resources, but lack sensitivity and specificity even under the auspices of skilled diagnosticians. Culturing the fungus from lung biopsies is no easier, as many patients are too fragile to undergo the procedure. Using these methods, diagnosis can take up to a week as physicians wait for results from specialist testing centers – and with the speed at which the disease can progress, this can be a dangerous delay. There's a clear unmet need, and in a bid to address it, an international research group including Christopher Thornton of the University of Exeter, UK, has developed an accurate, sensitive test based only on readily available imaging techniques.

Thornton initially met his collaborators at a conference, where they began talking about the possibility of such a technique. Despite their enthusiasm, it was only when the European Union put out a specific funding call in the area of imaging technologies for rare disease diagnosis that they were able to acquire the finances and assemble a consortium with the requisite skills. Thornton says, "As is frequently the case in science, there was a degree of serendipity – right time, right place, right idea." The group's test involves radioactively labeling antibodies to the fungus *in vivo*, then using a combination of positron emission tomography (PET) and magnetic resonance (MR) imaging to visualize the infection (1). So far, the technique has shown good results in mice (see Figure 1). "The next step is to adapt the technology for detection of human invasive pulmonary aspergillosis. To this end, we have 'humanized' the antibody tracer and are currently working through the regulatory framework to allow us to conduct human clinical trials in two years' time."

Aspergillosis isn't the end of the road for the test, either. By applying the right antibodies, it can be used to detect bacteria, viruses and even cancer cells. "At present, cancers are visualized using the radiopharmaceutical fludeoxyglucose (^{18}F)," explains Thornton. "However, we have shown that ^{18}F is not appropriate for infectious disease detection, as it can't discriminate between different types of pathogen, or between pathogens and general inflammation of the lung. By using our antibody-guided PET/MR imaging approach, diseases caused by fungi, bacteria and viruses will be detected and differentiated with a much higher degree of accuracy than is currently possible." Not only that, but they can be detected rapidly using tools already available in most hospitals – meaning that hopefully, patients will receive the right treatment faster. *MS*



Credit: Werner Siemens Imaging Center.

Figure 1. Infection of a mouse lung by the fungus *Aspergillus fumigatus* is shown by radioactively labeled (light-colored) areas (left). By contrast, infection with the bacterium *Yersinia enterocolitica* does not show any accumulation of the Aspergillus-specific radioactive marker (right).

Reference

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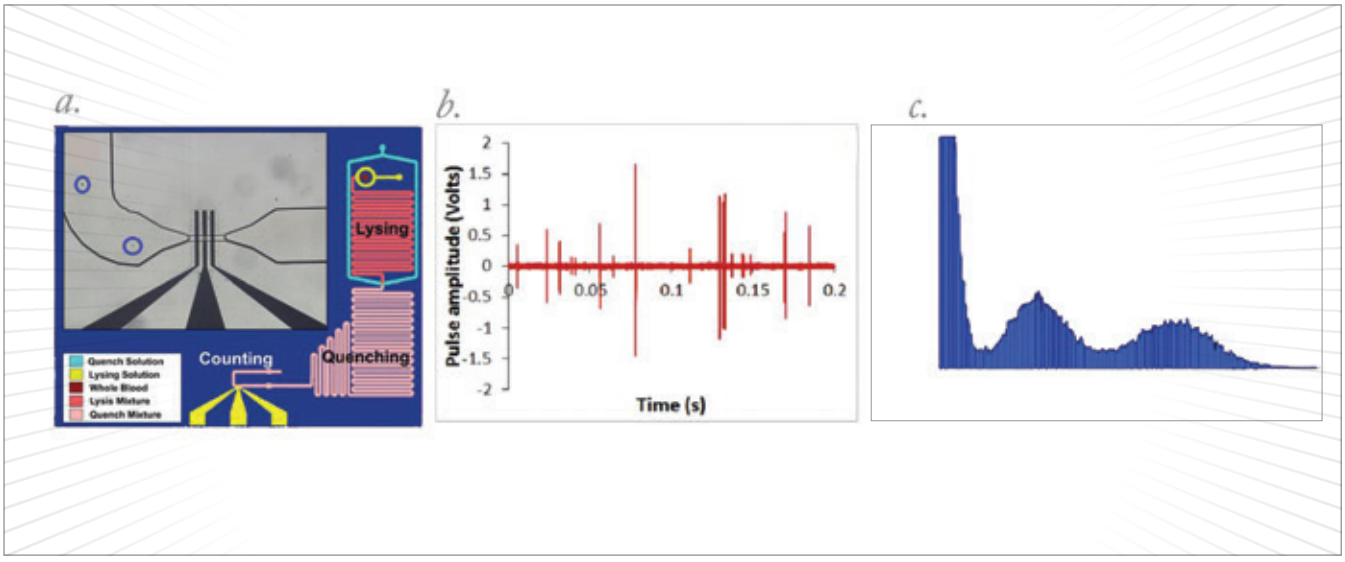

Credit: TECHNOLOGY.

a. Schematic of the biosensor, with inset showing the microfabricated coplanar electrodes aligned with the cell counting aperture. b. Representative voltage pulses generated as the individual cells pass over the electrodes. c. Pulse amplitude histogram showing distinct white blood cell populations.

Counting Chips

A new microfluidic device the size of a credit card may offer rapid, inexpensive blood cell counts that require only tiny samples

Overworked laboratories may soon have a new tool for tackling slow turnaround times. One of the most commonly used – and yet most resource-intensive – tests in clinical laboratories is the complete blood count (CBC). The routine test requires the use of a hematology analyzer to count the cells in a blood sample – but the machines are expensive, take up space in crowded labs, and require trained technicians to operate. With all these restrictions, patients must travel to hospitals or centralized laboratories to have these simple tests done. "It slows turnaround time, limits throughput in hospitals, and limits accessibility in resource-limited settings," says Umer Hassan, lead author of a paper that proposes a new type of CBC device.

Hassan's solution? An automated chip the size of a credit card that electrically detects the size and membrane properties of cells in a blood sample (1). The microfluidic device can use samples as small as a single microliter to count platelets and erythrocytes, or up to 11 µL for leukocyte counts and differentials. And it's not just the small sample size that makes the chip an intriguing new piece of technology – there's a significant time saving too. "Currently, in hospital settings, blood samples are transported to a testing laboratory and it takes a few hours to a day to get the results back to the patient," says Hassan. "Our technology can provide the cell count results in less than 30 minutes at the point of care, and completely eliminates sample transportation." The chips themselves are intended to be disposable, reducing the cost of a CBC to less than US$10.

One of the most compelling possible uses for the technology is in resource-limited settings where laboratory tests are often inaccessible due to cost, poor laboratory facilities, or the difficulty of follow-up after days spent waiting for results. The device's ease-of-use and the ability to interface with smartphones and tablets enable a point-of-care device that can rapidly transmit results to a provider anywhere in the world. This means that there's no need for a highly trained professional at the point of care – reducing costs and allowing the device to be used in all kinds of settings. For instance, a portable device carried on an ambulance could provide results that might shape the care a patient receives en route to the hospital or provide emergency departments with CBC results upon arrival. Certainly, once the prototype is complete and on-site clinical studies have been conducted, the chip holds the potential to improve the throughput of hospital laboratories and the speed at which patients receive care. *MS*

Reference

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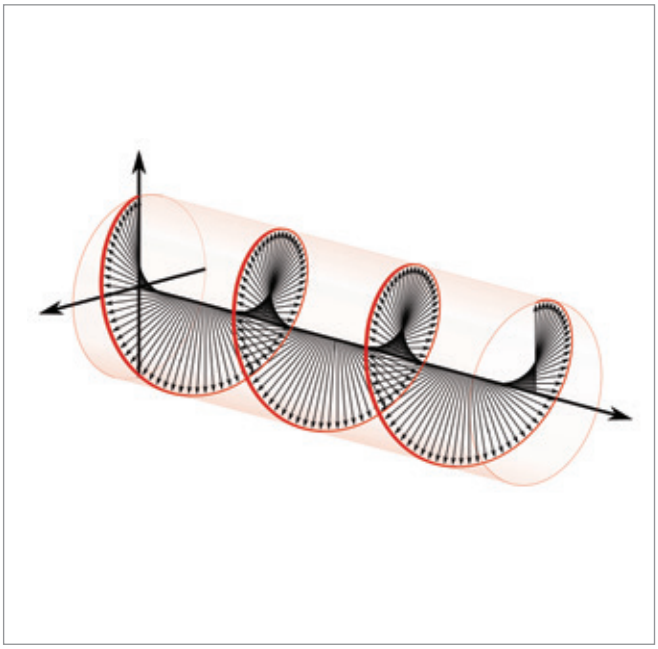
Cancer Polaroid

A new nanomaterial might be the key to portable devices that can rapidly detect cancer recurrence, replacing invasive tests

What?
A thin, elastic nanoparticle composite known as a "chiral nanostructure." It's capable of circularly polarizing light – coiling it into the shape of a helix (1).

Why?
The composite can be built into a portable device that can detect the early stages of cancer recurrence. Why is such a device needed? At the moment, cancer follow-up treatment and monitoring can include maintenance chemotherapy, blood draws, lumbar punctures, extensive diagnostic imaging and a host of other appointments designed to ensure patients never experience an undetected recurrence. But these appointments – however necessary – take time, cost money, and present an unwelcome disruption to patients' lives for years after they reach remission. And of course, they're not perfect; recurrences may not be detected until they're having a significant health impact. So a group of chemical engineers at the University of Michigan asked themselves, "Can we do better?"



A diagram of circularly polarized light.

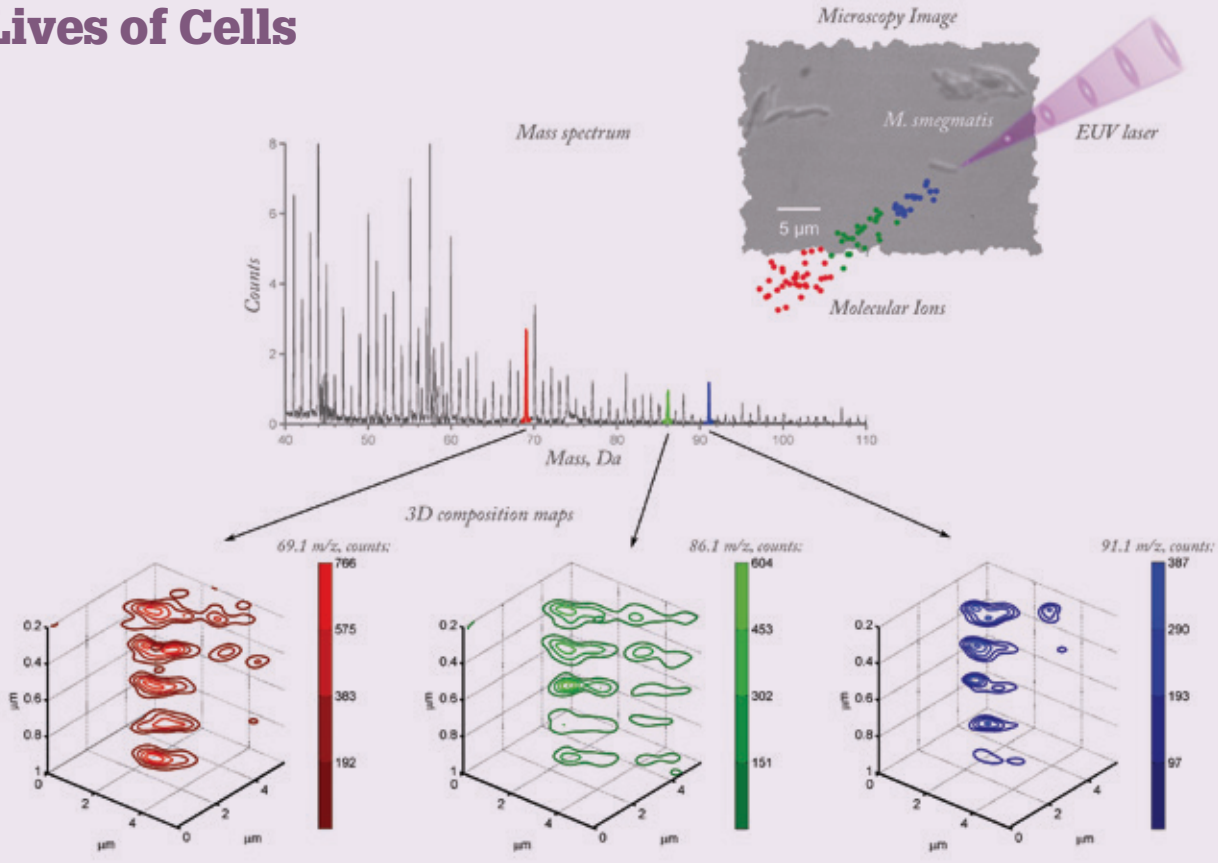
How?
They envision a process that begins with taking a blood sample and adding reflective synthetic particles that bind to natural cancer biomarkers. Viewing the sample under circularly polarized light would make the reflective particles visible – meaning that detecting even the earliest signs of cancer could be as simple as looking for them under the right conditions. With a device like this, a simple blood test could replace hours of invasive tests and spot recurrences even earlier.

When?
The material has only just been developed, so the research is still a long way from commercial availability – but it's possible that, in a few years' time, cancer specialists may be using portable polarization devices to quickly and noninvasively monitor their patients.

Reference

1. *Y Kim, "Reconfigurable chiroptical nanocomposites with chirality transfer from the macro- to the nanoscale", Nat Mater, [Epub ahead of print] (2016). PMID: 26726996.*

The Secret Lives of Cells



Mass spectrometry imaging can provide information on the spatial organization of chemical components in cells and tissues. Researchers probe a particular region by laser ablation, then analyze the ions produced. Colorado State University scientists have devised a one-of-a-kind instrument that lets them map cellular composition in unprecedented 3D resolution (1).

The device uses a laser that emits pulsed extreme ultraviolet (EUV) light at a wavelength of 46.9 nm. "The uniqueness of our approach resides in the fact that most materials are highly absorbing at 46.9 nm," explain researchers Ilya Kuznetsov and Carmen Menoni. "Therefore, the radiation can be confined to nanometer-thick layers and the beam focused to spots 1,000 times smaller than the diameter of a human hair" – about 100 times more detailed than was previously possible.

The image shows an isolated *Mycobacterium smegmatis* bacterium imaged in five passes and post-processed to produce smoothed profiles of each molecule's distribution inside the bacterium. For example, the 91 m/z ion (blue) shows similar distribution in all five layers, while the 86.1 m/z ion (green) varies widely. With improved sensitivity and extended mass range, the EUV laser ablation mass spectrometer may provide increasingly detailed information on the subcellular chemical organization of lesions and microorganisms.

Reference

1. *I Kuznetsov et al., "Three-dimensional nanoscale molecular imaging by extreme ultraviolet laser ablation mass spectrometry", Nat Commun, 6, 6944 (2015). PMID: 25903827.*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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 laboratory medicine. They can be up to 600 words in length and written in the first person.

Contact the editor at fedra.pavlou@texerepublishing.com

The Next Generation

We've come a long, long way already. Where to next for NGS?

By Benoit Arveiler, professor, medical genetics, University of Bordeaux and University Hospital of Bordeaux, France.

