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Case of the Month

A young man with a history of gastric bypass surgery presented with dyspnea, fever, and a history of recurrent pneumonia. On imaging, a solitary nodule was found in the right lower lobe, and a few tree-in-bud opacities were seen bilaterally. A surgical lung biopsy was performed.

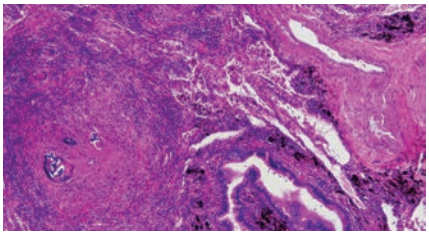
What is your diagnosis?

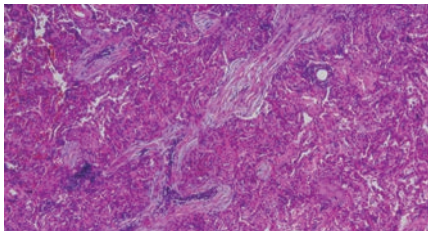
A. Talc granulomatosis (intravenous drug abuse)

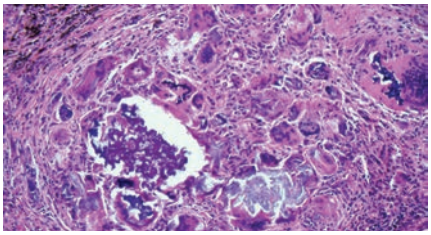
B. Particulate matter aspiration

C. Pneumoconiosis

D. Necrotizing sarcoid granulomatosis







To register your guess for this month's case, please go to http://tp.txp.to/1218/case-of-the-month
We will reveal the answer in next month's issue!

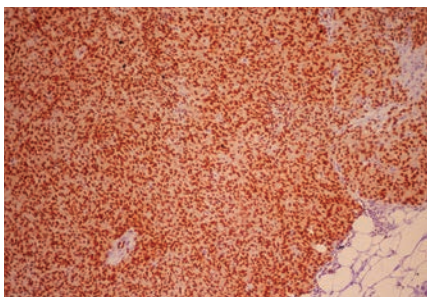
Answer to last issue's Case of the Month…

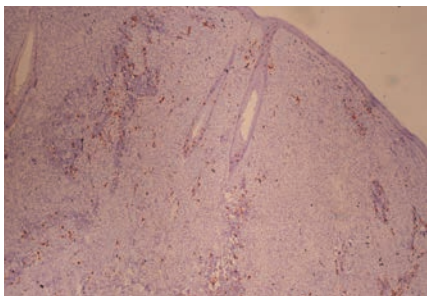
D. Granular cell angiosarcoma

There are only three prior cases of granular cell angiosarcoma in the literature (1,2). A 76-year-old female presented with a tumor in the parotid and temporal regions; a 72-year-old male with an ulcerated lesion in the nasal bridge that had spread to the cheek; and a 78-year-old male with a scalp tumor (2).

Common in elderly patients, angiosarcoma's microscopic features include abundant epithelioid cells mixed with some spindle cells, infiltrating the dermis and subcutaneous layer with moderately pleomorphic nuclei and distinct membrane. The granular cytoplasmic alteration occurs because of an overabundance of lysosomes (evidenced by CD68 and NKI/C3 positivity). Immunostaining is also positive for ERG, CD31, and CD34 – focal in some cases, but diffuse in ours. Key to the diagnosis are vessels in the deeper portions of the subcutaneous tissue or in the periphery of the lesion.

Submitted by César Augusto Alvarenga, Pathologist at the Instituto de Patologia de Campinas in São Paulo, Brazil.





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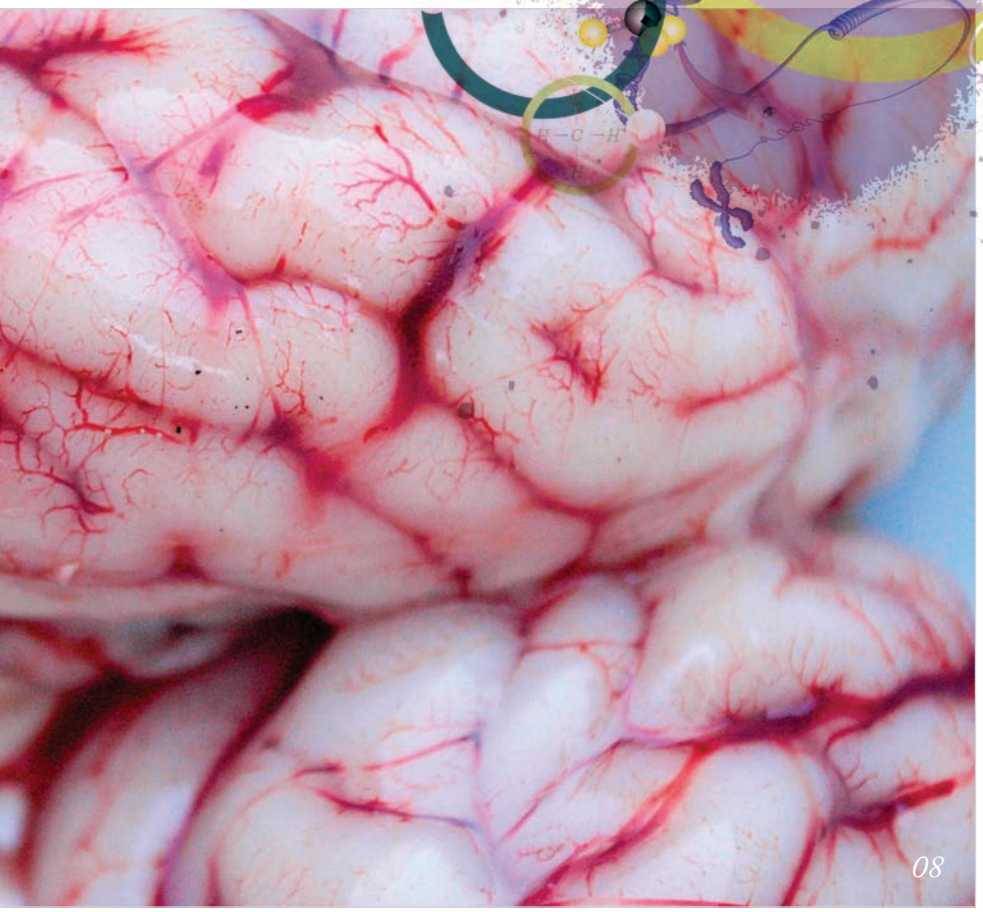

On The Cover

A collage of imagery relating to epigenetics and the laboratory techniques used to obtain epigenetic data.

Feature

Innovators

In Practice

NextGen

Profession

Sitting Down With

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A Roster of Unsung Heroes

An invitation to laboratory medicine professionals of every kind

Many of the professional conferences I attend, and the organizations I interact with, have "pathology" in their titles. In fact, it's rare to find one that doesn't. You could therefore be forgiven for thinking that such meetings and organizations are exclusively for those who hold the title of medical doctor and have trained in a laboratory specialty.

But that isn't the case. Two meetings I attended this year had a separate stream for medical students who had not yet chosen a specialty, and others had sessions for high school and undergraduate students to encourage their interest in medicine and life science – whatever discipline they ultimately desired. Another meeting this past summer had a stream specifically for pathologists' assistants – and, in fact, The Pathologist's booth at the American Society for Clinical Pathology annual meeting was set up next to one belonging to the American Association of Pathologists' Assistants.

A recurring theme amongst the pathologists I know is the need for better public relations – advocacy, outreach, and awareness of their existence and the role pathologists play in healthcare. But what about the pathology-adjacent disciplines? What laboratory can function without assistants, technologists, technicians, managers, and administrators? What about researchers who make breakthroughs in laboratory medicine without practicing it themselves? What about the allied health professionals who help obtain, process, store, and analyze samples?

We know now, more than ever, the need to shine a bright light on the achievements of pathologists – and the field of pathology. But we should widen the beam to include those in equally vital laboratory professions; without their dedication, the field of laboratory medicine would look very different today. In a comment on my previous editorial (1), a reader suggested speaking with laboratory technicians and technologists. So that's what I'd like to do.

What would your laboratory look like without non-pathologist laboratorians? I invite all of you – from every walk of laboratory life – to let us know. Post on social media (you can tag us at @pathologistmag) or send us an email (edit@thepathologist.com). If you are one of those professionals, tell us your story; share your experiences – we're here to make your voices heard as well.

Michael Schubert
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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Identifying Parkinson's Inhibitors

Screening nearly 750,000 molecules for inhibitors of α-synuclein protein aggregation

Parkinson's disease is the second most prevalent neurodegenerative disease and the most common movement disorder globally. Although its symptoms can be treated to an extent, there is currently no cure and no way to stop the progress of the disease.

A major contributor to the onset of Parkinson's disease is the aggregation of the natively unfolded protein α-synuclein (αSN), which forms both small oligomeric complexes and large fibrillary deposits. Attempts to identify compounds that inhibit this aggregation are hindered by its irregular and variable nature – but one research group may have found a better way (1). Daniel Otzen and his team stimulated αSN aggregation using sodium dodecyl sulfate (SDS) and then screened 746,000 compounds to identify the six most effective aggregation inhibitors using Förster resonance energy transfer (FRET).

"We show that these compounds truly do prevent αSN aggregation," says Otzen. "They just have the unfortunate side effect of also reacting chemically with αSN by forming covalent chemical bonds with it. Perhaps they can be modified to reduce their chemical reactivity while retaining their inhibitory role."

All six compounds are derivatives of (4-hydroxynaphthalen-1-yl) sulfonamide that share a core structure. Otzen thinks that their inhibitory qualities stem from interactions with the N-terminal region of monomeric αSN. "We believe that by binding to the N-terminal region, the compounds prevent the formation of intermolecular αSN interactions that are otherwise required for the aggregation process to proceed to form oligomers. Furthermore, the compounds can also bind to pre-existing oligomers where the N-terminal part is partially exposed and neutralize its toxic, membrane-binding effects."

Moving forward, Otzen wants to take the research in vivo and attempt to apply these findings on a practical level. "We would like to develop compounds that are truly specific to the αSN oligomer, so we can provide drugs for both diagnostics (early-stage detection of Parkinson's disease) and therapy (by binding and neutralizing toxic species). Currently, we are not limiting ourselves to small molecules; we are also looking at peptides, which are easier to manipulate systematically, and antibodies, which tap into the amazing selection system of our immune system."

Reference

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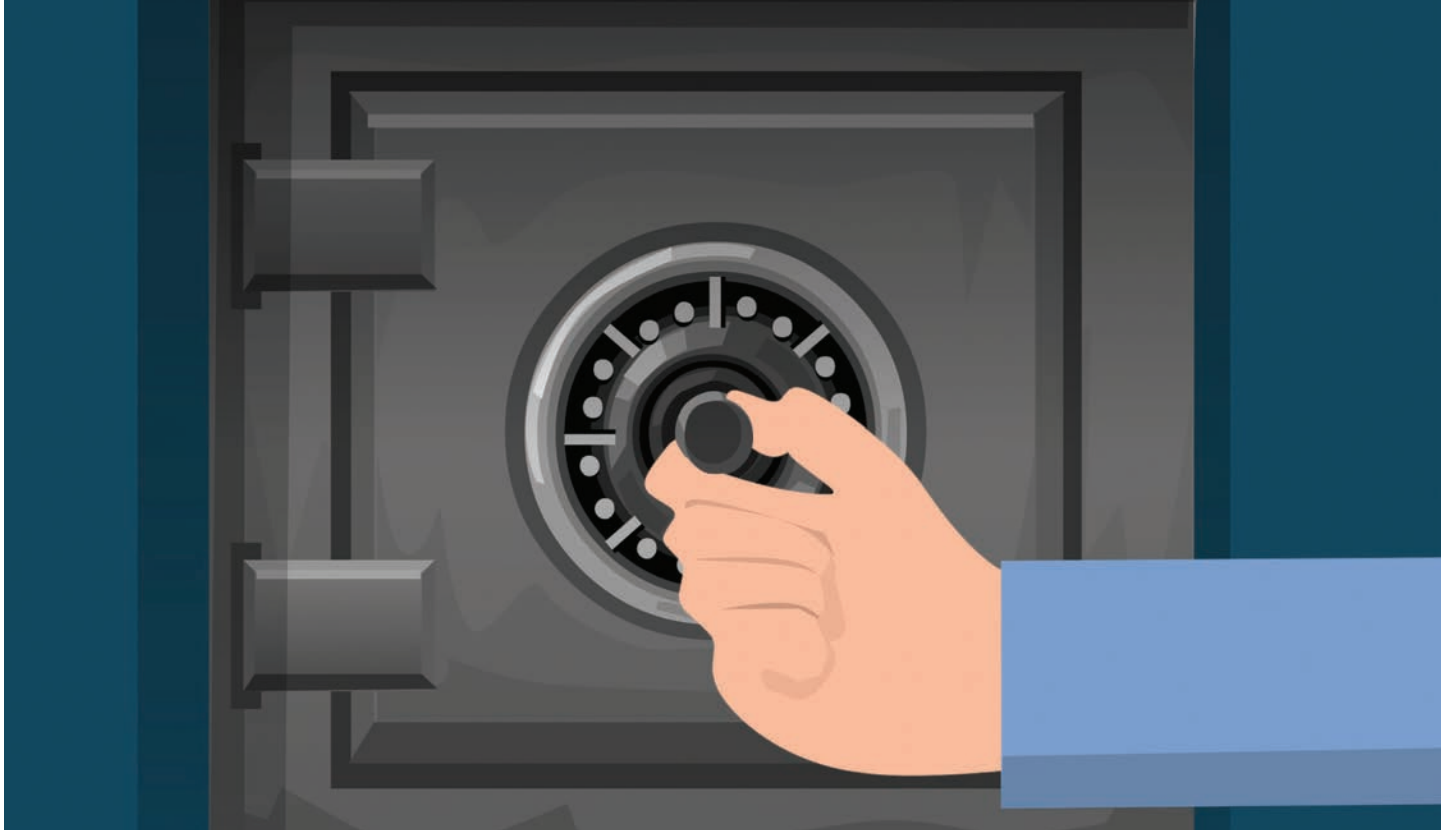


Nephrology in Your Pocket

Building a bank of interactive cases that nephrologists can use for teaching and learning

The American Society of Nephrology unveiled the winners of their annual Education Innovations Award in October, which aims to stimulate the development of innovative tools that inspire medical students to learn about nephrology in new ways. One of the winners was a mobile-friendly teaching tool called NephSim – a collection of cases and images that the user works through to achieve a diagnosis. We spoke to one of the app's creators, Rachel Hilburg, to discover what gave her the idea and how it can be used to build a community within the field.

What motivated you to create NephSim?

My colleague, Samira Farouk, and I both have a strong interest in medical education. We were aware of various programs in medicine that use case-based learning; however, we didn't think there was enough for the field of nephrology in that sense. We also found that many of the teaching methods within nephrology were individualized and disengaged, demanding isolation while going through a particular case. We were on the lookout for fresh ways to teach nephrology, and when we discovered the Education Innovators Award, we used it as a platform to develop a way to work through nephrology cases interactively and cooperatively.

What is the goal of the app?

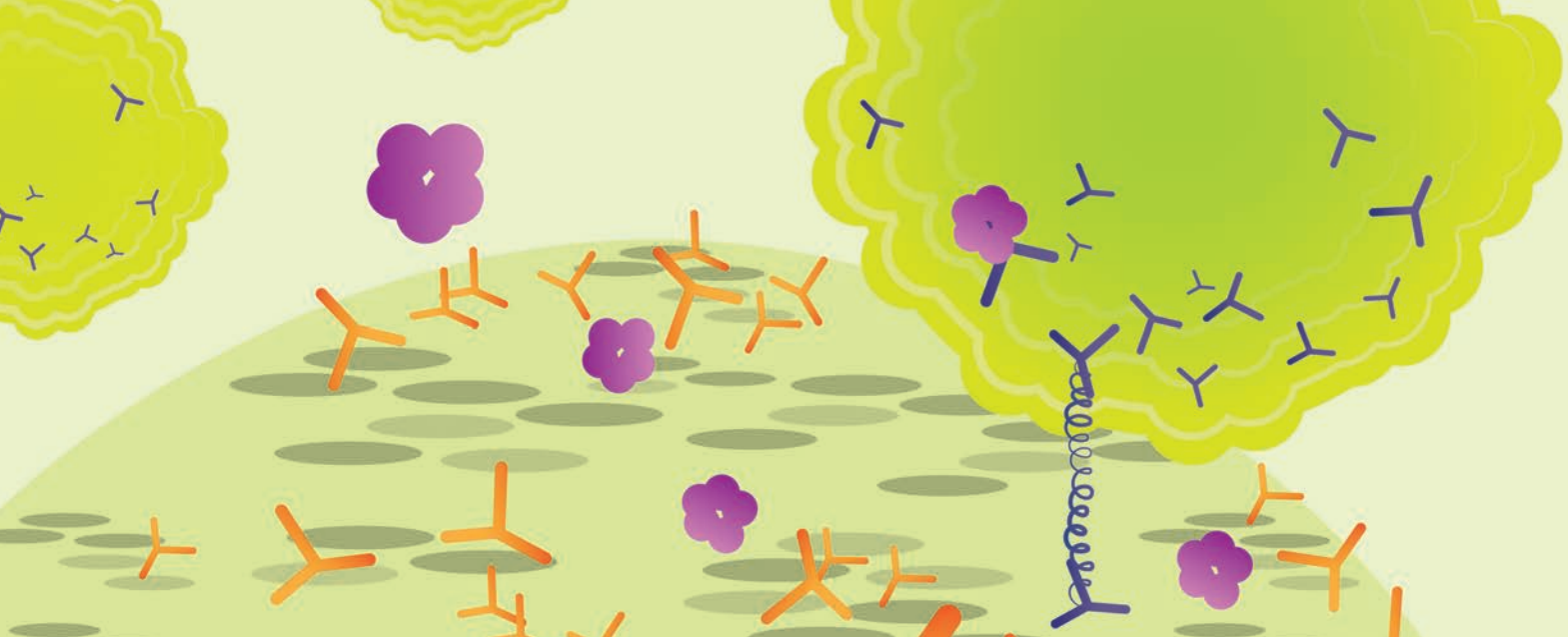
Our aim is to build a library of cases that people can use, both to learn themselves and to teach others. NephSim allows you to practice diagnoses in a variety of real-life scenarios, and we ultimately want it to spark interest in the field and create community. As residency can be such a busy time, the simplicity of the app will hopefully fuel small group discussions "on the fly," or while grabbing a coffee, because it's so easy to pull up a case quickly. We also want to provide a platform for young doctors who want to teach others, so each case contains prompts for the teacher to achieve this.

How did you find the development process?

I think the fact that Samira and I both have a limited understanding of technology has been helpful in terms of the app's usability. Initially, I was fearful of the implications of digital platforms in nephrology, but now that I have been more involved, it's really nice to see the unifying effect of such technology. In a small field like nephrology, being able to talk to other people about cases, both locally and globally, brings about a sense of community that can't be achieved by digging through textbooks.

What's next for NephSim?

In the short term, we're hoping to add a greater variety of cases to the app. We've also been reaching out to the nephrology community and are in the process of getting more people involved, because the whole app relies on people submitting their own cases. There has already been interest from various medical schools who want to incorporate NephSim into their curriculum and, in the future, we want to continue working with our colleagues to integrate the app with nephrology education more formally.



Sensing the Tiniest Change

Menno Prins describes a new biomarker sensing technology for sensitive, specific monitoring

Molecules that are essential for the body, such as proteins and hormones, can often yield significant insight into a patient's health status. But many of these molecules are present in the blood in pico- or nanomolar concentrations. The best-known assay to measure such low concentrations outside the body is an elaborate, multi-step process that yields a single concentration value: ELISA. In contrast, continuous monitoring dynamically follows biomarker concentration in solution, leading to a stream of data rather than an isolated result. For continuous monitoring to work, molecular binding must be reversible and lead directly to a measurable signal without consumption or production of chemical reactants. The sensing principle should be self-contained, reversible, and stable over a long period of time. Still, the assay should be as sensitive and as specific as ELISA. And that's the challenge my colleagues at Eindhoven University of Technology and I are addressing (1).

BPM refers to "Biomarker monitoring based on sensing of Particle Mobility." The technique exploits the fact that tiny particles in liquid are constantly in random motion because water molecules collide with them. What we did is couple the particles to a substrate via a flexible molecular tether, so that the particles wiggle back and forth. To detect a specific biomarker, the particles and the substrate are provided with affinity molecules; this enables specific, reversible interactions with the biomarker molecules in solution. When a biomarker molecule attaches to both particle and substrate, they form a molecular sandwich bond that greatly reduces the particle's mobility. When the biomarker is released, the particle regains its original mobility. So these mobility changes, which we detect via dark-field optical video microscopy, indicate the capture or release of a single biomarker molecule – and the number of changes per minute reveals, with high sensitivity and specificity, the concentration of the biomarker in the liquid.

The beauty of the BPM sensor technology is that increases and decreases in biomarker concentration can be precisely monitored over time. We have demonstrated its use in monitoring protein and DNA, but the technology is widely applicable; affinity molecules such as antibodies and aptamers are available for almost all biomarkers.

We think that BPM sensing can become an early warning system that signals patient deterioration – useful for postoperative, immunocompromised, or chronically ill patients, as well as those in critical condition. Furthermore, patients who receive potent drugs with a narrow therapeutic range might benefit from a sensor that enables rapid and robust dosing regulation. Before that can become a reality, though, we need to develop assays for several medically relevant biomarkers and demonstrate the required analytical performance. This will be followed by clinical proof-of-concept studies, which should give solid ground for subsequent development of a product. In total, we expect the process to take five to 10 years. We are now defining key applications and markets to determine our technical and clinical direction. Are we going to focus on measuring early warning markers, or on therapy monitoring? What patient group will we target? What value will we add? The answers to these questions will define our work in the coming years.

Continuous biomarker monitoring will go through several stages of maturity – and, in the future, may be as easy to perform as today's blood pressure or heart rate measurements. As technology development increasingly focuses on important medical needs, we have an interesting road ahead.

Reference

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Journey to the Center of the Cell

A new 3D model takes us on an unprecedented journey into the inner workings of living cells

Would you like to see the intricate, complex structures inside a living human cell? Until recently, this would have been unheard-of. Now, the Allen Institute for Cell Science has developed a comprehensive three-dimensional model of a live human cell that allows researchers to dig into our bodies' innermost secrets. The probabilistic model can accurately predict the shapes and locations of structures within a cell, a novel ability that the researchers hope will enhance our knowledge of cellular processes and facilitate a better understanding of human disease. To find out more about the mechanics and potential significance of the model, we went straight to the source.

What inspired you to develop this model?

Fluorescence imaging is incredibly powerful in that it allows us to see specific structures, but limited in that it only allows us to see a few components of living cells at any given time. We needed to develop methods that allowed us to integrate both of these abilities. Neural networks were a natural starting point because they permit us to scale the integration of dozens of cellular components, each learned from thousands of images, relatively easily.