Over the last 10 years, next generation sequencing (NGS) technologies have revolutionized genome analysis; we can now sequence the whole genome of an individual within a couple days for less than €5,000. To put that into perspective, it took several years and US $3 million to obtain the first draft of the human genome by Sanger sequencing!

"Massively parallel sequencing" is another term used for NGS, because it produces hundreds of reads of the same sequence and allows the sequencing of numerous fragments from different individuals at the same time. Whereas Sanger sequencing was able to produce 300,000 base pairs (bp) per run at most, NGS can sequence as much as 1,800 Gb of DNA (1.8x1012 bp) in one run. This is a very important development.

As the technologies evolve, the list of applications for NGS is continuing to grow; rare disease diagnosis being one area that has particularly benefited. In the case of genetically heterogeneous diseases, it is possible to sequence the exons of all known genes simultaneously. This allows completeness and substantially reduces turnaround time when compared with the consecutive analysis of different genes by Sanger sequencing. Medium (0.1-3 Gb/run) or high (10-100 Gb/run) throughput equipment is sufficient to analyze panels of 10-100 genes, or whole genes (exons, introns, regulatory sequences), in many (10-100) patients.

In the case of certain pathologies where a very high number of genes (100-500) need analyzing, or where defining a panel is too difficult or even impossible (for example, dysmorphology), sequencing a patient's exome (all exons of the 23,000 human genes) will provide a diagnosis in about 25 percent of cases. Sequencing the whole genome (3 billion base pairs) may therefore represent an alternative, because it provides a better covering of the exome as well as access not only to the gene coding regions but also to the introns, regulatory sequences, and intergenic regions where mutations with long-distance effects on gene expression can be located.

In addition to detecting point mutations, NGS is also able to detect and finely characterize gross gene or genomic rearrangements, such as deletions, duplications, inversions and translocations. So, we see NGS entering the field of cytogenetics where I believe it will eventually become a valid alternative to classical cytogenetic techniques. Here, copy number variations (CNV) are characterized by a drop (deletion) or an increase in the number of times a region of the genome is read in a given individual when compared with controls. Hence small (several kilobase pairs, typically comprising only one or several exons of a gene) or large (> 10 kilobase pairs or more) CNVs can be detected using NGS. Whole genome sequencing allows precise definition of the breakpoints of CNVs, inversions, translocations, and highly complex rearrangements that cannot be characterized by any other technological approach.

The power of NGS can be further seen in ultra-deep sequencing, where each DNA base can be sequenced many times (>1000). Using this approach, we can detect rare mutational events in a sample. This is

very interesting for oncology as it identifies mutations present in a restricted fraction of cancerous cells and in diseases where mutations are present as a somatic mosaic in subpopulations of cells.

Finally, NGS is a powerful approach for non-invasive prenatal testing (NIPT) of aneuploidies. Considering that fetal DNA represents five to 10 percent of freely circulating DNA in maternal blood, Trisomy 13, 18 or 21 can be diagnosed with almost 100 percent specificity and efficiency on a simple blood sample. This avoids invasive amniocentesis or trophoblastic samplings, which are associated with a one percent risk of miscarriage. This constitutes a significant progress for at risk pregnancies.

I have discussed just a few ways in which scientists and patients are benefiting from the incredible progress that has been made in molecular genetics. It's so exciting to be working in this ever-evolving field of healthcare, and there is still so much yet to come…

Color in Focus

Color calibration paves the way to large-scale digital pathology rollout

By Elizabeth A. Krupinski, professor of medical imaging, University of Arizona, Department of Medical Imaging, Tucson, Arizona, USA.

Digital pathology or whole slide imaging (WSI) is a valid and reliable method for interpreting images at a distance, enabling expert interpretation of pathology cases in remote and/or medically underserved areas. It is also an efficient and effective means of obtaining second opinions, and it's useful for teaching too. Although there is significant progress in the technologies used to acquire the images and the displays for viewing them, there has been less progress in developing methods for standardizing the presentation of WSI.

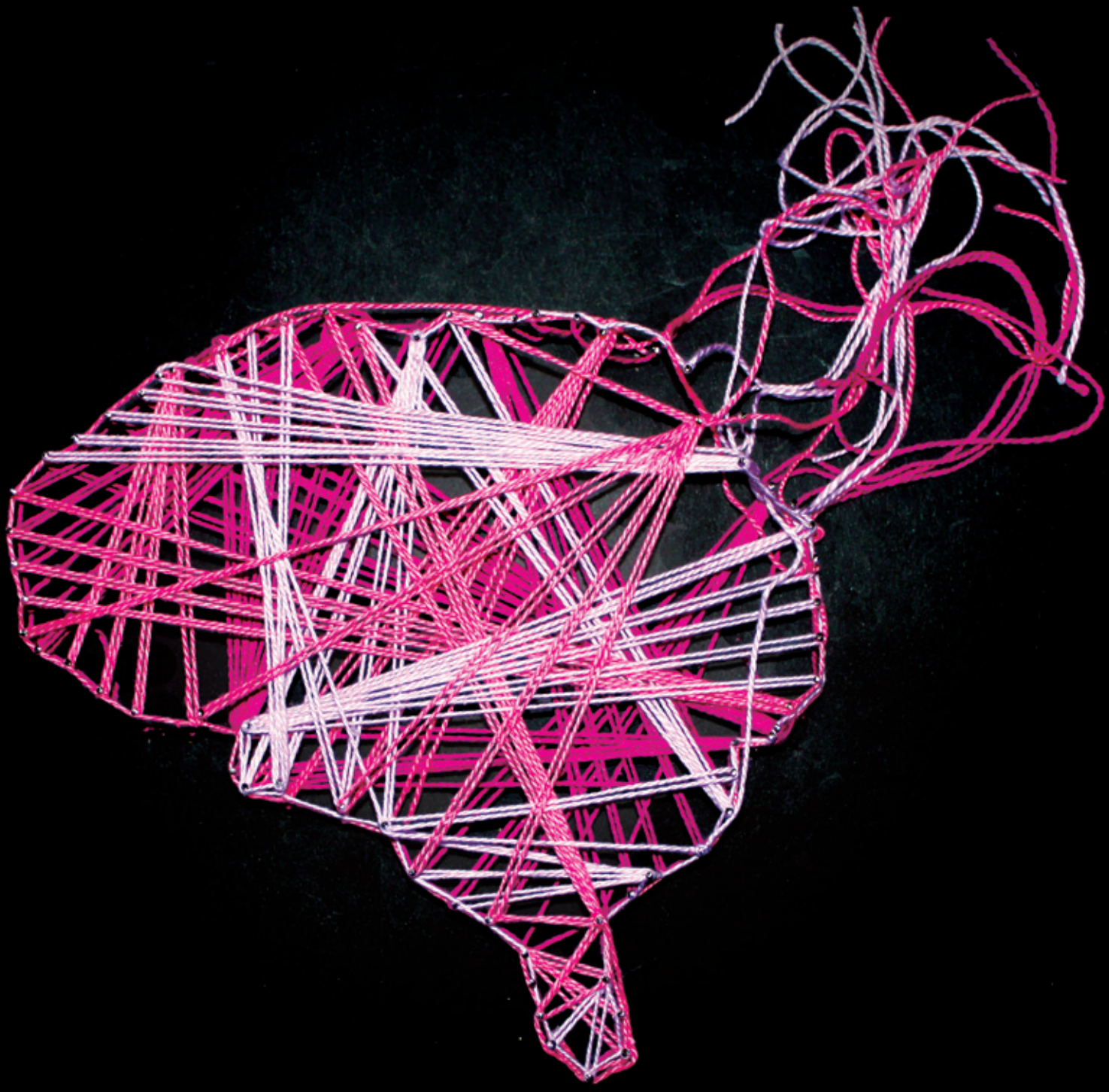
Radiology faced this problem when radiographic images first went digital. In a bid to solve the problem, the DICOM GSDF (Digital Imaging and Communications in Medicine Gray Scale Display Function) was developed to standardize the presentation of images so that, no matter what display was used, a given image would look the same. This has been quite successful for gray scale radiographic images, but pathology WSI (as well as other types of medical images, such as those from ophthalmology and dermatology) are inherently colored in nature and so require color not monochrome displays! Although there are methods available to calibrate color displays, there is no widely accepted or validated color calibration method for use in medical imaging.

Very few studies have assessed the impact of color display calibration on WSI interpretation, which caused us to ask the question: does color calibration actually matter? We conducted a study that developed a calibration protocol for color medical imaging applications using WSI as the application. We assessed the impact of this protocol on observer performance – pathologists viewing and providing diagnoses on a set of cases. We used 250 breast biopsy WSI regions of interest (half malignant, half benign) and showed them to six pathologists, once using the calibration protocol and once on the same display in its "native" un-calibrated state. We measured diagnostic accuracy and time to render a decision. With the calibrated versus un-calibrated display, we found that there was no significant difference in interpretation accuracy. However, when assessing speed of case interpretation, we found that this was significantly faster for the calibrated display compared with the un-calibrated display; cases were interpreted almost two seconds faster with the calibrated display.

Admittedly, there are several other factors that contribute to viewing and interpretation time of cases in clinical pathology, but if by simply using the proper calibration method you could reduce even one contributing factor, it should make a significant difference in overall efficiency and acceptance of WSI. There is also growing evidence in radiology that spending long workdays interpreting digital images increases fatigue and visual strain, which leads to increased errors. If WSI becomes more widely integrated into routine clinical practice, why wouldn't fatigue become a problem here as well? I think it will, and so we need to start thinking about ways to avoid reader fatigue in WSI applications.

We were rather surprised that the calibration method did not have that much of an impact on diagnostic accuracy but there are potential reasons for this. We only used breast biopsy specimens with hematoxylin and eosin staining. There are numerous other stains of various colors that are used on a wide variety of tissues. We may not have selected a tissue and stain where color contributes significantly to diagnoses – other image features may be more dominant. Other tasks do require careful color display calibration so we need to conduct more studies.

WSI is clearly going to be more widely adopted, especially if the FDA clears a path for approval of novel acquisition and display devices. Some of the concerns the FDA has are related to color reproducibility and standardization. In my view, we need more studies to assess the impact of display standardization on diagnostic performance to speed this process up and get WSI into everyday routine pathology as quickly as possible!



Unraveling Alzheimer's

Our understanding of the mechanisms that trigger Alzheimer's disease is growing fast, but many secrets still lurk in the shadowy recesses of the brain. How close are we to plotting a course through the dark matter – and ending decades of failure in diagnostic and drug development? Meet the researchers negotiating the tricky path to translation.

Dementia is a uniquely terrifying prospect for most of us, more frightening even than the dreaded C word. Cancer is an enemy to be battled, but Alzheimer's disease is a slow, creeping tide – there is no battle to be fought, and there are no survivors. It strikes at the heart of who and what we are – erasing our memories and stealing our very identity. As a society, fear (and even a little superstition) means that we often shy away from discussion of Alzheimer's disease, instead joking (nervously) about "senior moments." The reality is one we'd rather not dwell on.

As the population ages, Alzheimer's disease and other dementias are becoming increasingly hard to ignore. Already, Alzheimer's is the sixth largest killer in America, and estimates from the Alzheimer's Association put the number of Americans living with the disease at 5.3 million. Without a medical breakthrough to help prevent or slow the progression of the disease, this number is expected to rise to 13.8 million by 2050 – with similar trends predicted across the Western world. That's not just bad news for the millions of people affected directly or indirectly – it's a huge challenge for already overstretched healthcare budgets, as shown in "Counting the Cost" (page 26).

Alzheimer's disease is already costing some countries hundreds of billions of dollars, but comparatively little research funding is being dedicated to finding treatments. The US government allocated federal funds of around $5.4 billion in 2014 to cancer research, and $1.2 billion for heart disease – while research on Alzheimer's and other dementias came in at only $666 million. Director of the Massachusetts Institute for Neurodegenerative Disease, Bradley Hyman, who recently revealed another link in the chain of Alzheimer's pathophysiology (page 22), admits that any scientist will say that their field does not receive enough funding. "But given the impact that Alzheimer's disease will have on our society over the next 10 years, it is woefully underfunded. What is certain is that the cost of doing nothing is going to be immense."

Perhaps the lack of obvious progress makes funding Alzheimer's research a tough sell for results-focused politicians. After all, the history of the field is littered with failed research programs, with only a few approved drugs on the market, none of which can prevent progression, only temporarily reduce symptoms. Why is it so hard to develop treatments for Alzheimer's? Partly, it's because we lack a complete understanding of how the disease develops on a molecular level, says Michela Gallagher, a Professor at Johns Hopkins University who will shortly be initiating a Phase III trial with the aim of slowing progression of mild cognitive impairment (MCI) into full-blown Alzheimer's (page 27). "Much of the basic science has stemmed from genetic studies of inherited forms of Alzheimer's disease, which make up only one percent

of all cases," says Gallagher, "but in fact the pathology may look quite different to the more common sporadic form of the disease. We know a lot about the complexity of the players in the brain, but not how they all fit together."

While treatment remains elusive, researchers know more than ever before about how and why Alzheimer's occurs. Our understanding is moving fast, and some of our initial assumptions are being proved wrong, according to Ewan McNay, who tells us about his pioneering work on insulin in the brain on page 28. Take amyloid plaques, one of the defining features of the disease since 1906 when Alois Alzheimer reported "a peculiar severe disease process of the cerebral cortex," and still a diagnostic criterion for Alzheimer's disease today. It's becoming increasingly clear that plaques may not be what is causing damage. In fact, they may be evidence of a self-defense mechanism – the brain's attempt to sequester harmful extra amyloid protein. In other words, plaques are a sign – not a cause – of damage in the brain. James Connor's research into iron's influence in neurodegeneration may provide a crucial piece of the puzzle (see page 24). So what is it that starts the descent into Alzheimer's? Why is progression so unpredictable? And, above all, how can we stop it?

Many believe the answers now lie within our grasp. Author Terry Pratchett, who died aged 66 from a rare form of the disease, wrote in 2008, "I believe the D-day battle on Alzheimer's will be engaged shortly and a lot of things I've heard from experts strengthen that belief. It is a physical disease, not a mystic curse; therefore, it will fall to a physical cure. There's time to kill the demon before it grows."

Here, we speak with four scientists who are expanding our knowledge of Alzheimer's – in the hope of one day defeating it.

UNTANGLING TAU

As a neurologist, Bradley Hyman has seen first-hand the impact of Alzheimer's disease on patients and their families. His lab examines the genetic and neuropathophysiological factors that play a part in the disease, and recently discovered a rare pathological form of the tau protein, which may hold the key to how Alzheimer's spreads through the brain.

By Brad Hyman

Alzheimer's disease is a truly dreadful illness, but on a scientific level it is a fascinating one. The whole field of behavioral neurology – studying brain–behavior relationships – has learnt a great deal from these patients. Specifically, the way that people lose function during the course of the disease has taught us much about how the brain works, and raised many intriguing questions (many of which remain unanswered). An Alzheimer's patient can remember in crystal-clear detail something that happened a decade ago, but can be completely unable to recall what happened three minutes ago. Patients often lose very specific aspects of cognitive function (for example, their ability to do sums or understand language) while seemingly more complex faculties remain entirely intact. We are now starting to understand exactly why and how this happens.