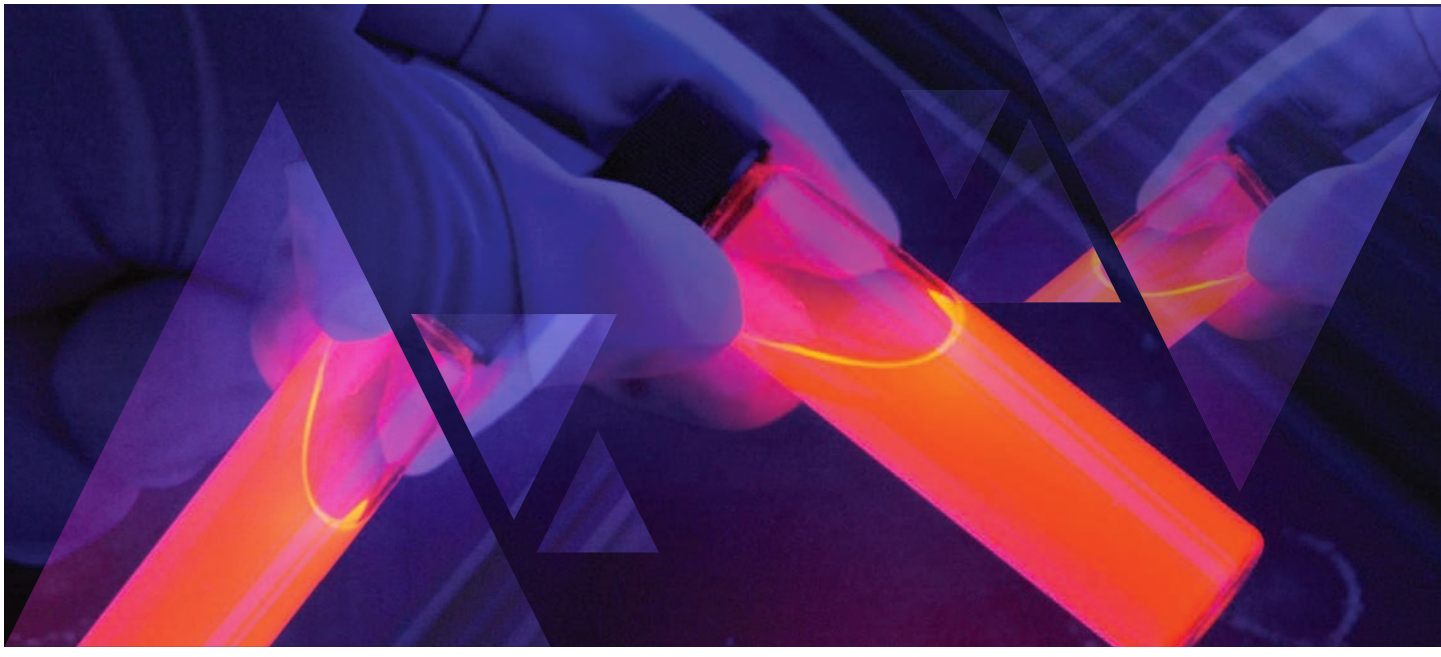
How accurately can the model visualize structures within a cell?

The accuracy of our prediction of structure location largely depends on the strength of the relationship between this structure and the cell and nucleus. For example, the location of the nuclear membrane is easy to predict when we see the nucleus, whereas cytoplasmic organelles, such as mitochondria or microtubules, may be highly variable in their localization and thus harder to predict. These relationships change as the cell grows and divides, and understanding the strengths of the relationships between different cellular structures under different conditions is crucial for understanding cell organization and behavior.

What are the possible applications in terms of disease research?

Although our models are powerful, the data on which they are built currently spans only a narrow range of cell physiology. As we collect data under different conditions and with more subcellular structures, we will be able to expand our understanding of the natural variations in cell biology and build more expressive and predictive models of how cells may respond to different stimuli.

A big challenge is to make our models as easy as possible for other scientists to use and interpret in their day-to-day work. We want to build more accurate models that allow us to see inside cells at higher resolution and subsequently use these models to identify and explore how important components reorganize as cells grow and mature.



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 editors at edit@thepathologist.com

Think Before You (Don't) Print

Consider the importance of specimen identification solutions in pathology digitization

By Tormod Schüßler, Primera Technology Europe, Wiesbaden, Germany

Digitization has found its way into many areas of our private and professional lives – and it certainly hasn't stopped at pathology. But how does digitization affect specimen identification? Where will its development take us? What advantages does it offer? And what are the requirements for the printing systems we use?

Digital pathology helps pathologists view, manage, share, and analyze tissue samples by converting glass slides into digital slides. Therefore, you need a high-speed, high-resolution slide scanner and an accurate barcode, which provides patient information – or, at least, the patient ID – throughout the entire process (from receiving the original specimen to digitizing the tissue section for diagnosis under a virtual microscope).

In addition to the ease with which digital slides can be shared, either for review or education, there are speed and safety advantages. Archiving and retrieving of slide images is faster using a digital "slide library" accessible with just a few clicks. Sample information, patient data, and full image history will be easier to find and work on, supporting an integrated diagnostic approach. Cases will no longer need to be assembled for pathologist review. These advantages deliver a vision of precision medicine that, thanks to fast and efficient data transfer, makes specimen identification more accurate and increases patient safety.

Indeed, one of the main goals of digitizing the entire process is to prevent misidentification, and to make connecting, reviewing, and managing patient data faster and more reliable. Let's take a closer look at the printing systems labs routinely use to label tissue sections, whether for glass or digital slides. In the past, most labeling was accomplished by handwriting information with a pencil or marking pen. However, handwriting tends to be difficult to read, may be inaccurate, and can rub off during processing. Even if the sample is not to be scanned and

"Digital pathology helps pathologists view, manage, share, and analyze tissue samples by converting glass slides into digital slides."

"One of the main goals of digitizing the entire process is to prevent misidentification."

digitized, a barcode – which can only be produced digitally – is a necessity for matching it to other samples and data in the laboratory information system (LIS). Fortunately, today's printing inks are chemical- and UV-resistant enough to withstand common laboratory processes. With the right solution, labs can print not only 2D barcodes, but also text, graphics, and logos to scan and apply directly to tissue cassettes and slides. Such an approach completely eliminates both handwriting and expensive, difficult-to-apply xylene-resistant labels – two things that decrease workflow efficiency and place patient safety at risk.

To understand the process even better, I want to give you a short overview of the printing systems most laboratories use nowadays. There are two types: cassette printers and slide printers.

Cassette printers are available as standalone manual printers or as complete automated systems. The standalone options are compact, robust, and small enough to fit next to a grossing station. They usually load one tissue cassette at a time through an operator. These modern manual printers can produce several cassettes per minute. Fully automated systems function at nearly the same speed, but use separate hoppers instead of requiring manual input. Some models even come with a robotic arm that picks each cassette from the top of the desired stack and places it into the printer.

Slide printers are designed for efficient, hands-free operation. Because slides are stored in easy-to-load cartridges that protect them from dust and other potential contaminants, they increase workflow safety and slide longevity. The devices can also print in a variety of hues and shades, reducing the need to purchase consumables in different colors. State-of-the-art systems are advanced enough to offer on-demand or batch mode printing and are also designed for very low noise emission.

Placing a cassette printer at a grossing station or a slide printer at a microtome station is an important step toward significantly increasing the lab's efficiency while reducing the risk of specimen misidentification. Even after several years, cassettes and slides with properly printed labels can be reliably identified. By printing on tissue cassettes and slides, we digitize the entire process chain, which not only facilitates LIS integration but also (when using the right printer) prepares the laboratory for a future in which the entire pathology workflow is digital.

"[Using printers] is an important step toward significantly increasing the lab's efficiency while reducing the risk of specimen misidentification."

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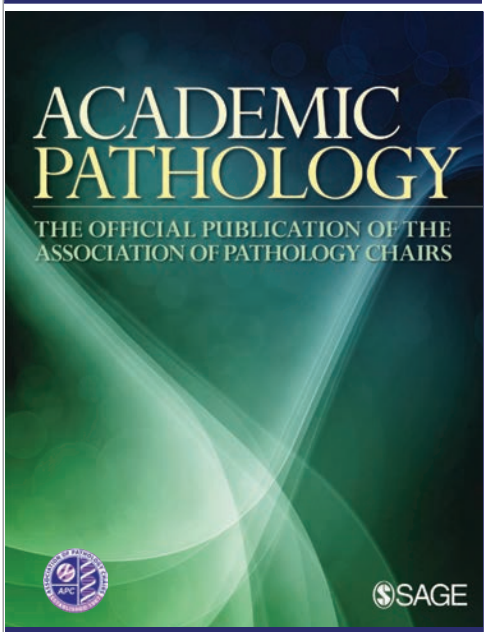

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People Power and Machine Learning

How collaboration could pave the way for super-efficient cancer testing

By Geoff Twist, Managing Director for Roche Diagnostics UK and Ireland, Burgess Hill, UK

The success of the National Health Service (NHS) is all about people working together in partnership. We've just celebrated the great British institution's 70th birthday this summer, and it's hard to imagine how we could have gotten here without everyone pulling together, from the doctors and nurses on the front line to the managers and administrators making everything work. And, of course, the pathologists and laboratory medical professionals diagnosing illnesses.

Seven decades on from the founding of the NHS, the technology available to us has moved on significantly – but the principle of working together still holds true. And so I am delighted that Greg Clark (UK Secretary of State for Business, Energy, and Industrial Strategy) has announced a new collaborative effort, the Northern Pathology Imaging Cooperative (NPIC). The initiative will bring together the NHS and academia to pioneer the use of artificial intelligence (AI) in digital pathology, and will be supported by £10 million of investment from UK Research and Innovation as part of the Industrial Strategy Challenge Fund, as well as a further £7 million from industry partners.

It's no secret that the NHS is facing unprecedented challenges: an aging population, long-term medical conditions, and significant funding issues. Leaders in the healthcare space have to find innovative ways to do more with less, all without compromising patient care. New technology, such as AI, presents an opportunity to help alleviate some of the pressure while also offering the potential for revolutions in what healthcare can achieve.

In pathology, AI can be trained to recognize the patterns of disease; for example, searching for small areas of cancer in a large sample. In short, pathologists will be able to use AI to diagnose cancer faster, better, and at lower cost – obviously an attractive prospect for the NHS.

But to work, AI needs to "learn" the patterns by looking at large numbers of images and becoming familiar with them, just like any human pathologist would. But there are no large collections of digital images that can be used for this – and computers can't learn from glass slides like we can. The NPIC brings together the NHS, industry, and scientists to solve this problem. To begin with, the collaboration will cover a network of hospitals serving a population of around 15 million people. It is led by the University of Leeds, working with another six universities and 10 industry partners. Together, we will work to make new AI systems that can analyze our images and make better diagnoses. Crucially, everyone involved in the project will ensure that AI systems can be used safely, and that doctors and the NHS are in control of how they are used. However, it's no less important for us to ensure that the public understands what we are doing and trusts that we are using their health data appropriately and securely.

"Earlier and more accurate diagnoses save lives, and AI has the potential to take us to the next level."

Our ambition is to develop world-leading methods for gathering and using data to make AI systems. It's the perfect balance – a collaborative partnership that uses "people power" to make new technology work for us. The project, which officially began on 1 December 2018, will continue for three years, so no time is wasted in taking the next step towards improving diagnostic capabilities for the NHS. Adopting cutting-edge digital technology will drive continued improvement in our ability to diagnose disease accurately and target treatment by pinpointing biomarkers in individual patients.

The future of healthcare is incredibly exciting, with potential for revolutionary digital pathways to support exceptional clinical decision-making. Earlier and more accurate diagnoses save lives, and AI has the potential to take us to the next level in this regard – good news for healthcare systems, for pathologists, and, most importantly, for patients.

Test Utilization – More Than Just a Buzzword

It's our job to make sure that patient testing is as good and efficient as it can be

By E. Blair Holladay, CEO of the American Society for Clinical Pathology, Chicago, USA

When I use the words "test utilization," what comes to mind? Is it "the right test for the right patient at the right time?" Or do you perhaps think of the Choosing Wisely recommendations – 25 published to date (1) – that caution against potentially overused tests? Test utilization is more than just a buzzword. What we're actually referencing is the opportunity to help you make laboratory testing more efficient so that your patients receive state-of-the-art testing using assays that are proven to be empirically data-driven and yield the best possible outcome. Often, that means using your expertise to drive behavioral changes both within the laboratory and throughout the entire medical system.

Within the laboratory, we've been practicing test utilization from day one. We study reflex protocols to find ways to save money while maintaining test quality and patient safety. We refine test menus by discontinuing old or inefficient methodologies. We also determine which tests can be performed in-house and which ones should be sent to a reference laboratory. We educate our staff so they're up-to-date on the latest methodologies and gold standards. We develop our own laboratory tests to better serve our patient populations. We analyze internal data and benchmarks to find inappropriate test-ordering practices so that they can be rectified through education. Although these daily tasks are important, educational activities for clinical staff outside the laboratory could have the biggest impact.

It can be as simple as designing a paper requisition that's easy to read and understand, making test ordering easier. Or we can go a little bit more in-depth by working with our institutions' IT departments to implement reminders within the electronic ordering system, ideally reducing redundant test orders and enabling clinicians to order the right test for their situation the first time.

We also have several opportunities for a more personal approach. We can consult with an ordering physician about patient results and discuss the best follow-up diagnostics. Giving presentations to clinicians can help them order the right tests for their specific situations. And we can discuss the utility of ordering morning labs each day for an inpatient whose condition has not changed – or who is leaving the hospital that day.

As pathologists and laboratory professionals, we have an obligation to provide excellent patient care as efficiently as possible. So "test utilization" is far more than just a buzzword – it's the essence of what we do.

"[Test utilization] means using your expertise to drive behavioral changes both within the laboratory and throughout the entire medical system."

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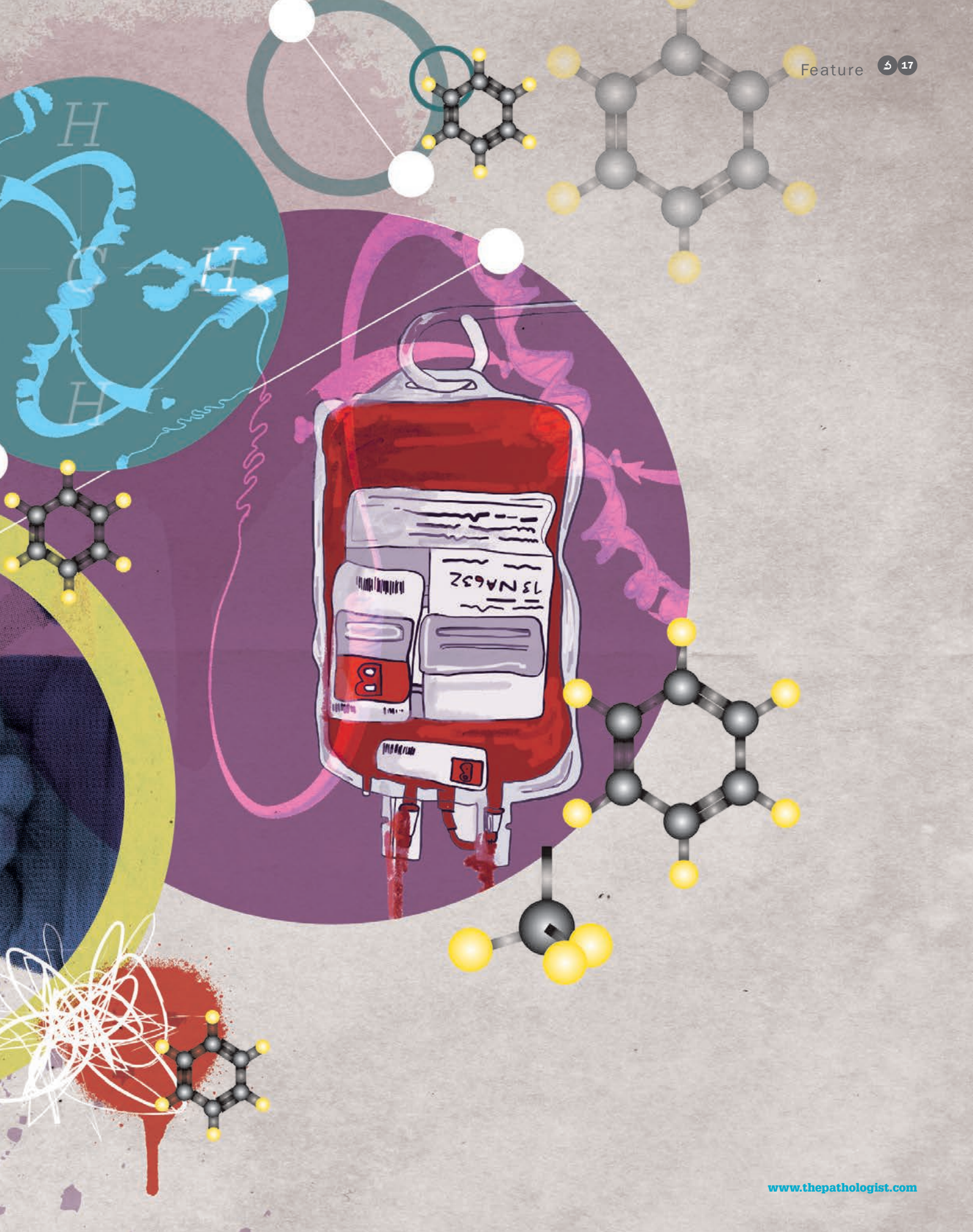
A LASTING LEGACY

In 1944, famine struck the Netherlands. Babies born soon after the famine ended initially appeared to suffer few ill effects but, as they reached middle age, they began to exhibit an unusual prevalence of heart disease and other metabolic disorders. Studies of the "Dutch Hongerwinter cohort" ultimately discovered that environmental factors can not only affect gene expression in an individual, but leave a lasting epigenetic imprint on their children. How can events that happened decades earlier affect later generations – and could we one day manipulate our epigenome to live longer, healthier lives?

By Mary J. Wirth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Over the past half-century, advances in the life sciences have been profound, and epigenetics is one of the most exciting new frontiers. The "epi" prefix is from the Greek for "above" – or, in this case, "in addition to." It was once thought that the genetic code with which we were born was a static blueprint for all that would happen in our cells throughout our lives. Epigenetics describes the revelation that, in fact, that blueprint can be affected to a large degree by the environment. Changes in gene expression dictated by the environment can even be passed down to the next generation and beyond. Many of us would have received a failing grade in high school biology if we had said that (information about) someone's life experiences could be passed down to their children. Now, epigenetics is recognized as a basic biological phenomenon.

One of the first documented manifestations of epigenetics in humans arose from dire circumstances: war and famine. During World War II, France was liberated after D-Day, but the Netherlands remained occupied by the Germans. To liberate the Dutch and hasten the end of the war, British Field Marshall Montgomery developed a plan that became known as Operation Market Garden. The Allies would drop 20,000 paratroopers into the Netherlands to seize a series of bridges along a highway leading to Germany, allowing subsequent Allied troops to move swiftly into the country.

If you have seen the movie "A Bridge Too Far," you will already know that the plan was a failure. The Allies encountered heavy resistance from German forces at Arnhem, the bridge over the Rhine River just before the German border. The Allied paratroopers were surrounded by German defenses and sustained heavy casualties – about 15,000 killed and many more wounded. Even before Operation Market Garden, food was in short supply and, with no reinforcements coming, the surviving Allied paratroopers were soon starving along with the Dutch.

One soldier's story

One of the paratroopers of the US 101st Airborne, a sharpshooter, flew out of England on September 17, 1944, and his division was dropped over Veghel under enemy fire. His pack was so loaded with supplies that his combat helmet popped off when his parachute opened. But it was easily replaced; so many of his fellow paratroopers were shot as they descended that the ground below was littered with their helmets. His division captured the bridge at Veghel and then marched to Nijmegen, where they helped take control of the bridge over the Waal River. Then came the order to march onward to Arnhem. With German panzer divisions defending Arnhem and no supplies coming, the Allies ran out of food. They had been told that the Dutch would feed them; some did, but they had very little food to share, and those who were caught helping the Allies were executed by German soldiers.

One day, the hungry sharpshooter and his best buddy, Bob Sherwood, were out scouting for enemy soldiers when they saw an apple tree laden with fruit. Delighted, they raced to the tree. Bob climbed and started shaking the branches to drop apples down to his friend below.

A single shot rang out.

Bob fell from the tree, killed instantly by a German sniper. Afterwards, the sharpshooter lay on the ground motionless for hours until he was able to escape under the cover of darkness.

The sharpshooter was not so lucky on October 5, 1944, when he ran in to replace a gunner who was killed by heavy German fire. Although he knew he would be the next high-value target, he quickly wiped out five gunners with five shots. But a sixth, hidden in the woods, fired a mortar shell that gravely wounded the sharpshooter with shrapnel. He lay bleeding in a wine cellar for days before being loaded into a truck overflowing with casualties. A week later, the sharpshooter was transported to the US by hospital ship – and, 10 months later, was finally released. For his efforts, he earned a Bronze Star for bravery. I am particularly thankful that he survived, because he is my father: Robert "Red" Wirth. Now 93 years old, he still tells stories of his harrowing wartime service.

The thrifty gene

During and after the battle at Arnhem, the Germans destroyed the transportation infrastructure to secure their hold on the Netherlands, cutting off the food supply just as an

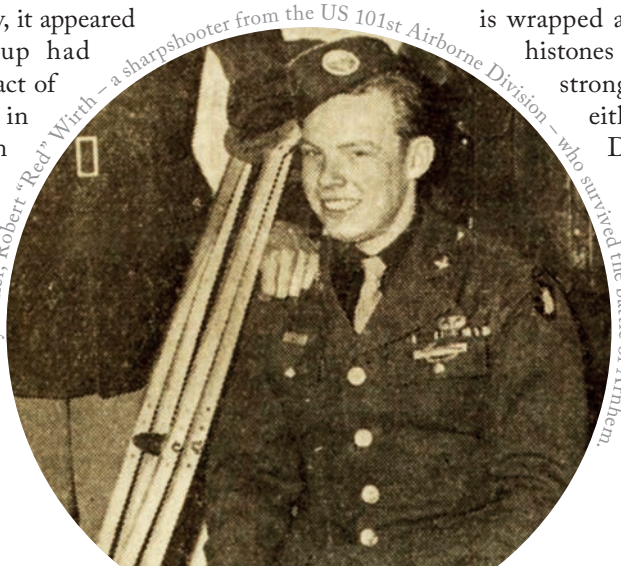
Paratroopers commemorate Operation Market Garden in the Netherlands.

"One of the first documented manifestations of epigenetics in humans arose from dire circumstances: war and famine."

unusually hard winter approached. The result was widespread famine.

The winter of 1944–1945 is known as the Hongerwinter –"the hunger winter." It was an inadvertent and tragic experiment on the long-term effects of famine. From December 1944 until Germany surrendered in May 1945, food was rationed to 1,000 dietary calories per day, and from February to May rations were cut again to just 580 calories per day. More than 20,000 people died of starvation.

Dutch babies born early during the famine were born underweight but, after the war, many grew to normal weight. Babies who spent only the first trimester in utero during the famine, born after the war, were born at a normal weight. Initially, it appeared that this latter group had escaped the worst impact of the famine. However, in the long term, people in this group were in fact some of the hardest hit. Far from being cushioned from the effects of the famine, their early exposure to starvation in the womb led to increased rates of obesity and a host of metabolic diseases affecting cardiac health once they reached middle age. They had somehow acquired what is popularly called the "thrifty gene." A half-century after World War II, Dutch people born just after the famine found that their bodies behaved as though they were still living under conditions of starvation. We now know that the malnutrition suffered by parents caused epigenetic changes in offspring conceived during the famine.