The past decade has seen an extraordinary series of breakthroughs. We have mapped at least a dozen genes that clearly impact the relative risk of Alzheimer's disease, although we understand the mechanisms of action of just three of them. When I started out as a neurologist, we could only diagnose Alzheimer's disease post mortem. Now we can use spinal taps to test for biomarkers or PET scans to look directly at the brain, and diagnose patients while they are alive. We have a much better handle on the biochemistry and molecular biology of the lesions visible in the brain, and we now know that there is more to the story than what we can see through the microscope. We have also come to appreciate just how long the pre-symptomatic phase of the disease is – around 15 years.

But there's still a lot we don't know. Some patients deteriorate dramatically over three or four years, while others decline very little over 20 years. We know a lot about how amyloid builds up and forms plaques, but we don't know what triggers progression from mild cognitive impairment to dementia. And we don't know how to prevent it.

There is a mountain to climb – but it is not insurmountable. We recently made a discovery that may shed light on how the disease progresses at a molecular level (1). Neurofibrillary tangles made up of an abnormal form of the tau protein are one of the hallmarks of Alzheimer's disease, disrupting the cellular microbtubule network and eventually killing neurons. They accumulate in a unique subset of neurons, spreading through the brain in a very distinct, hierarchical pattern, which maps to a stereotypical

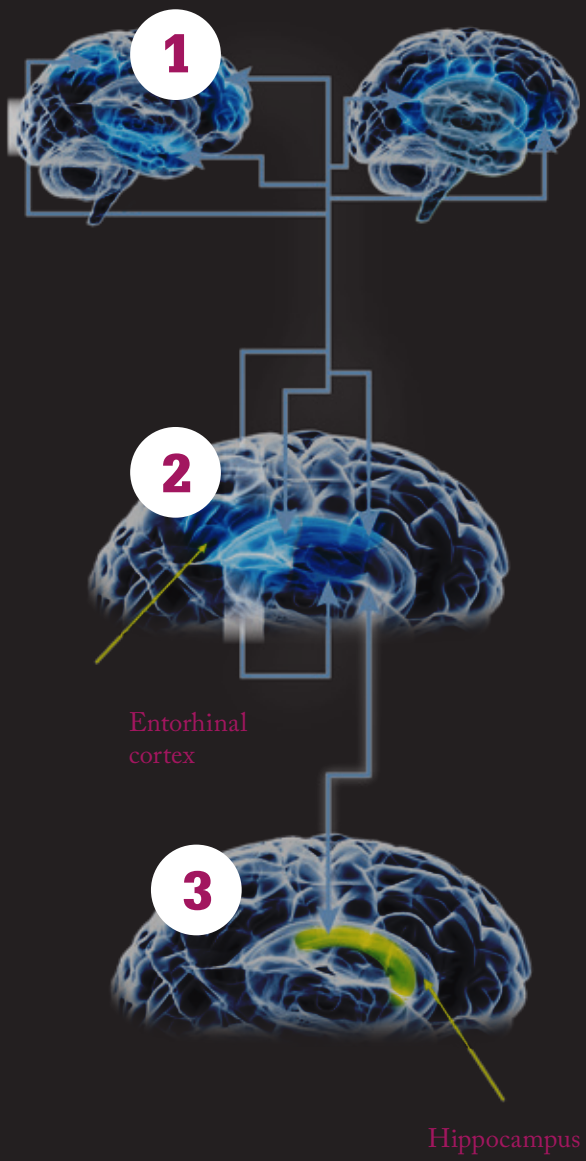
pattern of clinical symptoms. The hippocampus and entorhinal cortex – areas of the brain involved in memory formation – are the first areas affected, causing the typical early symptoms of forgetfulness or short-term memory problems. Then as the tangles spread into other parts of the brain, disturbances in language, spatial orientation and judgement start to appear. Our study was an attempt to understand the underlying biology of that progression. Neurons that develop tangles tend to be anatomically connected to each other, but the precise mechanisms behind this spread have been unclear. Mounting evidence suggests that tangles propagate via the transport of abnormal tau from an affected neuron to its neighbors. Once inside a healthy cell, abnormal tau can induce other tau molecules to assume the same pathologic form – like a prion. We have now identified a very rare form of tau that we believe is behind the propagation of abnormal tau between neurons.

We isolated various forms of tau from mouse and human brains, with and without Alzheimer's disease, and screened for the properties we hypothesized would be necessary for propagation between neurons. Eventually, we found a very rare species of tau which fit all the criteria. Normal tau is an unfolded protein with only a few phosphorylation sites and a molecular weight of 40K. The species we detected had many more phosphorylation sites, a molecular weight of 500K and a specific structure not found in normal tau. We also found that abnormal tau was transmitted along the axons to neighboring neurons.

It appears that this rare subset of tau could play a pivotal role in progression of the disease. If we could block the transport of abnormal tau between neurons, it could be a way to slow progression. The next step is to find out what makes this rare form of tau so unique and what aspects allow its uptake, transport and release. Neurons often create misfolded proteins under normal circumstances, but the abnormal proteins are usually picked up and rapidly degraded by specialized pathways. Why this form of tau escapes that process is a deep mystery. If we can find the answer, we might be in a position to intervene and shift that equilibrium, even a little, back towards degradation. Given how long it takes for the disease to develop, a small shift might be sufficient. After all, cutting your cholesterol by just 10 percent is enough to delay heart disease. We now have the tools to diagnose Alzheimer's disease at an early stage or even pre-symptomatically. If we could halt propagation between neurons at the first whisper of the illness, before it starts to cause symptoms, the hope is that we could stop the spread of tangles and hence the progression to dementia.

Bradley Hyman is Director, Massachusetts Alzheimer's Disease Research Center, Co-Director, MGH Memory Disorders Unit and John B. Penney Jr. Professor of Neurology, Harvard Medical School, USA.

The Hippocampus and Memory



1. Your experience and events of your life are represented in large-scale networks in your brain.

2. The entorhinal cortex is the conduit for the formation of episodic memories by the hippocampus.

3. The entorhinal cortex and hippocampus are early sites of Alzheimer's pathology and degeneration of neurons, coinciding with the signature symptomatic feature of Alzheimer's, in which episodic memory impairment worsens.

IRON'S INFLUENCE

James Connor's interest in iron's influence on neurodegenerative disease was ignited during his postdoctoral training. Since then, he has led teams who have pioneered the field of iron in neurobiology. His research has unearthed the most prevalent genetic mutation in a subset of Alzheimer's patients and his continued discoveries in genetic and environmental interactions in Alzheimer's could one day support the early diagnosis, and even the prevention, of the disease.

By James Connor

I first began studying the impact of enriched environments on aging changes in the brain during my PhD studies at UC Berkeley and it was as a postdoc at Boston University that I became particularly interested in iron in the brain.

Thanks to our early work, it is now generally accepted that iron plays a key role in the development of Alzheimer's. There are still, however, several key knowledge gaps that need to be filled.

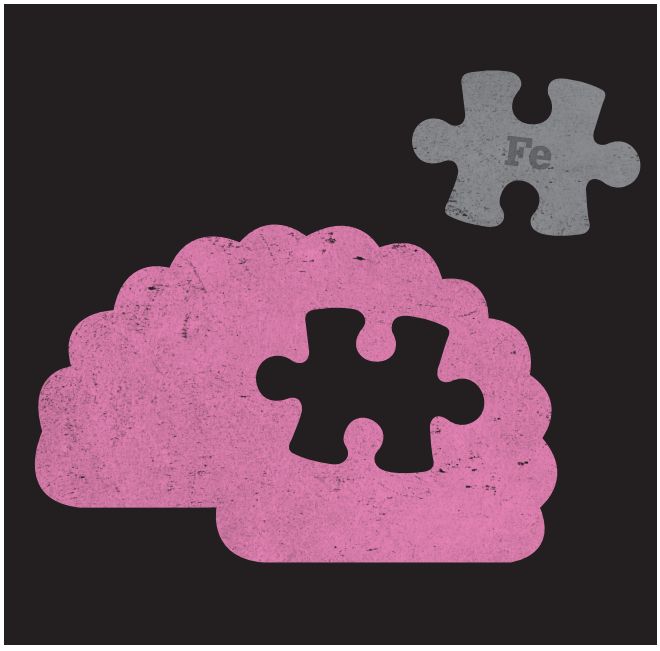
So let's look at what we know. We have shown that iron dyshomeostasis occurs in the brains of Alzheimer's patients and this excess iron promotes plaque and neurofibrillary tangle formation. Crucially, our work has allowed us to identify a mutation in the *HFE* gene that we now know to be present in around 30 percent of Caucasians with Alzheimer's. We began looking at the *HFE* gene and its mutations because of its association with the iron overload condition known as hemochromatosis. When we first started no one thought the *HFE* mutation would change brain iron status, but we and others have shown that it does. We also know that the mutation, in the presence of the *APOE4* allele, increases a person's likelihood of developing the disease. We have also identified that the *HFE* gene variant impacts white matter status, which has been shown to affect cognitive performance. These are groundbreaking discoveries. But we are faced with many questions: what genetic-environmental interaction predisposes a person to Alzheimer's? How can we restore iron homeostasis given the critical importance of iron in so many enzymatic reactions? How likely will someone with the *HFE* mutation go on to develop Alzheimer's? What processes are specifically disrupted that lead to iron dyshomeostasis? The field is so complex. What we need is to build a profile of the disease and all of the different influences that determine its development, and this is what I am focused on.

To demonstrate the challenges that we are faced with, let's hypothetically suggest that dietary iron intake is reduced, or iron chelators administered, in at risk populations. Studies have shown a correlation between anemia and cognitive decline in the elderly. So, although giving iron chelators is showing some promise in clinical trials in the UK and France in Parkinson's disease, this may not be right for Alzheimer's patients.

If we look at cholesterol; there are three steps in the formation of cholesterol in the brain that are absolutely dependent on iron, which means that its synthesis is going to be disrupted if there is iron dyshomeostasis in the brain – and there are good examples in the literature of the association between decreased brain cholesterol and Alzheimer's. As a result, we are now questioning if cholesterol-lowering statins should be used in those people who are at risk of neurodegenerative disease, and data are beginning to emerge that urge caution in their use in Parkinson's disease, multiple sclerosis and in ALS. Researchers haven't looked at this issue in Alzheimer's yet, but I think the iron and cholesterol story needs to be visited pretty quickly and aggressively in a population-based study. We are very excited by the outcomes of some of our recent work, which we will be publishing soon, where we have shown a disruption in brain cholesterol in an animal model of the *HFE* mutation with similar findings in humans that have the same mutation.

If we consider what we know about *HFE*; although we have shown that a mutation in this gene is present in 30 percent of people with Alzheimer's, around 10–15 percent of the general population also carry it, the majority of whom are unaffected by hemochromatosis, and this is puzzling. So we're working to understand if there is a gene-environment interaction that, somewhere along the iron pathway, puts a person in a position that they will develop iron dyshomeostasis and Alzheimer's at some point later in life. We know that this is not a condition that is caused solely by a genetic mutation.

So the $60k dollar question: what precise changes cause the brain to lose iron homeostasis as it ages? Is it genetic? Are there signals in the brain that become altered and make the brain think it needs more iron than it does? We just don't have a good answer yet.



We need to better understand the processes that lead to tangle and plaque formation, and iron's role, and my hope is that these processes will become our therapeutic target. Even though iron is involved in forming those pathogenic processes, we may want to leave the actual iron alone. Chelating iron is probably not the answer, and probably neither is limiting iron in the diet. The race is on to find an effective therapeutic target. Is it an iron chelator, an anti-amyloid vaccine, an anti-oxidant, an anti-inflammatory? Is it a combination of more than one? This is what we don't know. But what we do know from testing in clinical trials is that sometimes things work in some patients but not all. I believe at some point along the biological pathway to dementia, it becomes a common pathway, and the challenge for us as scientists is to find that common point. If we're lucky enough to do that, we'll have found our therapeutic target.

Our biggest opportunity to make an impact in Alzheimer's right now is to catch it early and to limit any cognitive decline before it becomes problematic. The way we'll do that is to combine genotyping, biomarkers and imaging, with iron and cognitive testing. A blood test alone won't cut it; there is a lack of agreement on the serum levels of iron and iron proteins and the impact of those levels in the brain. The same is true of copper and probably cholesterol. What's in the blood doesn't always inform us very well of what is going on in the brain, so we'll need all of those additional pieces of information.

My ideal vision would be for people from the age of 50 to get full genetic, image and cognitive profiling and for us to use the knowledge we gather from those assessments to highlight a person's risk of later Alzheimer's development. We can then provide the adequate guidance to delay and even perhaps prevent progression to full blown dementia. Right now, we're not able to do that. We need to define a risk profile first. It's the missing piece of the puzzle that's needed for early diagnosis and, importantly, prevention. I think we'll get there in the next 20 years though.

Looking back on my career, I feel proud that our research has defined a clear relationship between iron and Alzheimer's; when we first started out, nobody was looking at iron in the brain and its connection to neurodegenerative disease. What would really make me feel good at the end of all of this would be to know that we've really made a significant contribution to elucidating those biological processes that lead to dementia. I'm hoping that all of our work on genetic profiling, the influence of iron on tangle and plaque formation, will help us get to that therapeutic target. I wish I could say to someone that if you have the *HFE* mutation then you are going to get Alzheimer's and we can fix that, but that is totally unrealistic. I am absolutely focused on researching this gene-environment interaction. I want to find if there is some form of intervention strategy around iron that makes people better. If we do that, I will feel like our research has made a contribution and I will be happy.

James Connor is Distinguished Professor of Neurosurgery, Neural and Behavioral Sciences and Pediatrics, Vice Chair of Neurosurgery Research and Director of the Center for Aging and Neurodegenerative Diseases at Penn State University, MS Hershey Medical Center, USA.

Key Published Papers

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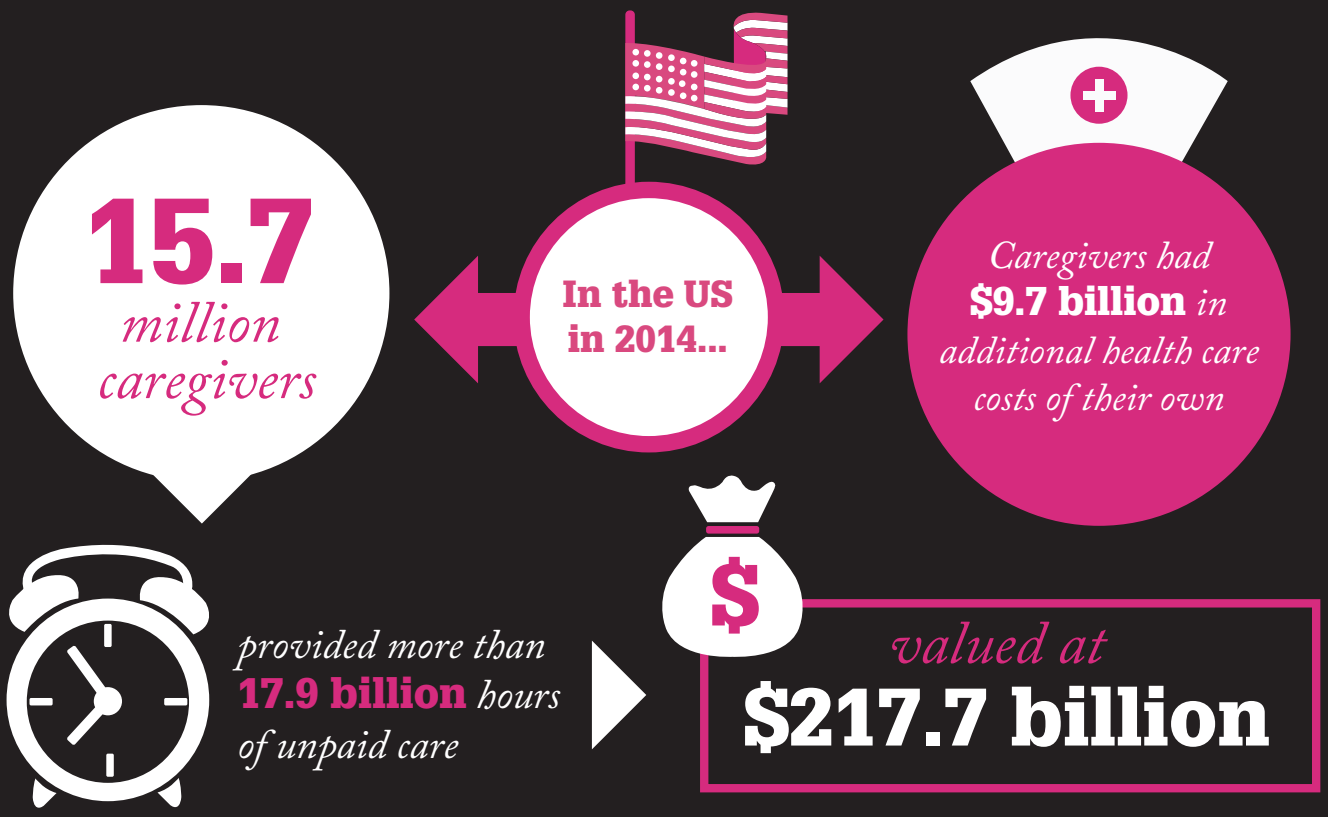
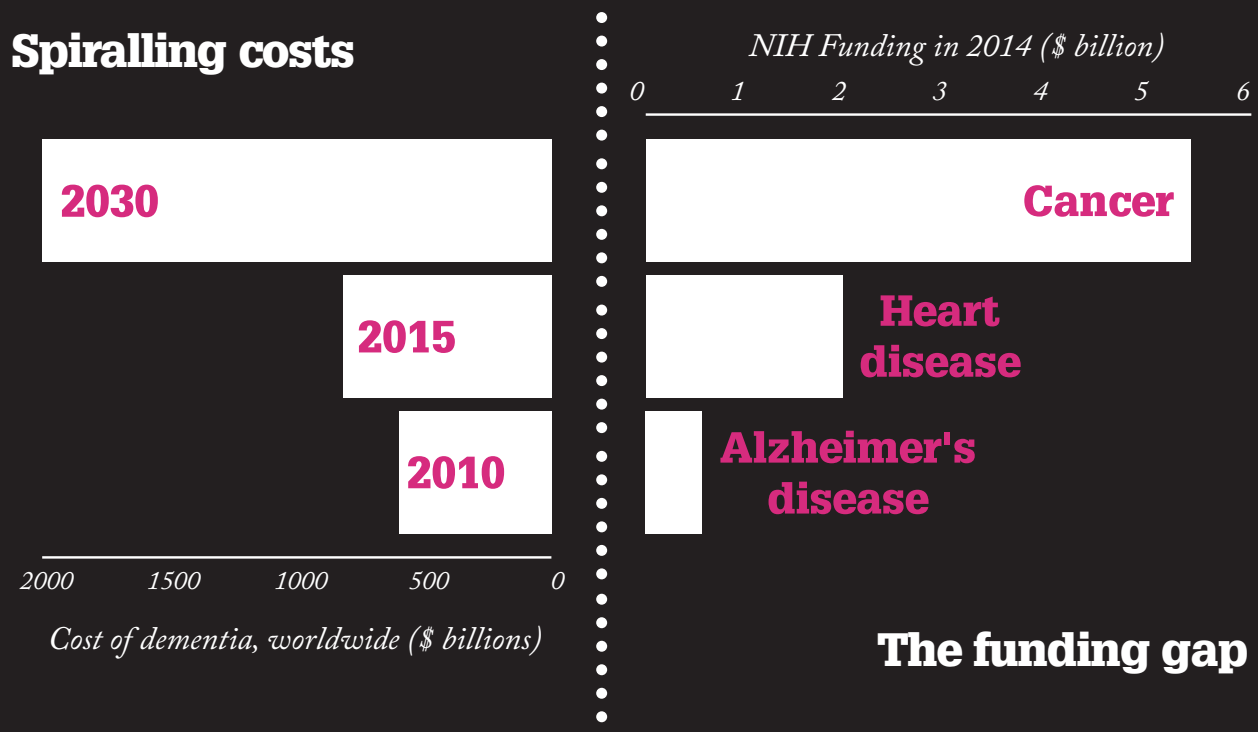
Counting the Costs





THE OVERACTIVE BRAIN

Michela Gallagher was studying aging in lab rats when an unusual finding launched her on a translational journey that now sees her poised to initiate a Phase III trial of a drug to delay the onset of Alzheimer's dementia.

By Michela Gallagher

In some ways, I've always been a neuroscientist – I was always very interested in memory and I became fascinated by the frequency and commonality of memory loss as people age. Today, my work lies at the intersection of normal age-related changes in memory, the pre-dementia state known as amnesic mild cognitive impairment (aMCI) and the full-blown symptoms that we recognize as dementia. Alzheimer's doesn't develop overnight – it's an exceedingly slow progression from normal aging to dementia. The transitional phase of aMCI in which memory impairment is somewhat greater than expected for a person's age can last a decade before progression to the point of a clinical diagnosis of early Alzheimer's dementia.

I didn't set out to study Alzheimer's disease. My work in this field hinges on a surprising finding from our studies on memory loss in aging rodents. In rats with memory loss, a subset of neurons critical for memory in the hippocampus are overactive. Other researchers made the same observation in patients with aMCI. This happens to some extent in normal aging but when Alzheimer's pathology begins to emerge, hippocampal hyperactivity is driven higher and higher. Initially, it was thought that this might be a compensatory mechanism – the neurons working harder to try to hang onto making memories and overcoming damage. But our work in rats told us that this wasn't true. To find out what the functional significance was, we had found ways to bring the activity down to normal levels. The result was to improve the memory deficits usually seen in those animals, suggesting that overactivity was causing rather than compensating for damage.

To translate the work into human patients, we needed to find a drug with a good safety profile that would dampen the overactivity in these neurons. After much searching, we found that levetiracetam – an FDA-approved and commonly prescribed atypical anti-epileptic – fitted the bill. Our initial studies in animal models and later in patients with aMCI have shown proof-of-concept and efficacy, with aMCI patients performing better in memory tests after treatment (2). Now, we have approval to start a larger-scale Phase III randomized clinical trial – HOPE4MCI - treating patients with aMCI with a low-dose, extended-release form of levetiracetam for 18 months (3). We're still in the planning stages but we hope to start recruiting patients this year. We are using a dose of levetiracetam 12-times lower than that used in epileptics, so toxicity is not a major concern, but this is the first drug to attempt to slow the progression of aMCI into Alzheimer's dementia by reducing overactivity in the brain.

> *"This is the first drug attempt to slow the progression of aMCI into Alzheimer's dementia by reducing overactivity in the brain."*

Most clinical trials of Alzheimer's therapies to date have involved people who already have a diagnosis of dementia, and almost all of those trials have failed. A few have an impact on symptoms, but at that stage of clinical dementia there is already a lot of irreversible damage in the brain. We're trying to slow or stop neurodegeneration at an early phase of the disease, rather than reverse it. The Alzheimer's Trajectory Report from the US Alzheimer's Association suggests that a drug that could delay progression to a clinical diagnosis of Alzheimer's disease by just five years would cut the prevalence of the disease by 40 percent and save the US economy over $300 billion.

It has been an amazing journey – from life as an academic scientist to founding a company and running a major clinical trial. I love science and making discoveries, and I find working with my students in the lab glorious; I often call the lab my playpen! The process of translation is sometimes a hard slog in comparison, but if we can make a difference to patients' lives, it will be worth it.

Michela Gallagher is Krieger-Eisenhower Professor of Psychology and Neuroscience, Johns Hopkins University and Founder of AgeneBio, USA.

FOOD FOR THOUGHT

When Ewan McNay was eight years old, he decided that he wanted to do two things: live forever and find out how the brain works. The secret to eternal life is a work in progress, but his research on insulin resistance in the brain has added a new dimension to our understanding of Alzheimer's disease.

By Ewan McNay

Alzheimer's disease is a story that goes from a single atom all the way up to a human and even societal scale. My work focuses on the link between Alzheimer's and insulin-resistant diabetes, which first came to light in the late 80s and has progressed over the past 20 years to a point where we have a good understanding of at least some of the molecular bases linking the two diseases, and can even take an educated guess on how we might be able to intervene. People with Type 2 diabetes are up to seven times more likely to develop Alzheimer's disease, but you don't have to be diabetic for insulin resistance to affect the brain. It's perfectly possible to have impaired brain insulin signaling, even if your body is not systemically insulin resistant – an observation confirmed in post mortem studies of Alzheimer's affected brains. We suspect that, in some patients, brain insulin resistance is a consequence of a whole-body disease, while other patients have what is effectively a brain-limited form of diabetes – sometimes referred to as "Type 3 diabetes".

Both beta amyloid and insulin are broken down by insulin degrading enzyme (IDE). In Type 2 diabetes, the body fails to respond to insulin, so the body produces more in an attempt to overcome this lack of response. IDE preferentially breaks down insulin over beta amyloid so beta amyloid builds up in the brain while IDE is occupied breaking down insulin. That accumulating beta amyloid causes several problems, one of which is to bind to insulin receptors and further dampen insulin signaling – a downward spiral. Specifically, beta amyloid impairs a glucose transporter called GluT4, which removes glucose from the blood in response to insulin. In the brain, GluT4 is found in a very specific subset of brain pathways – one of which is the hippocampus, key to memory – which matches up with where you see the deficits in Alzheimer's and some other dementias. The ability of the hippocampus to respond to intellectual challenges by increasing glucose metabolism is impaired or absent in the Alzheimer's brain, causing cognitive impairment in these patients.

"The antibody fragments mop up oligomeric beta amyloid – the toxic form that eventually forms plaques – and, as predicted, cognitive impairment was reversed."

We were the first to discover both that insulin acts to regulate hippocampal metabolism and memory, and that if you block endogenous insulin in the rat hippocampus, it causes a big memory lapse. We then showed that if you introduce excess beta amyloid, you see similar impaired insulin signaling, impaired metabolism, and impairment in memory. We predicted that if we examined the brains of rats with the equivalent of Type 2 diabetes, we would see elevated levels of beta amyloid, impaired brain translocation of GluT4 and reduced glucose metabolism. And sure enough, we saw all three.

Next, we asked whether blocking beta amyloid in the hippocampus could reduce cognitive impairments. In our most recent work, we showed that it did, at least in animal models. We used a rat model of Type 2 diabetes, feeding them a high-calorie, high-fat diet to induce obesity and insulin resistance: we had previously shown that this treatment also causes hippocampal accumulation of beta amyloid. As expected, the rats began to develop cognitive impairment. Working with a protein engineer at Rensselaer Polytechnic Institute we produced antibody fragments targeted against particular forms of beta amyloid. Delivered directly into the hippocampus, the antibody fragments mop up oligomeric beta amyloid – the toxic form that eventually forms plaques – and, as predicted, cognitive impairment was reversed (4).

Unfortunately, we are currently administering these antibody fragments directly into the brain – not a plausible intervention

route for humans. It's very unlikely that we could give that antibody fragment orally or even intranasally. If someone can crack the challenge of how to get the appropriate drug treatments to the brain then that conversation can start to take place. Right now, there is no way for us to do that, but our studies do suggest that there are lifestyle choices we can make that are likely to reduce our risk of dementia in later life. Your brain is not floating in a vat: it is part of the biological system of your whole body. Habits that affect the rest of your body can and do have long-term consequence in your brain. At the moment, the evidence suggests that you are less likely to get Alzheimer's if you:

- Maintain a healthy weight to prevent Type 2 diabetes
- Exercise regularly
- Drink coffee and red wine (in moderation) – both are linked to reduced risk of dementia, possibly because the antioxidants they contain protect the brain from damage

Right now, we're working on confirming our hypothesis that GluT4 is a point of common action by which insulin boosts, and beta amyloid impairs, brain metabolism and memory. If it is, there could potentially be therapeutic benefit in upregulating GluT4 to allow more fuel to reach your brain cells. Tight regulation of brain metabolism is essential – if you just put the brain into overdrive, you might get a temporary increase in function but end up causing oxidative damage to the cells – imagine permanently running your car at 7000 rpm... A better understanding of the molecular-level interactions will tell us whether there might be some way to improve function without "blowing the engine".

Ewan McNay is Associate Professor of Behavioral Neuroscience at the University at Albany (SUNY), USA.

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One to watch

Shraddha Sapkota, University of Alberta, caused a stir at the 2015 Alzheimer's Association International Conference with her work on analyzing saliva biomarkers to detect Alzheimer's disease. We caught up with her to find out more.

Could you briefly describe your work?

We used cutting-edge liquid chromatography–mass spectrometry at the University of Alberta to analyze saliva samples from patients with normal cognitive function, with mild cognitive impairment or with Alzheimer's disease. We were looking for perturbations in metabolites that represent early biological markers of disease processes. Metabolomic analyses allow us to sort through thousands of metabolites to determine the top biomarkers that discriminate different clinical groups. In this case, we were able to clearly discriminate patients with Alzheimer's disease from those experiencing mild cognitive impairment and the other comparable non-impaired older adults. Analyses also provided information about the possible biological pathways from normal aging to Alzheimer's disease. Finally, we used the detected biomarkers to predict preclinical manifestations of the disease – specifically, early cognitive decline.

What's next?

We are working towards the identification of key biomarkers that reflect the earliest changes indicative of preclinical Alzheimer's disease and possible mechanisms. These changes can begin to appear more than 10 years prior to clinical diagnosis, so we are aiming for tests that can be used in middle-aged adults, especially those experiencing some memory impairment. However, we are still at the early stages and much work is needed before we can include saliva tests for the general population.

Could it help advance research?

We have received a lot of interest from the research community. Aside from diagnosis, salivary metabolomic analyses could promote our understanding of the mechanisms from normal aging to Alzheimer's disease – and provide objective biological endpoints to compare different risk factors and interventions. Saliva is very easy to collect and transport, which could enhance participation in remote centers and diverse populations.

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Telepathology Heads Up

Wearable devices show promise for improving pathology workflow

By Liron Pantanowitz

The revolution in wearable technology has brought a surge of devices to the market, including Google Glass (Figure 1), Microsoft Band, Apple Watch, and gesture-controlled armbands. I'm particularly interested in Google Glass (Glass) having used the technology myself – they are eyeglasses (spectacles) with an optical head-mounted display that allow users to connect directly to the Internet or tether the device wirelessly to a cell phone.

Glass has a dual-core processor, touchpad control, microphone, camera and prism display. The optical display places a virtual screen in front of the user's face and glassware (software) installed on the device includes several well-known Google applications (for example, Gmail), as well as novel apps. Multiple users can connect, chat and share videos using Google hangouts too.