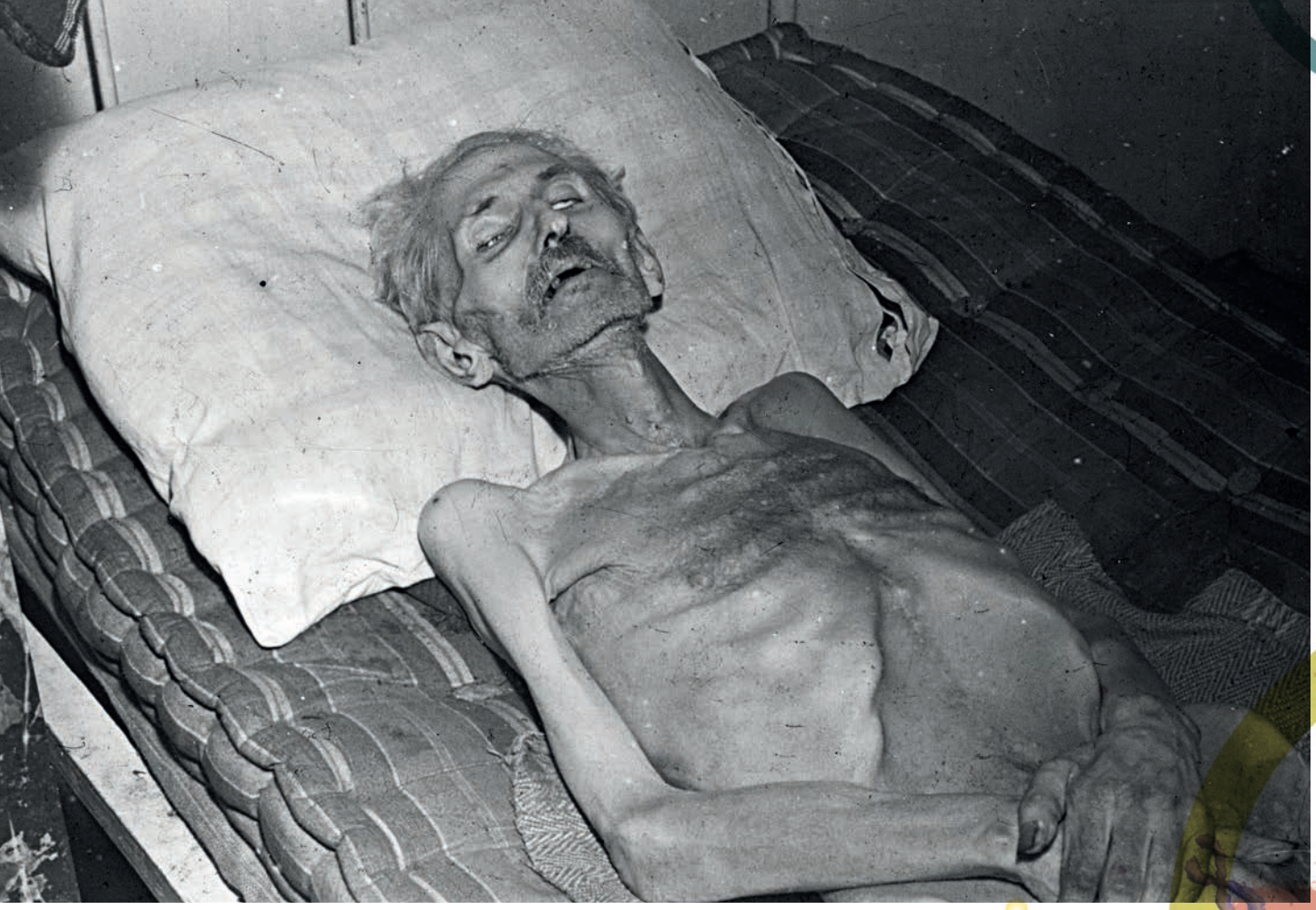


My father, Robert "Red" Wirth – a sharpshooter from the US 101st Airborne Division – who survived the battle of Arnhem.

Not quite a clean slate

The conventional understanding of molecular biology – its central dogma – was once simple: genes are transcribed to make mRNA, which is translated to proteins. In reality, though, things are not so simple. Transcription can be blocked by environmental effects, and genes that are supposed to be blocked can be unblocked. Two main types of gene silencing occur in cells: modification of the DNA and modification of histones. DNA is wrapped around histones like pearls on a necklace; histones are opposite in charge to DNA, yielding a strong electrostatic attraction. Modifications to either component can affect how tightly the DNA is held by histones.

In reproduction, the egg and sperm typically lose their DNA methylation, erasing any memory of the parental environment. The DNA is then re-methylated during development. Folic acid is a source of methyl groups for this critical process, which is why the mother's nutrition in the first trimester is critical. For



A victim of starvation in the Dutch Hongerwinter. Credit: Menno Huizinga.

the Dutch Hongerwinter Cohort, as researchers call them, maternal malnutrition meant that methylation was not completely erased, and the epigenetic changes caused by the famine were passed down to the children and, in some cases, even the grandchildren – imprinting them for life with the traits needed to survive conditions of starvation.

Scientists in the Netherlands and the USA recently pinpointed the mechanism behind the higher body mass index and elevated serum triglycerides seen in the Dutch Hongerwinter Cohort: DNA methylation at CpG sites in genes mediating energy metabolism (1).

Experiments on animals shed light on the epigenetics of starvation. The two mice pictured in Figure 1 are genetically identical; the only difference is in the diets of the mothers during pregnancy. Both mice were engineered to be predisposed to obesity, but the mother of the smaller mouse was fed a much more nutritious diet. Although the genetic blueprint of each mouse is identical, the expression of genes relating to metabolism is radically different, reflected in their disparate appearance. In other words, they have the same genotype, but a different phenotype. DNA hypomethylation even affects the coat color.

In this case, the mother's diet was responsible for passing on unfortunate epigenetic traits, but a study on extreme exercise (mimicking starvation) in male mice showed that the father can also pass down the so-called "thrifty" gene or genes through epigenetic changes in sperm, leading to obese offspring (2). But that doesn't mean that mothers are better off choosing a man who lies on the couch watching TV all day to father their children; too little exercise in male mice causes other epigenetic problems (3).

DNA methylation, once presumed to be a persistent gene silencer, is now appreciated to be a reversible, dynamic process; an individual's genome undergoes significant change over their lifespan (4). The average centenarian has significantly less cytosine methylation than a baby, with de-silencing of certain genes responsible for some age-related

Rewriting the Code

The key mechanisms through which genes can be silenced or amplified by the epigenome.

1. DNA methylation

A gene can be silenced by blocking the binding of its transcription factor through methylation of a cytosine in the promoter region, at a location where the C is followed by a G in the 5′-to-3′ direction (a CG pair, or CpG). Gene silencing is nothing new, of course, but it is only relatively recently that we have recognized that environmental factors can silence or de-silence genes through cytosine methylation.

2. Histone modification

The other main epigenetic mechanism involves chemical modification of histones in the tail regions, affecting their electrostatic interaction with DNA. There are many types of modifications; for example, lysine acetylation removes the charge on lysine to loosen the connection between histones and DNA for easier gene expression. The various histone modifications are dynamic and reversible, and can work in concert with DNA methylation.

3. Non-coding RNA

A third mechanism occurs later in the process, after the gene is expressed. At this stage, non-coding RNA can bind to mRNA to block its translation into protein.

conditions (5). Even identical twins have virtually identical epigenomes at three years old but, by age 50, their epigenomes significantly differ (6), which may help to explain why identical twins usually die of different diseases. For example, the famous American twin-sister advice columnists, Abigail Van Buren and Ann Landers, were amazingly identical when they were young, and even pursued the same career path. However, they died of completely unrelated diseases – Ann at age 83 from multiple myeloma and Abby at age 94 from complications of Alzheimer's disease. As well as this drift, epigenetic changes also lead to predictable effects. The epigenetic clock, for example, is based on a steady rate of DNA demethylation for a specific set of genes as people age – and it is even used in forensics to estimate the age of crime suspects (7).

Overcoming destiny?

Can we do anything about our aging epigenomes or must we make do with what we have inherited and put up with whatever changes occur over our lifespan? It appears that we can, in fact, manage epigenetic changes to some extent. A quick online search for "DNA methylation and exercise," for example, brings up a wealth of studies. Heart disease is the most common cause of death in industrialized countries, and we know that exercise helps prevent heart disease. Now we know why: because our epigenetics change with exercise to help lower our risk of heart disease (8). Even if your genetics predispose you to heart disease, your epigenetics can offset some of the risk.

The breadth of studies on epigenetics is vast. Though we have focused on metabolism thus far, DNA methylation plays a role in a huge assortment of diseases (9). The epigenome of a cancerous cell is very different from that of a healthy cell, and this fact is being exploited for new therapies (10). In other illnesses of old age – everything from Parkinson's disease to chronic obstructive pulmonary disease – you will again find epigenetic factors at work. The diseases that the famous advice column twins succumbed to are now both known to be associated with epigenetic changes.

How can science help us to understand – and maybe even control – our epigenome? The key ingredients for a major (or minor)

scientific advance are, first, asking the right questions and, second, having the ability to answer them. We can use analytical chemistry to measure DNA methylation and histone modification to answer a vast array of interesting biological questions. The method we presently use for detecting cytosine methylation was invented in 1992 by Frommer and colleagues, who demonstrated that bisulfite deaminates cytosine, converting it to uracil (11). Methylated cytosine (see Figure 2) is much slower to react with bisulfite; therefore, they are still visible as cytosines in DNA sequencing after bisulfite treatment.

"Can we do anything about our aging epigenomes or do we have to make do with what we inherited and put up with whatever changes occur over our lifespan?"

Analyses in epigenetics began with single-mode measurements; for example, detecting average methylation across all DNA or average modifications across all histones. Once scientists began to understand that DNA modifications and histone modifications work together, new methods were needed (12). The overlapping nature of epigenetic changes poses a measurement challenge, because there is just one copy of each nucleosome per cell. PCR does not amplify methyl modifications, and, of course, protein concentrations cannot be amplified.

As a result of the high sensitivity needed, single-molecule techniques are now widely used for these analyses. In a technique called ChIP-seq, a highly specific antibody for a histone modification (for example, lysine acetylation) selects the nucleosomes with this modification by chromatin immunoprecipitation (ChIP) (13), and single-molecule DNA sequencing using nanopore technology (14) identifies which cytosines in the chromatin are methylated. The nanopore technology sequences DNA and detects modifications without the need for PCR and does not require bisulfite treatment. Future measurement technology will need to address an even greater challenge: there are multiple modifications of histones and other modifications of DNA besides methylation of cytosine at CpG sites. The same histone can have multiple modifications, all of which work in concert with one another and with the DNA modifications. These comprise a complex epigenetic code that describes how our cells operate, how they respond to the environment, and how diseases arise.

The limitations of current technology are clear when considering the vast complexity in analytical measurements required to unravel this code (15).

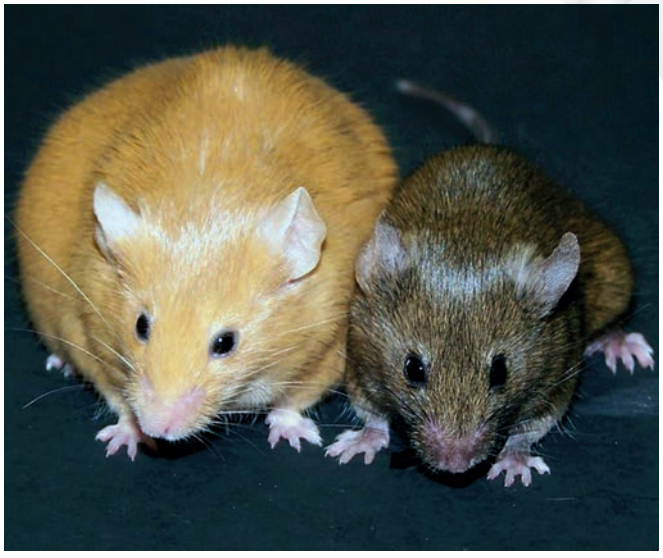


Figure 1. Obese and normal mice. Credit: Randy Jirtle and Dana Dolinoy

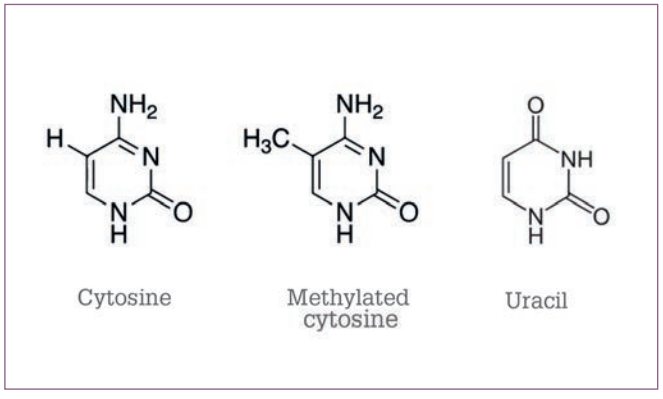


Figure 2. Chemical structures.

Beyond biomedical research

Epigenetics has captured the interest of social scientists, who are concerned about the epigenetics of social status. After all, human epigenetics came to our attention in part because of a social issue in the Netherlands: war and famine. It is striking that an environment that we have no control over can cause deleterious biological changes, and that this effect can be passed down to the next generation and beyond. The Hongerwinter demonstrated that there can be a critical window, such as the first trimester of gestation, that impacts one's entire life and sometimes the lives of one's children. Beyond heart disease, folic acid deficiency during gestation in the Hongerwinter has been associated with a higher level of schizophrenia (16). But malnutrition is just one factor affecting the epigenome – in mice, maternal care in the first months of life has been demonstrated to epigenetically affect stress response in later life (17). Plus, pollution, drug addiction, family dysfunction and stress may all play a part in our individual epigenetic code. These issues and more are discussed in a recent review in the sociology literature (15).

Epigenetics is a rapidly growing and expanding science, encompassing nutrition, exercise, disease, substance abuse, family life and socioeconomic status. There is much we still don't know, but one thing is certain: progress in all areas would be accelerated with better, faster laboratory tools and techniques.