Figure 1. Google Glass Explorer edition as acquired by the University of Pittsburgh Medical Center.

Where it all began…

The first "Glass-to-Glass" consultation first took place in 2013, when Dutch surgeon Marlies Schijven successfully communicated with American surgeon Rafael Grossman, live, while performing an operation. Grossman was in a conference center and the consultation was followed live worldwide via a YouTube broadcast. This became the first, proof-of-concept study of the device's use in a healthcare setting. Several studies have since followed and referred to the great potential of Google Glass in healthcare (1,2); supporting hands-free patient interactions, remote consultations, and even live virtual training. Preliminary findings indicate that wearing the device in a clinical setting is well-tolerated by doctors and patients; specific examples include streaming of live vital signs and alarms to a surgeon's Glass device during an operation, or levering real-time access with voice dictation to chart hands-free directly into a patient's electronic health record. Some practical challenges have, however, been noted including low battery endurance, picture quality, hygiene, limited availability of medical apps, and most importantly, data protection issues (3), causing some to question its current utility in the clinical setting and suggesting its confinement to the classroom.

But is Glass of any use in pathology? We decided to tackle the question in our department of Pathology at the University of Pittsburgh Medical Center (UPMC) (4); our aim being to use Glass to develop a hands-free imaging modality integrated into gross pathology workflow. We acquired an Explorer edition of Glass, connected it to the Internet using our institution's secure Wi-Fi system, and we tested it for use in autopsy, gross telepathology and telemicroscopy (Figure 2). And

At a Glance

- *Wearable, digital technology is supporting all areas of healthcare delivery, from pathology to surgery*
- *Google Glass is one such device, which is beginning to show potential in clinical settings*
- *In preliminary studies it has been shown to support telemedicine, hands-free patient interaction, education and training*
- *There are some downsides, though, and modifications are still needed, including issues such as data protection, before a role in clinical care can be clearly defined and accepted*

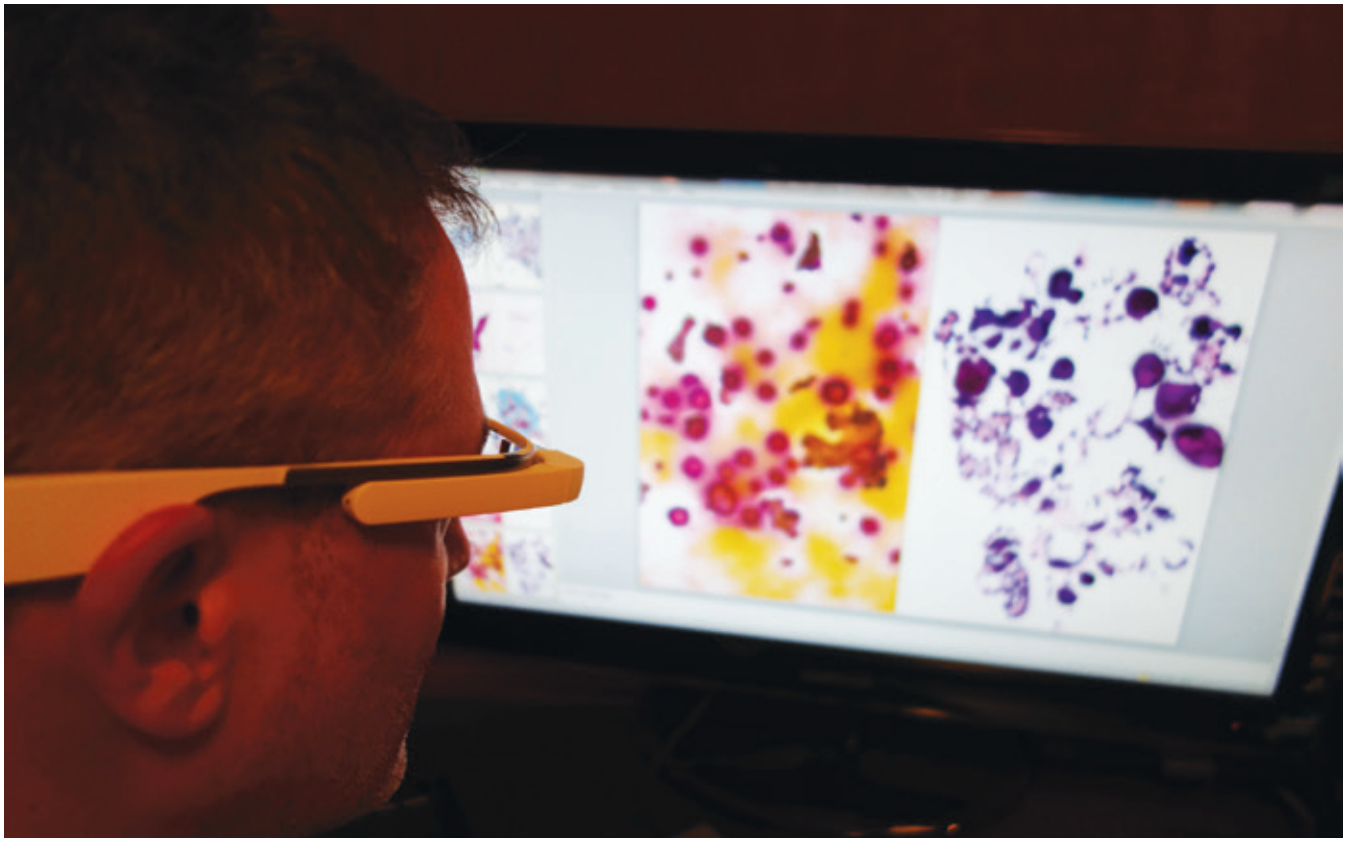

Figure 2. University of Pittsburgh Medical Center pathologist investigates Google Glass for telepathology.

we were pretty impressed. It allowed our pathologists to remotely stream live feeds from our autopsy suite and easily capture hands-free static images of specimens. It was also of value to prosectors who could remotely access pathologists for their assistance while grossing pathology specimens. There were some difficulties encountered in the pathology laboratory, though, including lighting problems, low image resolution, and distracting background noise. But the biggest issue with this technology centers around data security. When streaming data, security is a major concern when it comes to privacy of patient's personal healthcare information. Clearly, while Glass has much potential in pathology, more studies validating its role in clinical practice are needed.

If the aforementioned key issues get addressed, we would likely use Glass on a regular basis. It offers great applications for real-time sharing. Also, like everything else in pathology informatics, it is hard to keep up with the pace of technological advancements. Not only are there already several competitors to Glass (for example, Recon Jet, GlassUp, Epiphany, Telepathy One), but there is also Oculus Rift from Facebook and HoloLens from Microsoft. Only time will tell if these are just gimmicks or if they have true potential for pathology, and even if that potential is proven, will the laboratory community embrace it or see it as a technological leap too far for today's practice?

Liron Pantanowitz is a Professor of Pathology and Biomedical Informatics, Director of Pathology Informatics, Director of the Pathology Informatics Fellowship Program and Director of Cytology (Shadyside) at the University of Pittsburgh Medical Center, Pennsylvania, USA

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Predicting the Impact of Personalization

Kinetic models allow researchers to integrate multiple physiological responses into a single test that predicts patients' likelihood of drug side effects

By Neema Jamshidi

When you think of personalized medicine, what comes to mind first? If you're like many people, you envision a discipline designed to help doctors select the most effective course of treatment for a given patient. Understanding a patient's molecular profile allows therapy to be tailored to a patient's specific risk and response factors. But drug efficacy isn't the only purpose of personalized medicine – it's equally important for ensuring a treatment's suitability, including the potential for side effects.

At a Glance

- *Personalized medicine currently relies heavily on single-factor tests such as enzyme or metabolite measurements*
- *While vital to progress in precision medicine, such tests don't account for the complex behavior of multifactorial systems like cells or whole humans*
- *Whole-cell kinetic models integrate genomics and metabolomics to describe cell properties and predict patient outcomes*
- *These models are not yet clinic-ready, but may one day be powerful tools in personalizing patients' treatment plans*

That vital function is what inspired us to develop a new model for predicting the side effects of drugs, based on genetic and metabolomics measurements taken from patient blood samples.

The building blocks of kinetic models

The first step in our process was to select a cell type to serve as a test subject for our model. We chose the humble red blood cell. This was in part because it's the simplest available human cell, in part because it's so easily accessible from blood samples, and in part because researchers and healthcare providers have a tendency to underestimate the role that these cells play in systemic health and disease processes – including drug metabolism. Both our group and others have a long history of building models of the human erythrocyte to simulate and analyze enzyme deficiencies (1,2). In some of our earlier work, we noticed that these models were able to identify severity of enzyme deficiencies, such as glucose-6-phosphate dehydrogenase (the first enzymatic step in the pentose phosphate pathway) and predict the severity of a patient's susceptibility to hemolytic crisis (by examining their single-nucleotide polymorphisms) (3).

Given the findings from these previous studies, we hypothesized that individual specific models could be constructed using metabolomics profiling from volunteer participants' plasma and red blood cells (4). These were whole-cell kinetic models of erythrocyte metabolism, and we constructed them for 24 healthy individuals – based both on fasting-state plasma and erythrocyte metabolomics and on whole-genome genotyping. By gathering and incorporating both genetic and metabolic information, we were able to use the models to link genotype to protein function. Ultimately, we demonstrated that differences in patients' personalized kinetic rate constants were reflective of differences in physiology – allowing us to spot patients at risk for drug side effects. In our paper, for instance, we used our models to identify individuals at risk of ribavirin-induced anemia, then also identified a particular genetic variation, inosine triphosphatase deficiency, that may protect against it. Constructing similar kinetic models for other patients may give us the ability to characterize individual metabolic variation – helping us choose the most effective and least harmful drug for each patient.

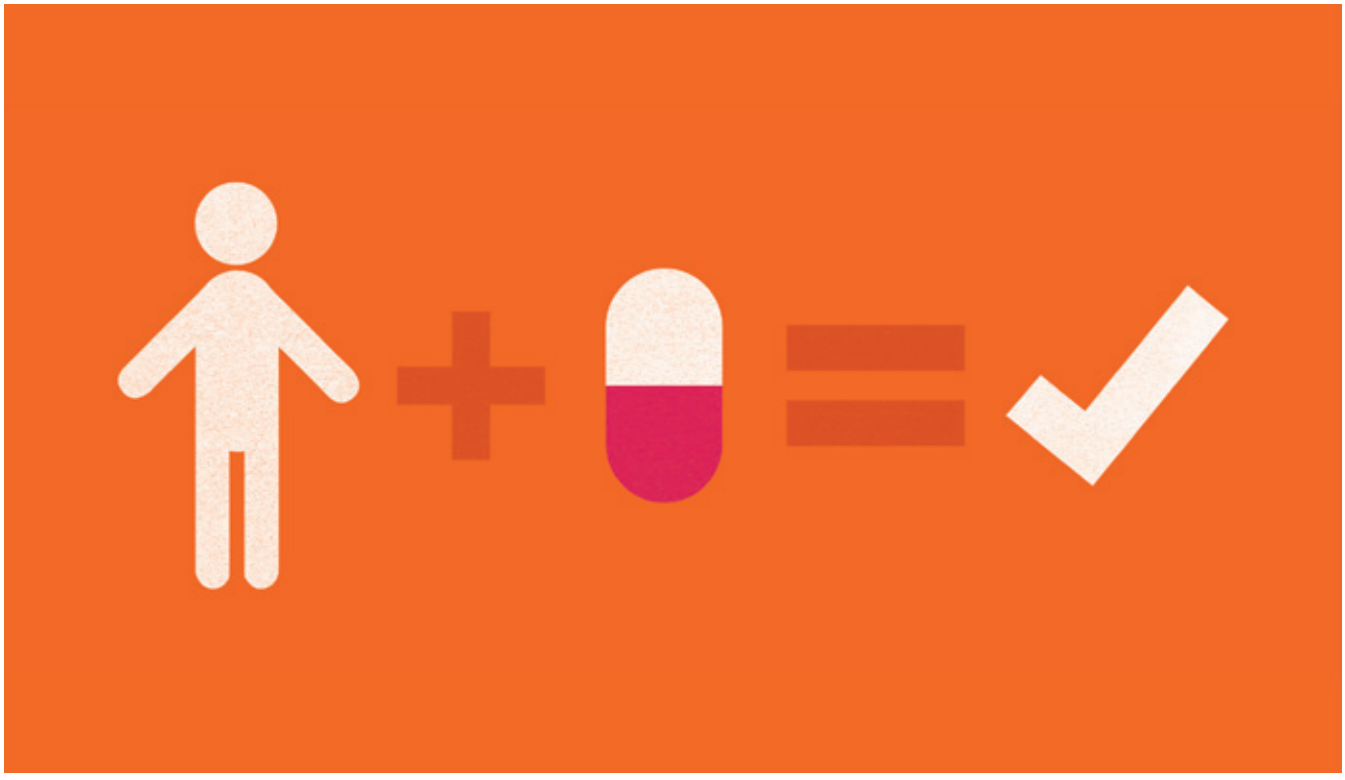
Weighing drug risks and rewards

There's no single biggest issue with individual variation in drug response. Rather, it's largely dependent on the disease being treated and the available medications. For example, at the moment there is a big push in oncology to move toward more personalized treatments, so of course inter-individual responses to medications is of prime interest. That's not to say that there isn't also a concern regarding adverse effects – but in many cases, those are anticipated and can be treated. The goal in furthering our understanding of and ability to predict inter-individual variation in drug response is to help define the risk/benefit ratios of prescribed drugs with greater specificity.

Of course, it's impossible to look at risk/benefit ratios without considering both sides of the equation – and that's where side effect investigation comes in. We need to identify patients with different susceptibilities, because the potential severity of a treatment's side effects can have a significant effect on doctors' ultimate decisions.

The computerized cell

To explore the pros and cons of treatments in individual patients, we created an "*in silico* cell" that reflects erythrocyte metabolism. We then



applied data to this *in silico* model and observed its behaviors as a prediction of how a red blood cell would react to treatment *in vivo*. Our approach uses direct metabolomics data to generate individual specific models of metabolism for the cell. These models simulate the way patients' erythrocytes will react to various conditions – in our case, tolerance to different doses of drugs like ribavirin.

Building the model is a multi-step process (see Figure 1). We started with our pre-existing knowledge of the enzymatic proteins and the biochemical reactions that occur in red blood cells – the biochemical network. Next, we collected metabolomics data by carrying out a series of small molecule measurements from the plasma and red cells of healthy individuals. We used these data to construct a differential equation model based on mass action kinetics (Mass Action Stoichiometric Simulation, or MASS models), from which kinetic parameters from each individual can be calculated. Conceptually this approach differs from some of the standard approaches for building models in that, rather than taking a series of measurements and fitting a model to the data, we move in the reverse direction and from a set of measurements we calculate parameters that satisfy constraints on the system (mass conservation, reaction directionality, etc.). There is an algorithmic procedure for the steps in the model construction pipeline, so although we still have a few steps that require expert curation, much of the process may eventually be automated.

From byte to bedside

Initially, we didn't expect to identify individuals with potential susceptibility to treatment side effects. Our goal was just to determine if we could differentiate between patient populations based on metabolic differences, and link these differences to the individuals' underlying genetics. Models aren't the only way to do this – supervised and unsupervised learning methods have been successful at differentiating samples from different individuals. But the advantage of models is that they have biological coherence, which means that we can interpret the observed behavior and predictions in the context of the metabolic network. Models also allow us to explore specific hypotheses for the observed results – that is, after we see the model's predicted outcomes for a particular manipulation, we can use it to investigate the potential causes of those outcomes. The results of our initial study suggest that it's possible to apply this methodology to other cells in order to achieve personalized

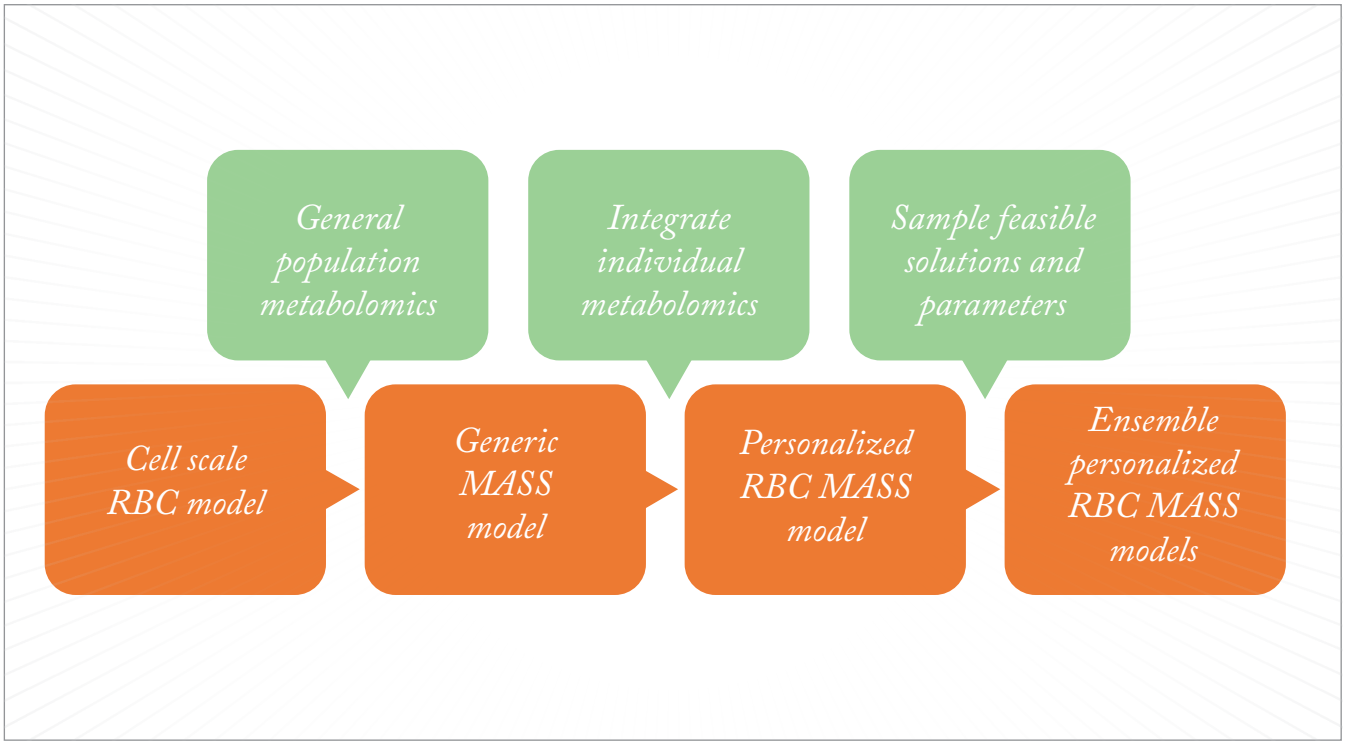


Figure 1. Generation of a MASS personalized kinetic model for erythrocyte behavior. The model allows investigators to predict red blood cell reactions to various conditions, such as drug treatments. MASS: mass action stoichiometric simulation; RBC: red blood cell.

diagnoses and treatment plans.

That's not to say that it's ready for the clinic just yet. For clinical use, there would likely need to be simplified versions of the models targeted at capturing only the features necessary for a specific application (like spotting potential adverse reactions to a particular drug). Such models would also enable simultaneous interpretation of multiple measurements – so, for instance, doctors who order metabolite panels won't have to struggle to consider the implications of 10 or more variables at once; instead, they can simply use the model to integrate the data and provide a single "functional" interpretation. At the moment, the model we've developed is a general one that can accommodate multiple variables, but that adaptability makes it too complex for easy clinical use. With time, though, we can design models that address a single test's parameters – making them useful tools for refining personalized patient care.

Kinetic models like this are only just beginning to find a place in laboratory medicine. The possibilities for exploration are still wide open, but for us, the next step will be to further test our models' predictions of susceptibility to drug side effects. We'd also like to expand the model to include other cell types, such as platelets and hepatocytes.

With the ability to integrate multiple "-omics" into a single source of information, kinetic models may soon be the tool of choice for studying variations in physiology and drug responses – and we hope that, one day, they will become a standard part of patients' personalized treatment plans.

Neema Jamshidi is a visiting scholar with the Systems Biology Research Group at the University of California, San Diego, USA.

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The Cathepsin Key

Testing for cathepsin C in urine samples may offer a quicker, cheaper way of diagnosing Papillon-Lefèvre syndrome

By Brice Korkmaz and Francis Gauthier

Papillon-Lefèvre syndrome (PLS) is an inherited disorder that occurs in about one to four in every million people. The disease, which occurs equally in both genders and more often with parental consanguinity, is characterized by palmoplantar keratoderma (an abnormal thickening of the skin on the hands and feet) and early, severe periodontitis that causes loss of both primary and permanent teeth. Other features of the disease may include intellectual disability, intracranial calcifications, recurrent skin infections, hyperhidrosis, and liver or cerebral abscesses. Although the palmoplantar keratosis may be visible at birth or shortly thereafter, it's most common for all of the symptoms to develop in parallel sometime between the ages of six months and four years – often along with teething – and become apparent by the time the child is five years old. But what gives rise to this unusual disease? Researchers were able to clearly establish its genetic etiology by sequencing the *CTSC* gene (1–3).

At a Glance

- *Papillon-Lefèvre syndrome (PLS) is a rare inherited autosomal recessive disorder characterized by palmoplantar hyperkeratosis and severe periodontitis*
- *The disease-associated gene codes for cathepsin C (CatC), a dipeptidyl peptidase belonging to the papain superfamily of cysteine peptidases*
- *Immunochemical and enzymatic detection of CatC in urine may offer a new method of early diagnosis of PLS and address an unmet need*
- *The absence of urinary CatC activity soon after birth allows patients to be identified and treated before the onset of symptoms*

Pathological protein

CTSC encodes the protein cathepsin C (CatC; see Figure 1a). It's also known as dipeptidyl peptidase I, a lysosomal cysteine exopeptidase belonging to the papain superfamily. As its alternate name suggests, CatC cleaves two residues from the N-termini of proteins and peptides (4), giving it an important role in the activation of immune system enzymes. The 46 kb gene is located on chromosome 11q14, and so far, 75 different PLS-causing mutations (50 percent missense, 25 percent nonsense, 23 percent frameshift, and 2 percent other) have been reported.

Most diagnoses of PLS nowadays are based on clinical signs and confirmed by genetic testing. But the sequencing needed to confirm PLS has several drawbacks: high costs relative to the low socioeconomic status of patients from countries with frequent intrafamilial marriages; the uncertain interpretation of rare benign mutations; and the lack of an appropriate platform for DNA preparation and sequence analysis. Not only that, but assays at the functional protein level identify pathological, clinically relevant *CTSC* mutations – as opposed to the mutations of low or no impact that may be spotted in DNA. On the other hand, pathological mutations are not restricted to the coding region, and therefore not limited to the protein sequence. They can affect transcription, splicing, tetramerization or downstream CatC maturation.

Timely treatment

It's vital to diagnose PLS patients as early as possible; early treatment helps patients avoid or slow the progression of periodontitis, which can preserve their teeth (otherwise typically lost by age 17) and improve their quality of life. But with so many different mutations in the *CTSC* gene – and so many possible flaws in the protein –is it possible to devise a simple, low-cost screening method?

> *"If true, its absence in PLS patients' urine would give us a reliable test that could be used early and easily with very little expense."*

My colleagues and I hypothesized that active CatC is constitutively excreted, and can therefore easily be traced in the urine of normal subjects. If true, its absence in PLS patients' urine would give us a reliable test that could be used early and easily with very little expense (especially when compared to a genetic screen). To test our hypothesis, we developed a method of CatC detection that was able to spot the protein in 100 percent of urine samples from about 80 healthy controls, regardless of age or sex. Through a combination of kinetic analysis and immunochemical detection, we ascertained that all of the samples contained both proteolytically active CatC and its precursor. Next, we obtained urine samples from 31 patients

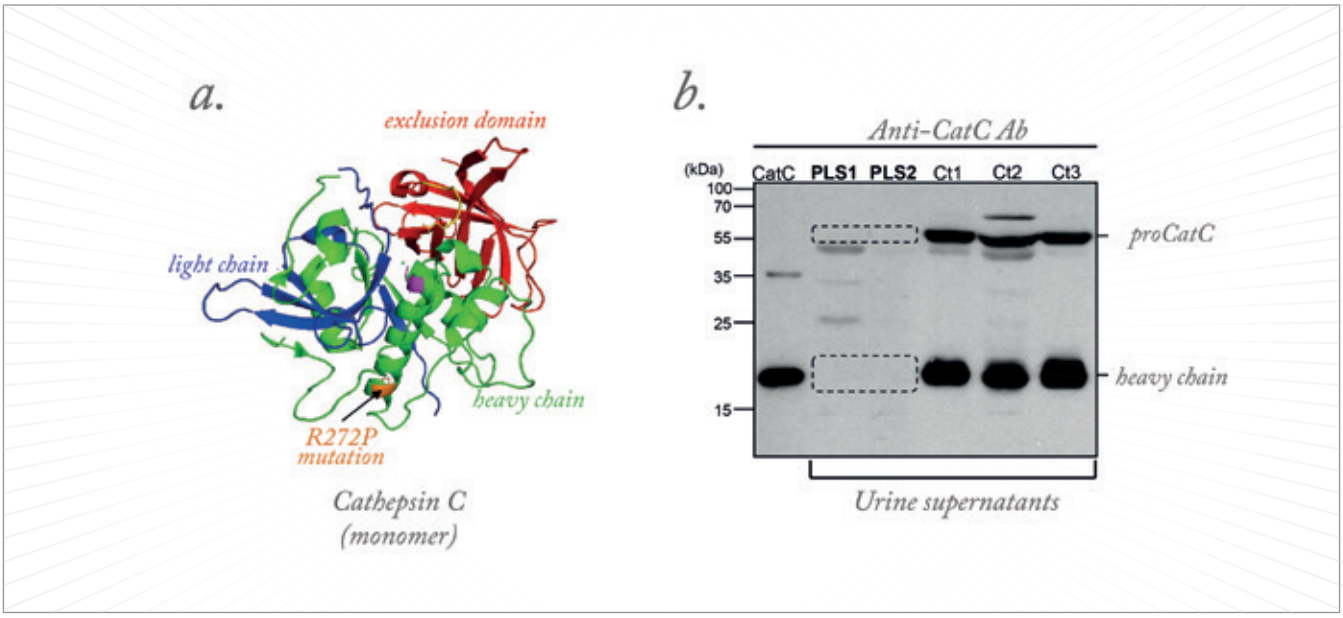

Figure 1. a. The location of the *R272P* missense mutation (orange) shown on the crystal structure of cathepsin C; red: exclusion domain, green: heavy chain, blue: light chain. b. Western blot analysis of CatC in the concentrated urine of two PLS patients with the *R272P* mutation, compared with three healthy control subjects.

with a PLS phenotype; of those, 29 contained neither proteolytically active CatC nor its antigen, confirming the patients' PLS diagnoses (see Figure 1b). In the remaining two samples, we detected CatC and followed up with a genetic analysis that revealed no loss-of-function mutation in *CTSC* – indicating that those patients actually have a PLS-like condition that is not PLS (5).

From testing to treating

Our work is the first step toward a simple, easy-to-manage test for PLS. In my experience, urine collection is much easier and less painful for children than venipuncture for blood sampling. And it's a test that can be used on all members of an at-risk family, including the very youngest children; we have established clean catch collection techniques for babies – including newborns – and there are dedicated urine collectors for them. The test can save patients and healthcare systems money, too. The cost of genetic analysis ranges from €300–600 in Europe, depending on the country. In the United States, patients' insurance providers may not cover the test, or may decide it isn't medically necessary. It's in situations like these, where genetic testing may present a financial hardship or be altogether impossible, that the urinary PLS test might address an unmet need.

We plan to develop a strip that can indicate the presence of both active CatC and its antigen. Such a test should only take a few minutes, and any laboratory technician should be able to administer it. Ultimately, we hope that a test based on the absence of urinary CatC activity will facilitate phenotype-genotype correlation in PLS and overlapping syndromes – and allow us to provide early diagnosis, treatment and symptom prevention to the patients who need it most.

Brice Korkmaz is a Senior Research Scientist at INSERM, the national institute of health and medical research in Tours, France.

Francis Gauthier is an Emeritus Professor at the University François Rabelais, Tours, France.

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Conquering the Challenges of Colposcopy

A device that uses electrical impedance spectra to characterize abnormal cervical tissue may improve on the subjective nature of colposcopy and reduce the rate of cervical biopsy

By Ursula Winters

One in every 135 women will be diagnosed with cervical cancer during her lifetime – and in recent years, incidence of the disease has increased in younger women (1). Early diagnosis is key, with 96 percent of patients diagnosed at Stage I surviving beyond five years, compared with only 5 percent of those diagnosed at Stage IV (2). This striking difference in survival rates makes it clear that early, accurate diagnosis and long-term survival are two sides of the same coin – and yet our testing methods are flawed, subject to false positives, false negatives, and differences of opinion between differently trained practitioners. But when discussing cervical smear tests and colposcopy, we frequently encounter two arguments: that they save lives, which is certainly true, and that we have no better way of screening. It's that second argument that my colleagues and I decided to address with a new device that uses electrical impedance to detect and measure cervical tissue changes in women presenting with abnormal smears.

At a Glance

- *Both cervical smears and colposcopy are subject to interpretation – and confirmation of abnormality often means one or more cervical biopsies*
- *The ZedScan device uses electrical impedance spectroscopy (resistance to flow of alternating current) to examine irregularities in cervical cell and tissue structure*
- *The more abnormal the tissue, the more its structure breaks down and therefore the less resistance it offers to the flow of an electrical current*
- *The device can also pinpoint the location of abnormal tissue, leading to more accurate sampling with fewer overall biopsies required*

Colposcopy challenges

Colposcopy – the traditional next step after an abnormal smear test – relies on the presence of visible indicators to detect atypical cells on the cervix. Unfortunately, these indicators are not specific to cervical intra-epithelial neoplasia (CIN), especially low-grade CIN, which means that interpretation is subjective. That's why we take biopsies to confirm the presence of disease before offering treatment. But biopsies are invasive – and as it can be difficult to judge the best location, we sometimes need to take more than one tissue sample, which can add to patient discomfort. Even after the biopsy is complete, it can take up to two weeks to receive the histology results, making waiting patients understandably anxious. In the majority of cases, the abnormal tissue will regress naturally, so to avoid unnecessary and potentially harmful treatment, we tend to recall these patients for repeat colposcopy at six- to 12-month intervals – only increasing the time and effort, in addition to uncertainty for the patient.