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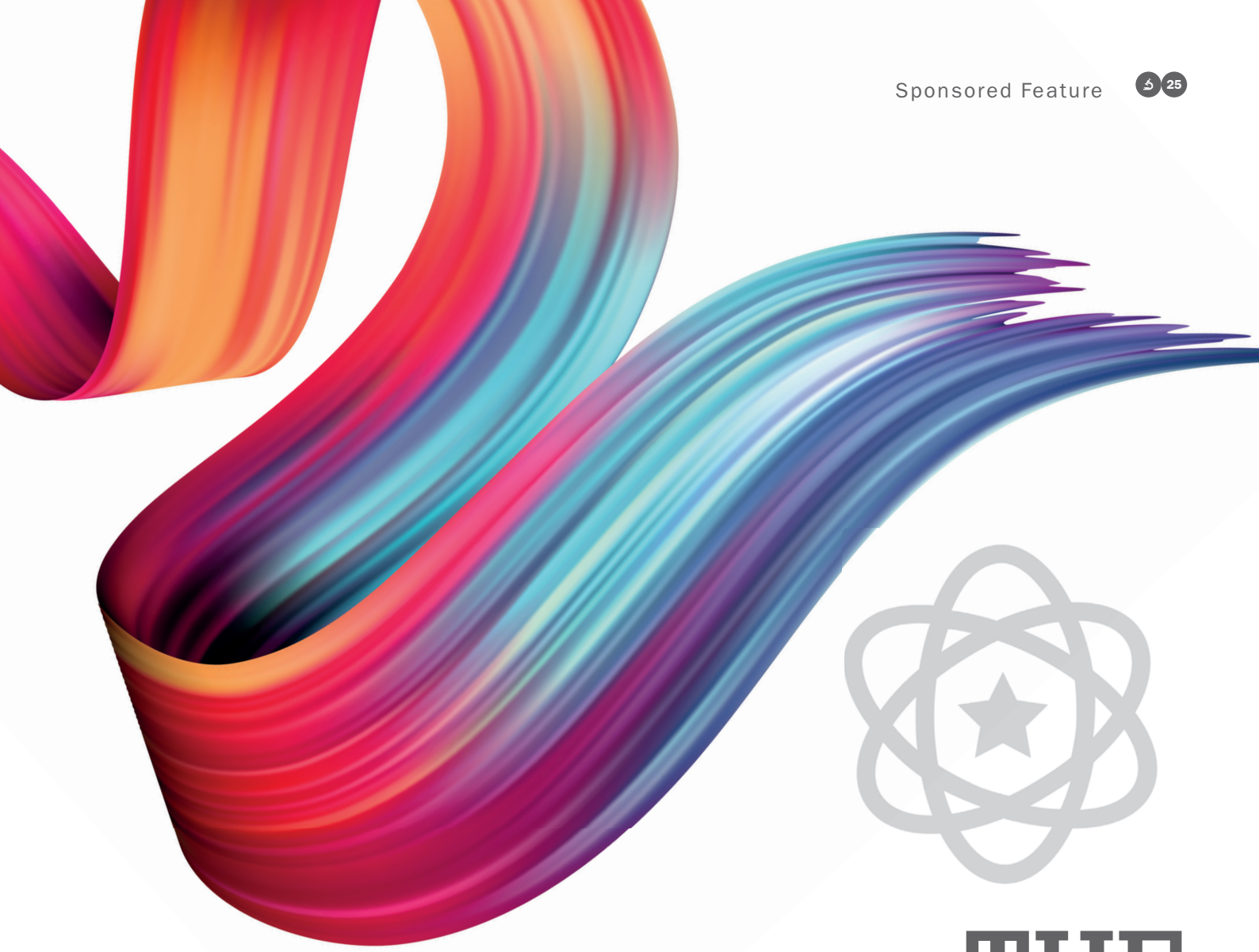

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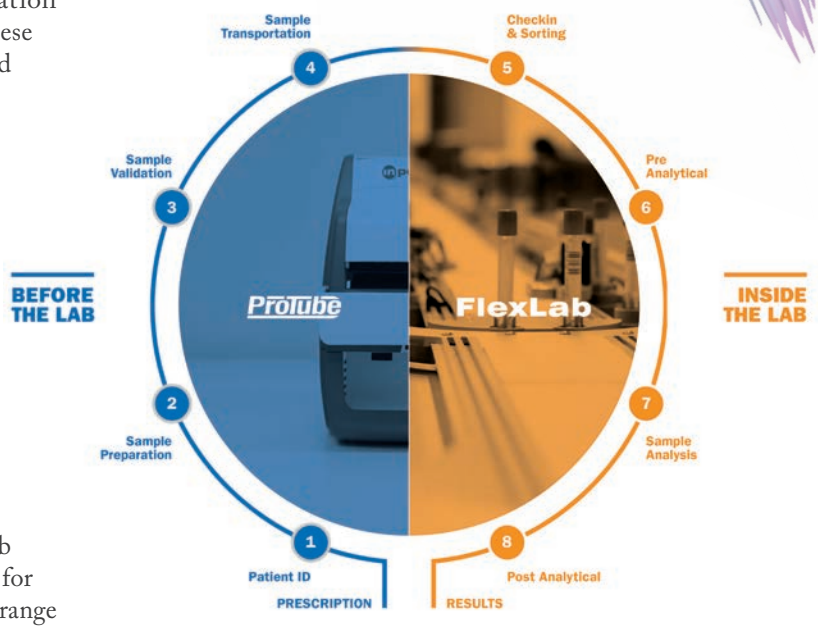

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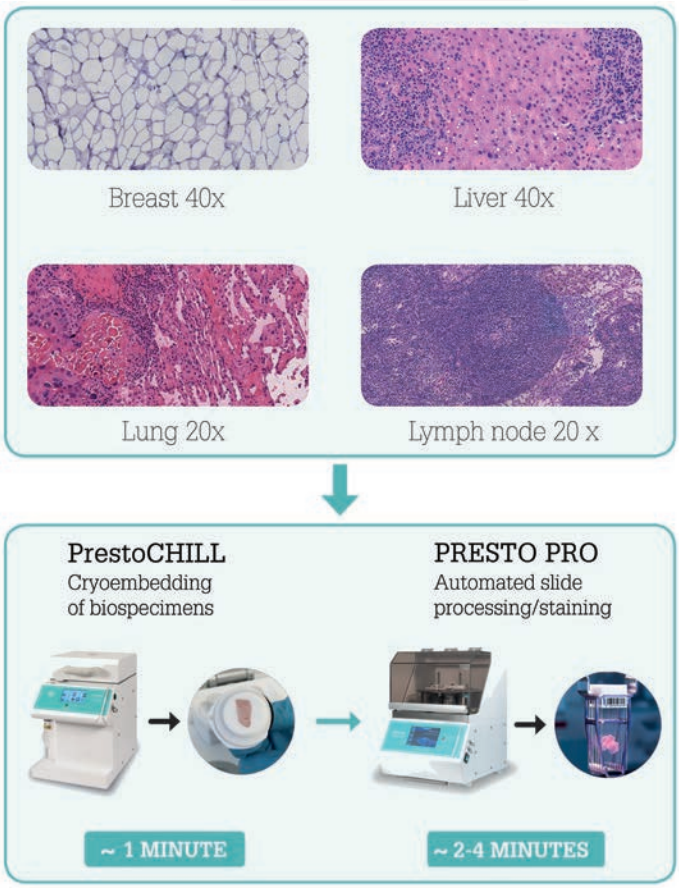

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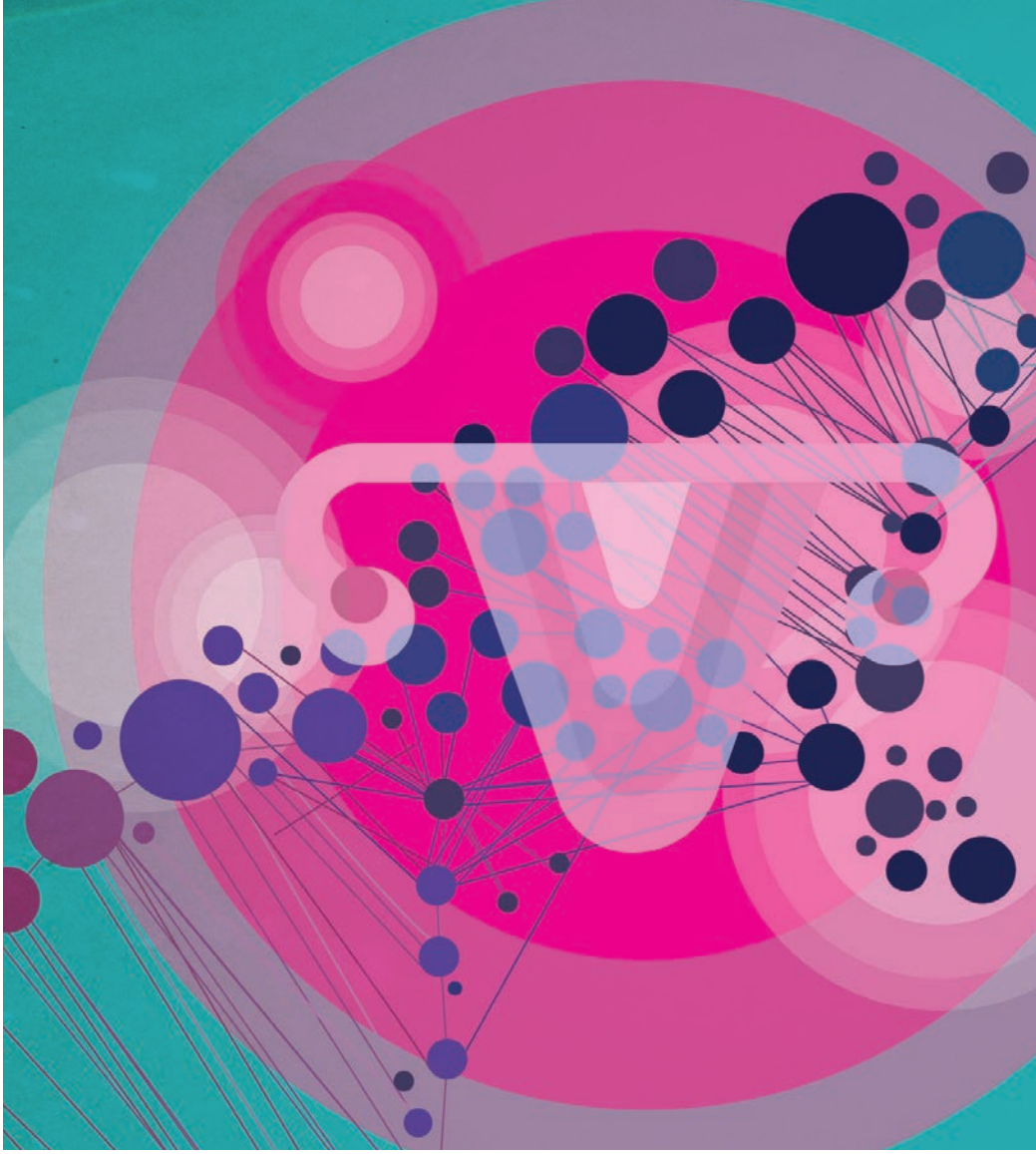


The Evolution of Epigenetic Biomarkers

Methylation and similar DNA biomarkers, combined with liquid biopsy, may offer better testing options for difficult cancers

By Niamh Buckley, Laura Feeney, James Beirne, and Paul Mullan

No part of the diagnostic route to cancer is pleasant – the initial symptoms, the scans, the waiting, the uncertainty. But of all the undesirable experiences, it's likely that tissue biopsy ranks highest on the list of things patients hope they never need to repeat. And yet, tissue biopsy certainly offers important insights into an individual patient's disease, which is why we continue to request such invasive, and occasionally risky, tests. But tissue biopsy presents problems beyond just obtaining the sample. It's a painful procedure for patients and, depending on the tumor's location and characteristics, may require general anesthetic, specialist imaging, or other interventions. This lack of accessibility can make retesting difficult and put the quality of the sample at risk. And a successful sample isn't the end of the story; some may be too small for all of the required tests, or too difficult to preserve well. Still others may not contain examples of every cell type and mutation in a heterogeneous tumor – and there's no way of finding that out by examining the sample we get.