An alternative solution

It's for these reasons that I am particularly interested in exploring alternative diagnostic methods – and we may have found something that can help… ZedScan allows us to assess the structure of the cervical epithelium based on its electrical impedance spectrum. Normal epithelial tissue has a very regular and structured architecture with tightly packed cells, so it exhibits a high impedance (resistance) to the flow of an electric current. The more abnormal the tissue becomes, the more this architecture starts to break down. That makes it easier for an electric

current to pass through the tissue, so the impedance drops. The ZedScan device applies a small current across the cervix and measures the impedance at 14 different frequencies to generate a spectrum.

During a colposcopy procedure, we take 10–12 readings from around the transformation zone, which takes about three minutes in total. Each measurement is compared to a standard, allowing the device to characterize the tissue and identify areas of high-grade dysplasia. Once the examination is complete, results are immediately displayed on the handset. Areas of high-grade disease appear as a red or amber dot on the screen, indicating where the measurement was taken so that we know straight away whether to offer treatment, take a biopsy or discharge the patient.

Electrical impedance spectra improve our team's ability to identify and treat women with high-grade precancerous CIN – important because the risk of development into cancer is significantly higher than with low-grade dysplasia. ZedScan also yields an objective, reliable and reproducible result – and because we get it in real-time, we have greater ability

Ever-Expanding Applications

Zilico, the Manchester company behind ZedScan, was founded in 2006 after a successful collaboration between the University of Sheffield and Sheffield Teaching Hospitals NHS Foundation Trust to develop a more accurate means of diagnosing cervical intraepithelial neoplasia (CIN). Brian Brown of the University of Sheffield and John Tidy of Sheffield Teaching Hospitals developed Zilico's patented electrical impedance spectroscopy (EIS) into a platform technology for cervical cancer diagnosis, and the company is now expanding its product development into other clinical areas. We interviewed Sameer Kothari, Chief Executive Officer at Zilico to find out more…

What are the current unmet needs in cervical diagnostics?

The first stage of screening has improved significantly in recent years, but it remains a subjective test. Cytopathologists and their colleagues assess the cells present in a cervical smear for the presence of changes suggestive of dysplasia. Despite improvements in education, training and the organization of screening programs, all cervical screening programs based on cytology are subject to both false positives and – more worryingly – false negatives. The next stage of testing is colposcopy – and here too, results depend on what an individual colposcopist judges to be normal or abnormal. A colposcopic diagnosis of abnormality is subjective, and can sometimes result in differences of opinion. In addition, it's very difficult to get representative images or video that can allow the diagnosis to be "quality-assured" by colleagues. The final stage is biopsy and, as in screening, results are interpreted in the laboratory. There is room for error in each of these stages, and the entire process can take a long time when patients have to wait for the results of each test.

What are the next steps for the development of this technology?

ZedScan's sister product, currently under development, is positioned within the cervical screening pathway. It will provide a real-time diagnosis at the point-of-care, thus reducing traffic between primary care, cytology laboratories and colposcopy clinics – and resulting in a more effective application of limited healthcare resources. Using the same EIS technology as ZedScan, this product discriminates underlying tissue in the same way and will be subjected to a screening trial, which will determine whether it will be used as a triage system or as a co-test with either cytology or human papillomavirus (HPV) molecular tests.

In countries where the testing infrastructure – cytology laboratories, sample logistics, and administrative support – is lacking, women have very restricted access to cervical cancer screening. Our EIS products will provide an opportunity to screen and manage patients at the point of care, instead of transporting them to urban centers for tests that can take several days.

The technology is applicable to a wide range of cancer types with significant changes to the cells and target tissue structure. In collaboration with the University of Sheffield's School of Clinical Dentistry, we've completed a proof-of-concept clinical study using EIS to discriminate between oral tissues, including neoplasia – and we have further studies planned in the areas of anal, esophageal, thyroid and bowel cancer.

to employ the "see and treat" method, where women with severe abnormalities can be treated immediately without requiring biopsy. That also eliminates the need to bring patients back at a later date for treatment, minimizing the cost to them in terms of time, effort and anxiety. And negative results are just as useful; we spend a large proportion of clinic time on follow-up appointments to monitor women with low-grade abnormalities. A negative ZedScan result reassures us that no severe abnormalities are present, letting us safely discharge those women to routine screening and free up appointments for new patients.

Anticipating advancement

We're just starting to adopt ZedScan into routine use at our clinic, but based on feedback from other centers, we anticipate our service will become more efficient, and consequently, more cost-effective. There were some initial concerns that the examination would add to the appointment time for each patient – and when we first began using the new device, it did take a little longer. But with every patient, we became quicker.

In my opinion, the biggest impact on pathology will be a change in the type and number of biopsy samples generated. As we become increasingly confident in offering "see and treat," the proportion of tissue excisions is likely to increase. I don't see this increasing the overall pathology workload, though, because it will be offset by a decrease in diagnostic biopsies. Neither women who are both ZedScan and colposcopy negative, nor those undergoing "see and treat" will require biopsy. Even in cases where biopsy is indicated, the new device will help to pinpoint the best site, so that clinicians can take fewer biopsies and – as indicated by clinical studies, feedback from other centers and a health economics study – reduce the burden on histology by as much as 60 percent

Addressing unmet needs

In my opinion, ZedScan addresses the current unmet needs in colposcopy by providing a more objective and reliable diagnosis and helping to identify the optimum site for biopsy.

The technology also supports detection of high-grade cervical intraepithelial neoplasia which may be less obvious on colposcopic examination, so is effective in patients with non-HPV16 disease which may be associated with less aceto-white change. This is increasingly important as HPV 16/18 vaccination will change the epidemiology of disease.

The detection of glandular disease in colposcopy continues to be challenging, as there are no specific colposcopic features on which to base a diagnosis. Anecdotal evidence from other centers suggests that the device can detect glandular disease even without co-presenting squamous disease – and if further studies bear that out, it would be a real bonus for colposcopists.

Women with a type 3 cervix (one where the transformation zone has an endocervical component) also present a challenge. The squamocolumnar junction, where disease tends to develop first, is located within the endocervical canal and is therefore not visible under colposcopic examination. To accurately detect and diagnose disease in this type of cervix, we would need to be able to take measurements from within the endocervical canal. At the moment, the ZedScan device can identify ectocervical lesions that extend into the endocervical canal, but isn't able to examine lesions without an ectocervical component.

The introduction of electrical impedance spectra will allow us to assess women presenting with abnormal cervical smears more quickly and accurately. That means we can offer our patients rapid access to appropriate treatments when they need them, reassure those with no evidence of disease, and reduce the overall number of biopsies needed.

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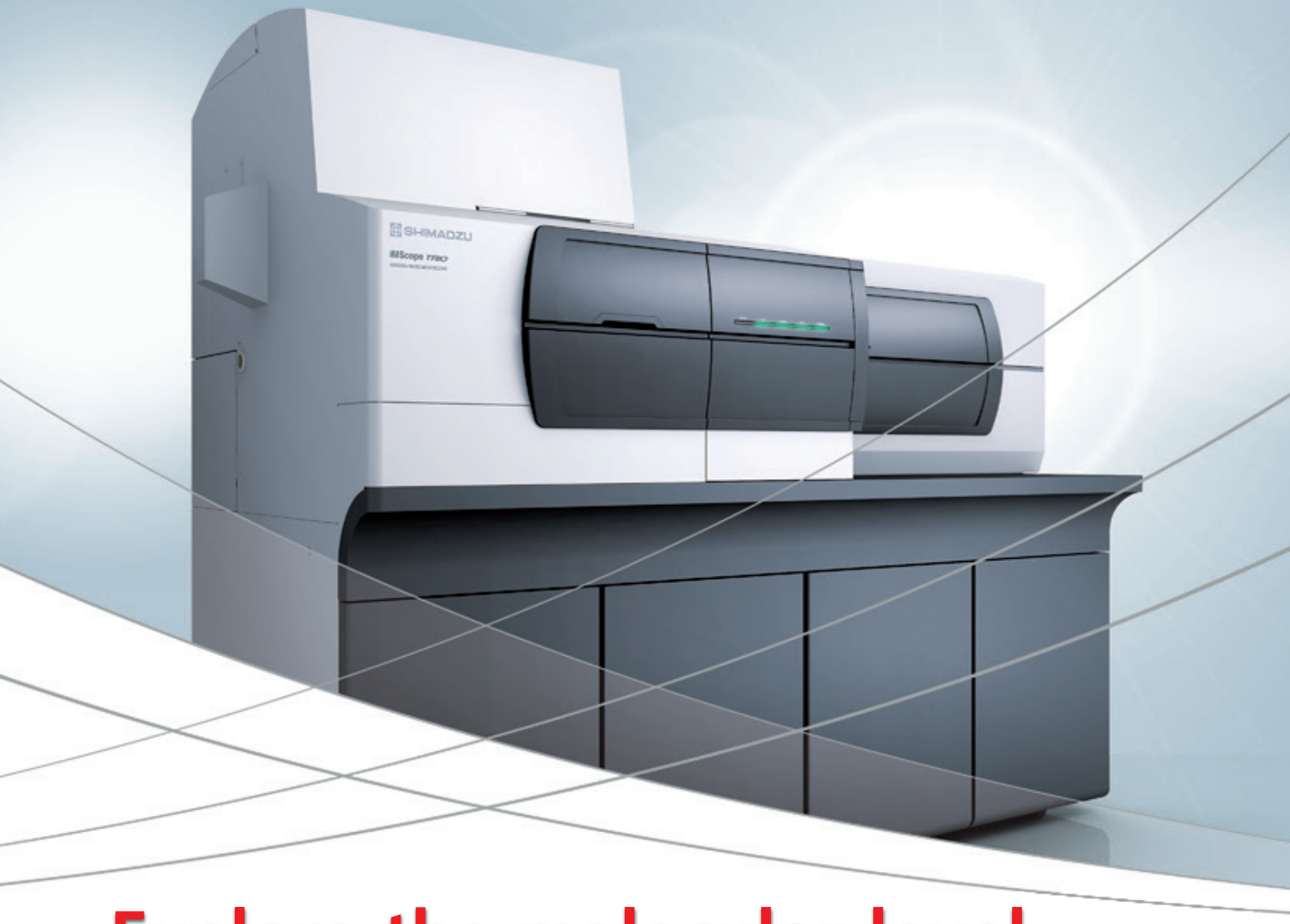

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Interpersonal Pathology

Pathologists play a significant role in patient care – and part of that role should involve speaking to patients about their diagnoses

Michael Schubert interviews Marc Rosenblum

Publicizing pathology remains one of the discipline's greatest challenges. Many pathologists agree that it's important to teach the general public what the "people behind the microscopes" actually do, but fewer seem clear on how to accomplish this. And fewer still are targeting the people for whom this kind of education would be most useful: the patients themselves. With a constantly growing, constantly aging population, pathologists are processing more diagnoses than ever – so why not step out from behind the microscope and speak directly to those patients? Marc Rosenblum, director of neuropathology at Memorial Sloan Kettering Cancer Center, spoke to us about his experiences with patient interaction.

At a Glance

- *Patients are often unaware of the role pathology plays in their care, and most lack the opportunity to speak directly to pathologists*
- *Hospitals and clinicians should make patients aware of expert pathology services as they would any other patient care asset*
- *Pathologists themselves should consider it a professional obligation to interact with patients who wish to discuss their diagnostic findings*
- *Communicating with patients allows them to better understand their diagnosis and the care they receive, and allows pathology to become better recognized*

Are your patients aware of pathologists' important role in their care?

I don't believe there are many patients who comprehend the extent to which pathologists guide their "hands-on" colleagues in medicine and surgery. To take the area of neoplastic disease as an example, I imagine that few patients realize pathologists are responsible for so many different tasks. We identify tumors, classify them, indicate their biologic potential, determine the extent of disease, assess the adequacy of surgical excision by inspecting tissue margins, and often interpret immunohistochemical, cytogenetic and molecular genomic tests. It's important for patients to understand that we're not only involved in the diagnosis and monitoring of disease, but also in prediction and prognosis – some examples include hormone receptor (estrogen, progesterone, HER2) status in mammary carcinoma, or isocitrate dehydrogenase (IDH) and chromosome 1p/19q status in diffuse glioma.

So you feel that pathologists should make themselves available to patients?

Yes. To those who wish to discuss their findings, but I think that a large responsibility for educating the public about pathology also rests with clinicians, and with the medical and administrative personnel who lead their institutions.

When clinicians discuss tissue diagnoses – or any other laboratory-based information – with patients, it's important to acknowledge the efforts of the pathologists involved. Medical and administrative leadership should ensure that patients have access to written or visual material explaining the roles played by specialists of all disciplines, including pathology, in their care. Accuse me of tribalism if you like – but if you can boast a seasoned pathology staff experienced in the application of state-of-the-art methods to tissue and fluid analysis, why wouldn't you want to make the public aware of expert pathologists as an integral hospital resource?

Can you give us an example of successful patient contact?

One of my patients had back pain and myelopathic signs secondary to a vertebral tumor compressing their spinal cord. A biopsy of this mass was interpreted (quite understandably, given the histologic picture) as demonstrating a metastatic, mucin-producing adenocarcinoma. But the patient didn't have a history of neoplastic disease, and extensive radiologic and serologic investigations proved unrevealing. I was asked to review the case in consultation, at which point I suspected that the lesion was a chordoma. Immunohistochemistry confirmed my suspicion, because I was able to demonstrate nuclear brachyury expression in the tumor cells.

The patient contacted me to discuss how I had arrived at my conclusion. I explained that the diagnosis they had initially received was entirely reasonable – but that, over the course of three decades of practice at a busy cancer center, I had become familiar with chordoma in its unusual, as well as typical, morphologic guises. This grateful and generous person actually offered to help disseminate awareness of brachyury as a diagnostic "marker" – as it was then relatively new as a commercially available reagent – and even offered financial assistance if necessary. Since then, the patient has made sure to keep me apprised of their treatment and progress.

You were involved in the treatment of a patient with the melanoma drug vemurafenib, which had a very successful outcome; how did your expertise influence the patient's care and their personal journey, in particular since the trial the patient was involved in was histology-independent?

Although the patient in question was enrolled in a histology-independent "basket" trial, their eligibility was in fact based on a histologic observation. It fell to me to analyze their neurosurgical specimen and I was struck by certain resemblances between their brain tumor and an uncommon cerebral neoplasm, known as pleomorphic xanthoastrocytoma, that usually presents in childhood or adolescence. That type of tumor often harbors a *BRAF V600E* mutation. Accordingly, I ran immunohistochemistry for the mutant antigen – and when it turned out positive, I ordered molecular profiling to confirm its presence. That mutation rendered the patient eligible for treatment with vemurafenib, a BRAF inhibitor most commonly used to treat a subset of melanomas. I think it's noteworthy that this trial demonstrated the non-uniform responses of *BRAF V600E*-mutant neoplasms to targeted therapy, and found that histology also had an effect on the likelihood of treatment responsiveness.

How has direct involvement with patients benefited you and your patients?

Beyond the personal gratification that I derive when patients express thanks for my services, I find that conversing with them underscores – often dramatically – the stakes involved in what I do as a pathologist. The many discussions I have had with patients and family members convince me that pathologists can help them understand how we arrive at our conclusions, and clarify just what those conclusions mean. At Memorial Sloan Kettering Cancer Center, where I work, this is our way of life as pathologists – and my colleagues and I regard it as a professional responsibility to talk to any patients who want explanations of their findings, or who want to discuss the implications further. Doing so has also helped our relationships with clinicians, who are clearer than ever about the role our assessments play in rational practice.

Direct communication between pathologists and patients is not generally encouraged by healthcare departments – in fact, it's sometimes actively discouraged. Pathologists in some parts of the United States face regulatory restrictions that bar them entirely from discussing laboratory reports with patients. I think this an unfortunate state of affairs, because direct patient contact can only increase our profession's visibility and our patients' understanding of their own health. Open communication brings benefits to us all.

Marc Rosenblum is Director of Neuropathology, Chief of Autopsy Services and a Founder's Chair at Memorial Sloan Kettering Cancer Center, New York City, USA.

The Path(ology) to Quality Improvement

What labs need to know about the importance of ISO 15189 accreditation

By Paul Stennett

Pathology service accreditation has come a long way in the last 25 years. Although the principles of accreditation haven't changed since its roots were established back in the 1960s, the process is constantly evolving as a source of confidence to regulators, commissioners, laboratories and – most importantly – patients. The catalyst for the most recent changes was the Report of the Review of NHS Pathology Services in England, chaired by Lord Carter of Coles (1). Aside from criticizing the low number of fully accredited laboratories, the report had two key recommendations: first, that "objective and measurable quality standards should be developed," and second, that "pathology service providers… should be subject to mandatory accreditation by an [independent] organization."

Why is accreditation so important? It addresses the primary concerns of everyone involved in medical laboratory services: accuracy, reliability and safety. It means that medical laboratory services have been assessed against internationally recognized standards to demonstrate their competence, impartiality and performance capability. The Pathology Quality Assurance Review, chaired by Dr Ian Barnes in 2014 (2), explains that "the main purpose of pathology laboratory accreditation is to assure the quality of the service being provided." Further, it is "a tool to demonstrate the competence of medical laboratories and ensure the delivery of timely, accurate and reliable results."

"It addresses the primary concerns of everyone involved in medical laboratory services: accuracy, reliability and safety."

Who's responsible?

The United Kingdom Accreditation Service (UKAS) is the UK's national accreditation body. Appointed by, but independent of, government, its role is to assess the competence of organizations that provide conformity assessment services (like certification, inspection, testing and calibration) against international standards. One such organization, Clinical Pathology Accreditation (CPA), has provided pathology accreditation to over 1,250 laboratories since its formation in 1992 – and after the emergence of the Carter of Coles report, the Department of Health supported any merger between CPA and UKAS to provide the necessary independence and transparency to adhere to European accreditation laws. As a result, in 2009, CPA became a wholly-owned subsidiary of UKAS.

But creating an independent accreditation body was only the first step. Next, we had to ask the question: what's the most appropriate accreditation standard to use for pathology labs?

Implementing ISO 15189

A key part of modernizing UK pathology services is transitioning all CPA-accredited laboratories to UKAS accreditation against the internationally

At a Glance

- *Accreditation gives laboratories, regulators and service users confidence in pathology*
- *National accrediting bodies like UKAS are responsible for ensuring that service providers uphold the internationally recognized ISO 15189 standard*
- *In the UK, the previous CPA standard will be withdrawn in 2018, so ISO 15189 accreditation is a priority for NHS England*
- *Accredited labs are able to reduce risks, control costs and stimulate innovation, providing patients with high-quality services*

recognized ISO 15189 standard. We chose this standard because it was specifically created to help medical laboratories develop their quality management systems and assess their competence. It's concerned with improving patient safety, mitigating risks and increasing operational efficiency in areas where medical laboratory practices directly impact the continuum of care. It can even be used as the basis for accrediting point-of-care testing (POCT) providers when applied in conjunction with ISO 22870 (which gives specific requirements for POCT).

As noted in the Barnes review, "the new ISO 15189 standard has increased emphasis on continuous improvement" and differs from the CPA standard in a number of important ways. Broadly speaking, new requirements regarding information management, evaluation and risk assessment, equipment records, staff suggestions and service agreements have been introduced. In addition, the criteria for meeting staff competence, EQA/IQC, purchasing, verification/validation, uncertainty, traceability have been modified from CPA standards.

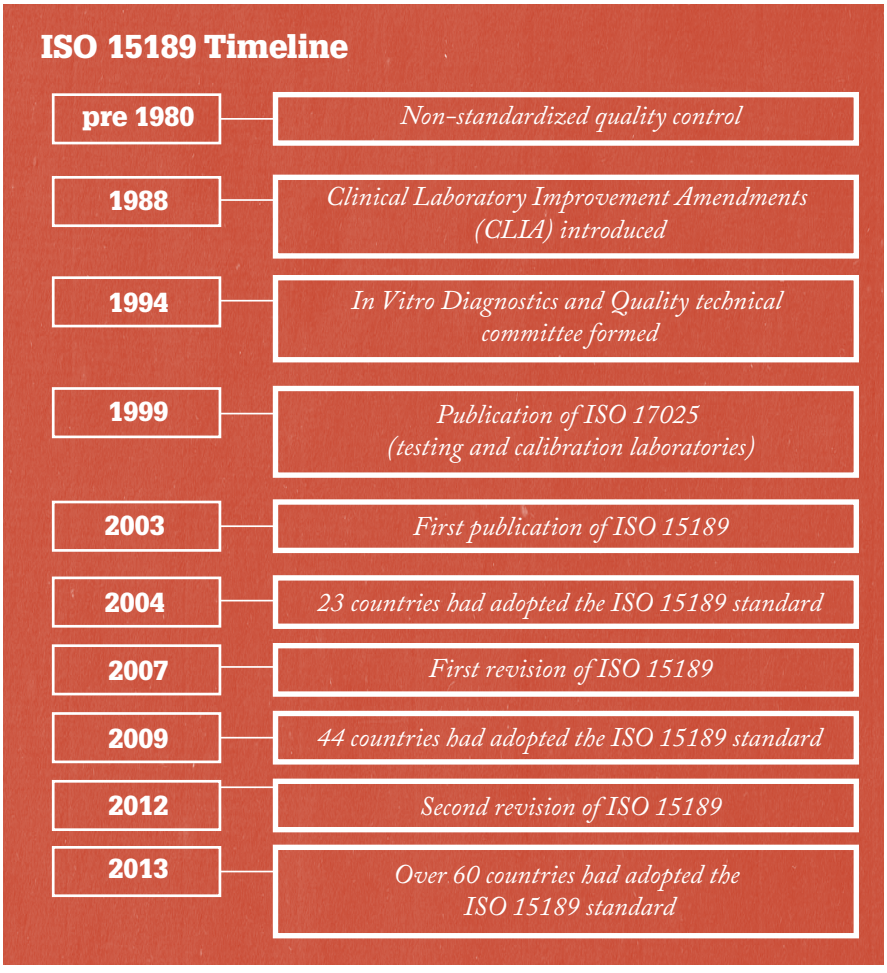
Overall competence is no longer restricted to the technical competence of staff. It also now includes the environment, handling and sampling systems, as well as the validity and appropriateness of methods. Similarly, management competence encompasses the qualifying of external services and suppliers and the management of patient feedback, in addition to internal audits of quality management systems and methods of controlling documents and records.

ISO 15189 looks at end-to-end processes in laboratories, both pre- and post-examination. The investigations are underpinned by UKAS-accredited external quality assessments, which themselves involve a more rigorous assessment of data and statistical evaluation, leading to greater confidence in the ultimate outcome. The ISO 15189 standard is also more patient-focused; it's not just about "following the process," but about ensuring the methods used are suitable for the diagnosis in question. During assessments, assessors will seek to determine how the laboratory has established that the examination procedures used are clinically suitable for the patient demographic. The clinical suitability of examinations offered by the medical laboratory must also be regularly reviewed.

Despite the differences between the standards, the basic principles of accreditation remain unchanged; namely it is an assessment (rather than an audit) that looks for multiple ways to establish conformity. The assessment team adopt a holistic approach to their work, where individual components

Fast Facts About ISO 15189

- *A comprehensive laboratory quality standard*
- *One of the fastest-growing international quality standards in the world*
- *National standards bodies can and should participate in ISO 15189 development*
- *Based on ISO 9001 and ISO 17025*
- *Quality and CLSI QSEs include:*
  - Structure (organization, management responsibility, personnel/human resources)
  - Oversight and improvement (assessment, occurrence management, process improvement, customer satisfaction)
  - Operations (purchasing and inventory, equipment, documents and records, process control/management, safety and facilities, information management)
- *Customer-focused:*
  - Awareness of users (clinicians) and ability to meet their requirements
  - Laboratory advisory panel consisting of users
  - Mechanism for user complaints
- *Reproducibility-focused:*
  - Personnel competency, procedure processes and documentation, reagents and materials, equipment, environment and conditions
  - Thoughtful lab design to avoid interference with workers or samples
- *Available in full at: bit.ly/1SORdLC*



of the standard are assessed together (where appropriate) instead of in isolation.

Making the standard switch

In October of 2013, the approximately 900 CPA-accredited laboratories in the UK began transitioning to UKAS accreditation. Nearly two years later, about 250 of those labs had been assessed against the ISO 15189 standard, with 60 awarded accredited status and a further 90 close to achieving it (3). And it's spurred pathology service providers to get organized, too. At UKAS, we've observed that many service providers are now "joining up" individual laboratories into a single UKAS accreditation covering each lab's individual scope. This consolidation will lead to the accreditation of as many as 350 laboratory units by 2018, when the CPA standard will be withdrawn.

The accreditation of all diagnostic services is a business plan priority for the National Health Service (NHS) in England and is strongly supported by the National Clinical Directors for both Pathology and Diagnostics. The Barnes review also recommends UKAS accreditation to ISO 15189. On an operational level, accreditation encourages the sharing of best practices and prevents unnecessary duplication of performance information gathering for Care Quality Commission registration.

It's not just about being organized, though; through embracing targets like fitness for purpose, value for money, and reliability, the goal is to improve outcomes for patients. Through providing independent assurance of quality and safety and providing a mechanism for measuring improvements, accreditation can enhance both the quality and value of patient care. Accreditation brings all quality assessments together in a single package – reducing risks, controlling costs and stimulating innovation. It enables cultural change and gives organizations a competitive edge – which is why Sue Hill, Chief Scientific Officer for NHS England, said, "… the standards, approach and protocols of the accreditation process itself do an enormous amount to embed a quality culture within each individual service," (4).

Paul Stennett is Chief Executive of UKAS and its wholly-owned subsidiary, CPA. He is also an Honorary Fellow of the Royal College of Pathologists, a member of the BIS Measurement Strategy Board, and was appointed MBE in 2008 for services to business and pathology.

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Rolling With the Punches

Sitting Down With…
Michael Laposata, Chairman of the Department of Pathology at the University of Texas Medical Branch at Galveston, Texas, USA.

What inspired you to develop a system to help clinicians understand diagnostic tests?

As a clinical pathology resident, a bright internal medicine resident asked me a simple lab test selection question; he didn't know how to further evaluate an abnormally prolonged clotting test known as the PTT. It surprised me. I realized I had knowledge about making a diagnosis that he, and most residents in other specialties, didn't have. I asked him what he would have done if we hadn't spoken. He told me he would do what every other doctor did – guess which test to pick and guess what the results meant!

So you realized something had to be done, but then the first barriers came up?

Yes. I tried to set up a program to provide patient-specific, expert-driven narrative interpretations of complex clinical lab evaluations while I was a resident. Unfortunately, I could not interest the faculty in participating. I was told by one assistant professor that becoming an associate professor was required, or he would lose his job. And "you do it not by taking care of patients, but by publishing papers!" As a trainee, that was quite a shocking revelation to hear from a mentor.

Undeterred, when I got my first job at the University of Pennsylvania, I created a page to be inserted into patient charts, with blanks for lab test results and a box at the bottom for our interpretations. It was incredibly well received, and patients started to get their diagnoses quickly, rather than needing multiple rounds of tests. But a senior hematologist told me to stop because "my hematology fellows are not seeing enough patients, so my revenue is down." I knew that was a game I couldn't win.

I then moved to Massachusetts General as director of clinical laboratories. We initiated interpretations for complex coagulation cases and the program became a source of national and international attention because of its clinical value in establishing rapid, accurate diagnoses. Oddly enough, the interpretations in the chart led to patient referrals to pathology from other doctors in the area (most hematologist-oncologist doctors!), and we developed a widely respected clinical practice for patients with bleeding and clotting disorders. Some patients were very confused, and asked if they were referred in preparation for an autopsy! I assumed they were kidding.

Even then, someone in the hospital's hematology–oncology division told me that he didn't need my interpretation. I told him that the other 2,000 doctors in our healthcare system did. He crumpled up the paper and threw it at me.

But you still persisted?

Absolutely. I realized the higher you are in an organization, the fewer people there are to kill your innovations – and the more people there are who are willing to believe they're worth a try. When I went to a higher position at Vanderbilt, we initiated the same service, and it developed a name – the diagnostic management team or DMT.

DMTs were created for coagulation, transfusion medicine, microbiology, hematopathology, and a few other clinical areas, and each claimed a triumph in improved patient care and decreased cost. Papers were written, and I was asked to speak about the DMT in at least 50 major institutions and many societies. It became abundantly clear that, with thousands of diagnostic tests available and the cost and complexity associated with them, diagnostic experts had to become involved. If they didn't, somebody was going to get hurt.

Why has nothing changed?

The biggest reason? Financial incentives in the United States. According to statistics from the College of American Pathologists, an average pathologist works about 48 hours per week, makes around $200,000–400,000, and does work almost exclusively in anatomic pathology. There is substantial payment for anatomic pathology services, but almost no payment for clinical pathology advice on test selection and result interpretation. For decades, this misalignment of incentives has, expectedly, greatly minimized the number of experts in clinical pathology disciplines. But the external environment is changing and, thanks to our work with the Institute of Medicine, diagnostic error is no longer flying under the radar.

I'm now at the University of Texas, where we are hoping to create a state-wide group of experts in all major areas of clinical and anatomic pathology. We plan to have DMTs for dozens of clinical areas to bring diagnostic experts electronically to the bedsides of all 26+ million patients. The endgame is better patient outcomes. We shouldn't allow our patients to die because there is no expert in their immediate environment. Once this is available all over the world, I will have completed the task that started with a simple PTT evaluation query in 1984.

Adopting the DMT model won't be easy for pathologists, though, especially those who have been working with a different focus for years. Somebody has to provide the example, and we plan to show others how it's done. It's going to take the next generation of residents who are trained to provide diagnostic consultations covering all areas of pathology to effect the major change. And I haven't lost my enthusiasm. It may take a while until the change comes, but I'm going to do this until I can't do it anymore.

Any regrets?

Grandpa was a sulfur miner, and dad a barber – it's hard for me to complain.

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