At a Glance

- *Tissue biopsies serve a valuable purpose – but they are far from perfect*
- *Liquid biopsy may be a better option, if we can overcome its technical limitations*
- *Ovarian cancer is one disease that can benefit from better biomarkers and better assays*
- *Epigenetic biomarkers, such as methylation, may improve sensitivity and specificity in the diagnosis of ovarian cancer*

A new kind of sample

By now, most – if not all – pathologists have heard of liquid biopsy, and some of you may already be using it in your work. What makes liquid biopsy a better option? The approach measures biomarkers in bodily fluids, which are limitless resources and, in most cases, accessible in a minimally invasive manner. Blood, for instance, requires a simple draw, whereas saliva and urine are even easier to obtain. It's true that certain fluids, such as cerebrospinal fluid or pleural effusions, are trickier to access, but even those carry fewer risks than traditional tissue biopsy or tumor resection. As a result, the liquid biopsy procedure is not only safer, but also less expensive for both patients and healthcare systems. It's not completely free of limitations – for instance, target material often appears in only small concentrations in the sample, meaning that tests may be less sensitive or require advanced sampling techniques – but the advantages are promising.

From liquid biopsy samples, we isolate or interrogate circulating tumor cells (CTCs), circulating tumor DNA

"The liquid biopsy procedure is not only safer, but also less expensive for both patients and healthcare systems."

(ctDNA), cell-free DNA (cfDNA), and exosomes so that we can analyze biomarkers such as gene fusions, point mutations, methylation changes, circulating mRNA, and exosomes. This variety of options means that we can screen patients at risk of disease (or even, one day, perhaps the entire population). After diagnosis, we can stratify them for treatment, monitor their response to therapy, and keep an eye out for minimal residual disease (MRD) – all without the need for invasive surgical procedures.

My analyte in liquid biopsy is cfDNA – short (150–180 bp) fragments of DNA. cfDNA tends to be present in higher abundance than CTCs and can be assessed from frozen plasma, which broadens the available testing options. But it's not only tumors that release cfDNA into the blood; all cells can do it, which means we can examine genetic and epigenetic changes in tissue symptomatic of other diseases as well.

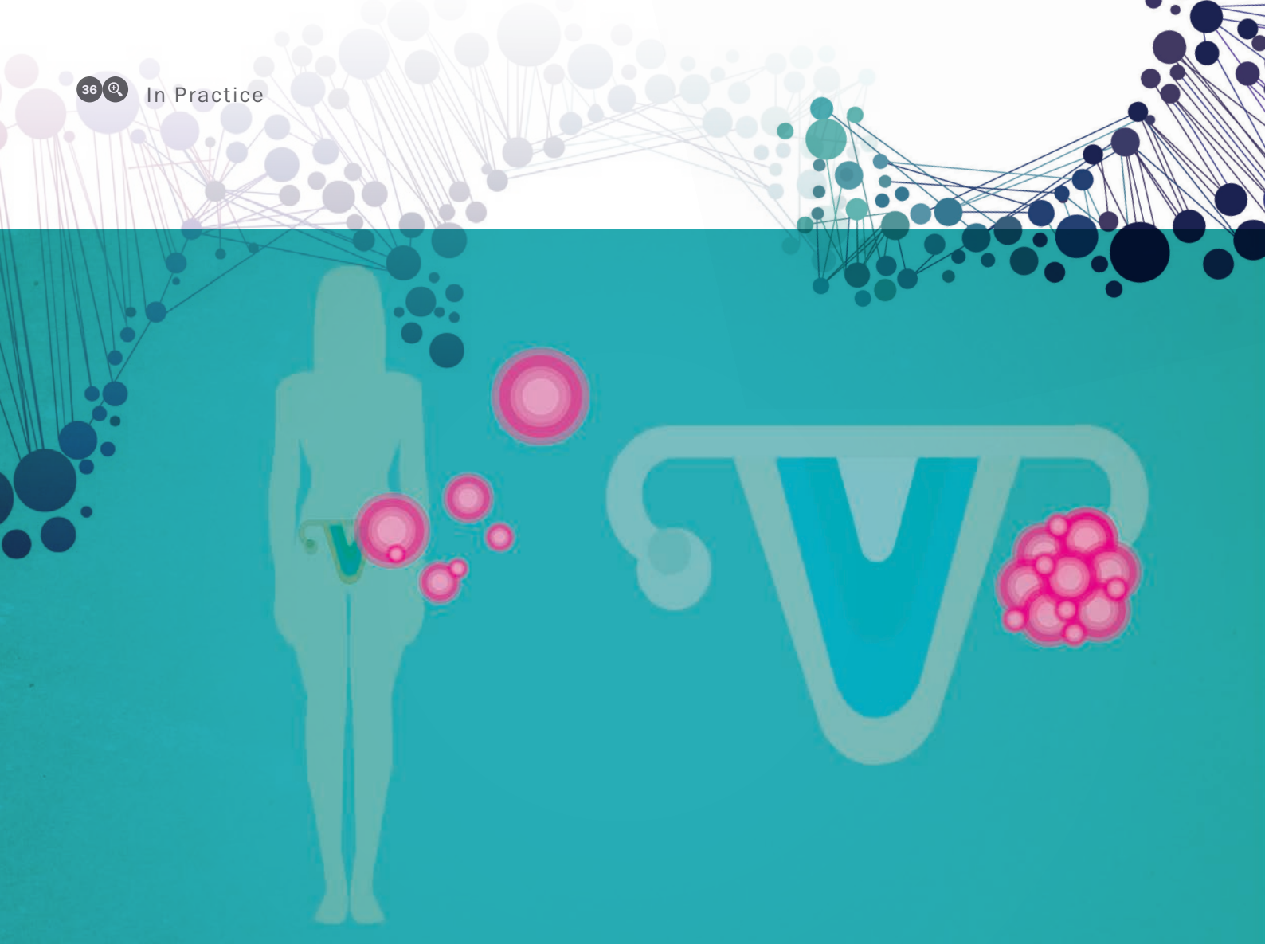
There are a number of tests on the market to measure genetic alterations in blood samples. The first, approved by the US Food and Drug Administration in 2016, searches for *EGFR* exon 19 deletions and exon 21 substitutions in non-small cell lung cancer to select those who may benefit from inhibitor treatment (1). Newer tests are not limited to one or two mutations; in fact, they aren't even limited to one or a few cancers. One recent test reported in the literature looks for 16 gene mutations in cfDNA, as well as abnormalities in eight circulating proteins, to detect eight different common cancers (2). Its overall sensitivity ranges from under 40 percent to nearly 100 percent, and its specificity sits around 99 percent. It is important to note, however, that non-cancer samples were not analyzed in this study, so this specificity needs further investigation. Nevertheless, it's clear that these tests have huge potential – and that's why they hold such promise for ovarian cancer.

Tackling ovarian cancer

Ovarian cancer is the third most common gynecological malignancy worldwide (3), with nearly 300,000 new cases diagnosed each year. Unfortunately, it's also the second most lethal, claiming nearly 185,000 lives – almost two thirds of those diagnosed. This is partly because the disease is so often detected late, and partly because it frequently shows resistance to standard chemotherapy.

At the moment, CA-125 is the gold standard circulating protein biomarker for the management of ovarian cancer. But despite its status as the main single biomarker in diagnostic testing, it's not elevated in up to half of early cancers – and it can be elevated in a number of non-cancerous conditions, including benign tumors, follicular cysts, endometriosis, infection, and even pregnancy. As a result, it has been deemed unsuitable for screening, and its capacity for cancer diagnosis is limited. Potential new tests reported in the literature, evaluating liquid from Pap smears, show promise; one that analyzed 18 genes and aneuploidy was able to detect 33 percent of ovarian cancer cases, including early-stage disease (4). When combined with ctDNA, the detection rate increased to 63 percent – still low sensitivity, but with 100 percent specificity.

It's this potential that prompted me to investigate liquid biopsy's potential in high-grade serous cancer (HGSC), the most common – and most aggressive – form of ovarian cancer. Recent research shows that HGSC actually begins in the distal fallopian tube as serous tubal intraepithelial carcinoma (STIC) before seeding to the ovary (5). My colleagues and I developed a bespoke clinical cohort of six patients with matched tissue from normal fallopian tube, STIC, ovarian cancer, omentum, and normal ovary, with the goal of identifying novel epigenetic biomarkers.



Epigenetic advances

Why epigenetics? These markers boast several benefits, such as stability and frequency of occurrence. People often think that mutations are the easiest cancer-associated change to test; however, this is not always the case. Although p53 is mutated in approximately 95 percent of ovarian cancers, the specific mutation is not always the same, and potential mutations are spread over kilobases of DNA sequence. Epigenetic changes tend to be more conserved. They also offer noninvasive accessibility (for instance, via blood) and reversibility – all of which mean they may be not only diagnostic, but also potential treatment targets. We opted to look specifically at DNA methylation because it's the most widely studied genetic alteration, tends to be highly concentrated in CpG islands within and near promoters, and has known behavior: hypermethylation is associated with chromatin condensation and gene silencing.

How do you identify methylation markers worthy of investigation? Genetics and omics studies can reveal them in specific tissues. Once a candidate gene is identified, it is validated in its native tissue and then migrated to liquid biopsy. An example is the *SEPT9* gene, which is hypermethylated in many colorectal cancers. A discovery project looking for a blood test for these cancers examined tumor tissue, normal colon, non-colonic control tissue, and peripheral blood lymphocytes to identify this candidate gene (6), which was then further validated by a retrospective trial in symptomatic patients. After prospective trials that compared methylation testing with colonoscopy and the fecal immunochemical test for population screening (7,8), an epigenetic screening test for colorectal cancers was approved in 2016.

One approach to epigenetic biomarker identification is the "candidate gene" approach in a disease with known biology. The gene is typically a novel oncogenic driver or tumor suppressor. Its altered expression signifies its potential importance in disease, leaving investigators with questions like: how is its expression regulated? Could it be used as a biomarker? If so, what kind – diagnostic, prognostic, or predictive? My colleagues and I recently identified

"People often think that mutations are the easiest cancer-associated change to test; however, this is not always the case."

just such an alteration, present in up to 15 percent of ovarian cancer cases, and are currently investigating its possibilities as a methylation-based biomarker for the disease.

Another approach is the "discovery" approach, in which a methylation array highlights differentially methylated CpG islands, whether or not they are easily associated with a specific gene. Regardless of how these potential methylation marks are identified, they must then be validated using assays such as bisulfite pyrosequencing. This test determines methylation levels by converting unmethylated cytosine to uracil; methylated cytosine remains unchanged. Thus, differential levels of methylation in control and tumor tissue can be measured to validate potential epigenetic biomarkers. In the case of the marker we discovered, its sensitivity is comparable to the gold standard CA-125, but its specificity is much higher. In combination with CA-125, it yields improvements in both sensitivity and specificity.

The translation equation

It's clear that these biomarkers have real potential for use in the diagnosis and management of ovarian cancer. But how do we make this a reality? We need to translate our current knowledge to liquid biopsy, but that won't be easy. Bisulfite conversion is very harsh on DNA and pyrosequencing lacks sensitivity – so we'll need a lot of material to analyze.

There are no standard methods for processing blood or extracting DNA, although ASCO/CAP guidelines recommend collecting samples in cell stabilization or EDTA tubes and processing within six hours. But is there enough ctDNA in a standard blood sample – especially if the tumor itself is small? If we assume that the tumor is approximately 1 cm in diameter (thus consisting of about 500 million cells) and 0.01 percent of genome equivalents are present in circulation, then there will be between 17 and 20 tumor genomes (34 to 40 copies of any sequence) per milliliter of plasma. The limits of methylation detection are reported to be as low as one or two copies (9), so the assay should work. However, it does need to be very specific to differentiate normal from tumor DNA.

Are there any other assay options? Methylation-specific restriction digestion may offer an alternative, although it would be necessary to ensure the optimal conversion of unmethylated DNA. Some techniques also offer improved sensitivity over pyrosequencing: methylation-specific PCR (with droplet digital PCR), PCR with high-resolution melting, and COLD-PCR (co-amplification at lower denaturation temperature). All of these assays hold promise and have the required level of sensitivity – a sign that, despite the technical challenges we have yet to fully overcome, it's time to explore our options in the realm of liquid biopsy.

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Thinking Outside the (Genome) Box

How minimally invasive epigenetic technologies pave the way to early and accurate detection of cancer

By Jason Mellad

Across the globe, cancer causes one in every six deaths. Mortality is most commonly a consequence of malignant disease of the lung, colon, stomach, liver, or breast tissue (1). Disease progression, accompanied by the metastatic spread of tumor cells to other tissues within the body, remains the primary cause of death and disability among those with cancer (2). To reduce this burden, early detection and intervention are critical. We must improve survival rates and lower morbidity – but we must do so wisely, optimizing the use of expensive specialist medicines, health resources, and procedures.

At a Glance

- *Access to accurate and effective cancer diagnostic technologies is variable, leading to delays in detection and treatment and poor clinical outcomes*
- *Conventional diagnostics are costly, invasive, and time-consuming, often requiring complex specialist procedures or hospitalization*
- *Epigenetic biomarkers are highly specific for disease state and tissue type and can be detected upstream of genetic alterations*
- *By combining minimally invasive sampling techniques with the precision and sensitivity of epigenetic analysis, we can detect cancer early and begin timely therapeutic intervention*

A diagnostic deficit

Conventional diagnostics are limited by suboptimal accuracy. They are also expensive to deliver, requiring specialist skills and services. Inadequate access to resources or technology and the intrusive nature of many investigative techniques (such as tissue biopsy, colonoscopy, or pleural fluid sampling) hinder disease detection and significantly impact patients' quality of life. How can we improve upon these approaches? Minimally invasive techniques that employ highly sensitive biomarkers for early-stage disease and allow regular screening using a simple blood or saliva sample would support a simple, practical, and cost-effective approach that might be more acceptable to patients. And with increased access to testing and increased willingness to be screened, such a technique could potentially identify cancers early enough to improve the odds of treatment success. It would also facilitate ongoing monitoring during or after treatment – giving patients the best chance of having disease progression identified and halted, or receiving prompt treatment in the case of relapse.

In recent years, the rapidly evolving field of epigenetics has driven transformative advances in research regarding the fundamental biological processes controlling human development, disease, and aging. Innovations in this area have the potential to revolutionize cancer diagnostics beyond the capabilities of traditional genetic screening, delivering exceptional levels of accuracy and enabling detection ahead of symptomatic disease.

Better biomarkers for disease

Epigenetic modifications are highly potent and specific chemical groups within genomic DNA or RNA nucleotide sequences and histone proteins (3). These modifications, which include methylation, phosphorylation, acetylation, and more, do not alter the underlying sequence of genomic DNA itself; rather, they influence the behavior and regulation of genes.

"Disruption or dysregulation of the epigenetic machinery can have disastrous consequences."

Epigenetic modifications are heritable and can also be added, removed, or altered in response to external factors. Lifestyle (for example, smoking or diet), environment (for example, pollutants), and other stressors can influence the dynamic structure and function of the epigenome. Why? Epigenetic changes may provide an evolutionary advantage or drive molecular processes and cascades associated with aging and disease. The epigenome maintains a delicate balance of chemical modifications essential for multiple cellular processes in healthy biological systems. However, disruption or dysregulation of the epigenetic machinery can have disastrous consequences; mutations within epigenetic regulators are prevalent across all cancers and have also been linked with a wide range of neurological, immunological, and metabolic diseases (3,4).

Specific epigenetic patterns or signatures have become important biomarkers for disease. Pioneering technologies that allow these stable biomarkers to be mapped and quantified have revealed that it is possible to detect high-intensity signals from

clinical samples. Epigenetic biomarkers are also highly specific for disease state and tissue type, allowing accurate assessment of disease progression and tissue of origin.

Among the most important epigenetic modifications characterized to date, 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) (see Figure 1) have been proven to play a pivotal role in the development of cancers and other serious diseases (5–8). Regulation of the molecular pathways associated with these chemical changes is critical to maintaining normal cellular function and avoiding pathogenesis. For example, the Ten-Eleven Translocation (TET) family of enzymes converts 5mC to 5hmC in DNA. Loss of function or changes in the expression of TET enzymes correlate with abnormalities in cytosine methylation that are associated with a number of aggressive cancers (7,8).

Changes in 5mC and 5hmC are also predictors of very early-stage cancer transformation. Research in glioblastoma cells has demonstrated that epigenetic alterations associated with oncogenic pathways can be detected in neighboring non-tumor cells, even when these cells appear genetically "normal" (9). This discovery highlights the potential value of epigenetic signatures in cancer screening programs – as robust biomarkers for early-stage disease that can be detected well before genomic changes are apparent (see Table 1).

In addition, epigenetic biomarkers can provide insights into the risk of metastasis, tumor recurrence, and overall prognosis. Further research, again in glioblastoma cells, has shown that levels of 5hmC are important in the regulation of disease-critical genes. Global reduction in 5hmC across the genome tends to be associated with poor clinical outcomes and reduced survival (5). Using such information, clinicians may be able to stratify patients according to risk and epigenetic profile to offer more accurate prognoses and guide appropriate treatment.

Therapeutic resistance is an ongoing challenge in the successful management of cancers. Specific signatures associated with drug resistance have been identified within the epigenome and can occur in the absence of genetic alterations (3,10). Individuals exhibiting resistance biomarkers may have a greater risk of treatment failure and therefore be better candidates for alternative therapeutic options. Regular testing would allow resistance issues to be identified quickly, allowing patients to move to the most effective treatment options as soon as they are needed, rather than having to work through a rigid treatment regimen after it has lost its

effectiveness. This kind of personalized approach would allow patients to avoid chemotherapies that are associated with significant toxicities but, due to the individual's molecular/epigenetic profile, offer little or no therapeutic benefit. The ultimate outcome? Healthier patients, easier treatment, and better use of costly health resources.

Harnessing the power of epigenetics

Over time, cancers tend to evolve with disease progression or in response to environmental factors and the body's own immune response (11,12). Disease heterogeneity presents a further barrier to appropriate treatment selection and tailoring of management strategies. Traditional approaches to sampling and diagnosis, such as solid tumor biopsy, are generally unable to provide a full picture of heterogeneity because analysis is limited by the range of cancerous cell types present in the "snapshot" sample. In contrast, analysis of circulating tumor DNA (ctDNA) or cell-free DNA (cfDNA) from liquid biopsy samples taken at regular intervals could provide a more comprehensive view of cellular evolution. After all, liquid biopsy makes serial sampling and screening easier to implement in routine practice, allowing samples to be taken during treatment or follow-up to guide disease management, gain a better understanding of how a cancer is changing, and identify issues or concerns while they can still be addressed. To obtain valuable diagnostic information from these samples, we need meaningful signals from even small quantities of cfDNA or ctDNA – so technologies must offer a high level of sensitivity and specificity. Notably, not all biomarkers can be identified and measured using traditional genetic research techniques; critical epigenetic markers or signatures are missed when laboratory scientists look solely at the genome. To detect them, we need epigenetics-focused technologies with adequate signal strength to find markers in samples containing exceptionally low ctDNA concentrations.

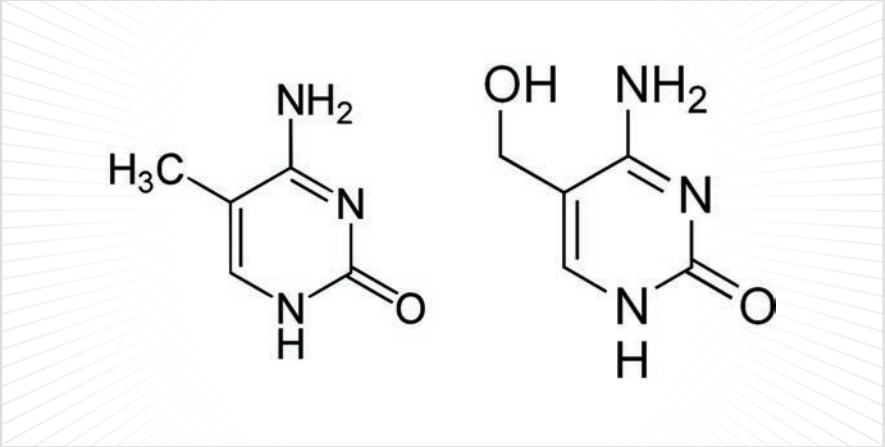


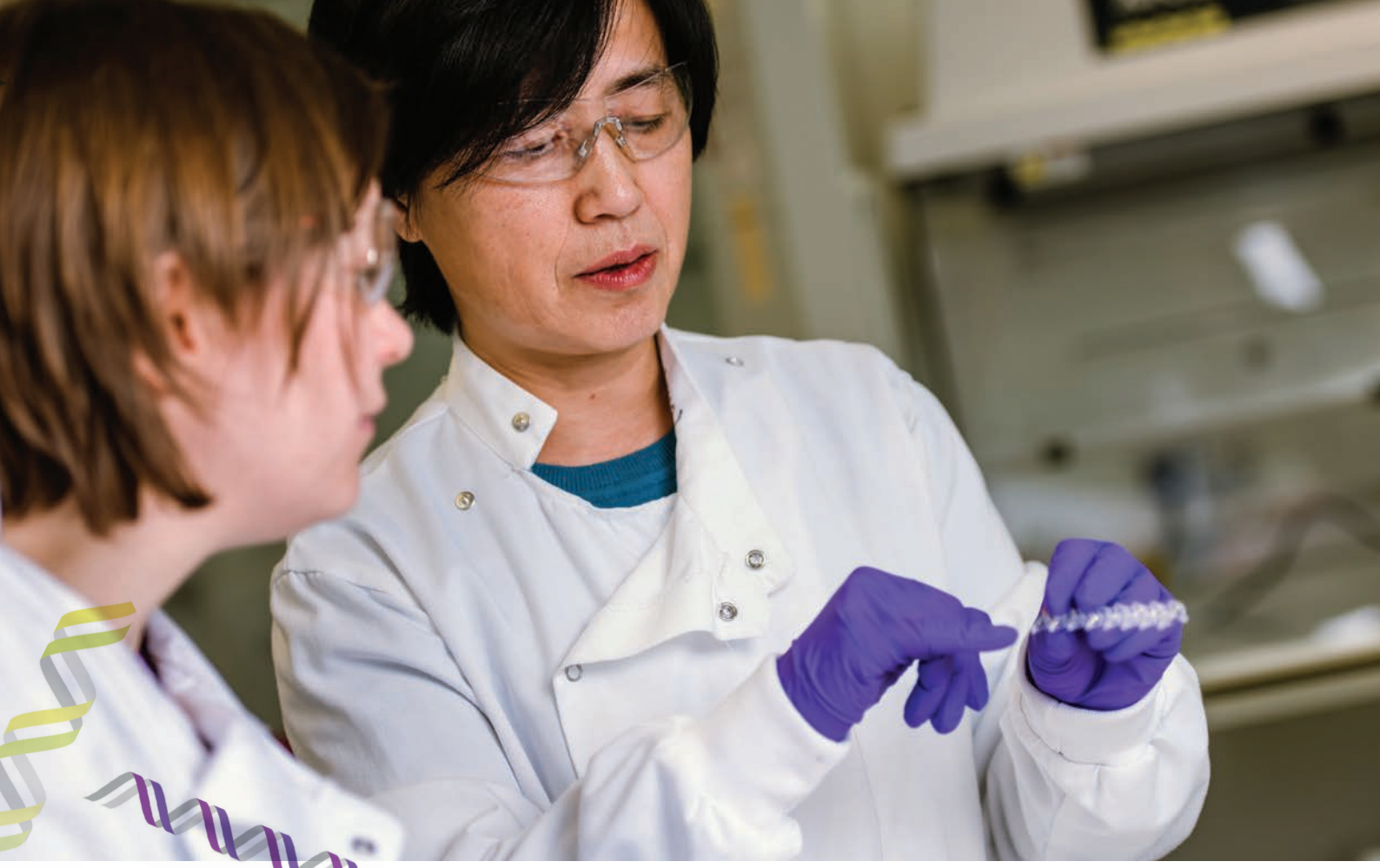
Figure 1. 5mC (left) and 5hmC (right) are critical epigenetic modifications involved in the regulation of molecular pathways required for normal cellular function. Dysregulation of the 5mC and 5hmC pathways is associated with pathogenesis and a number of aggressive cancers.

Feature	*Diagnostic or clinical benefit*
Stable, with high signal intensity	Accurate mapping and measurement using novel technologies
Highly specific and accurate	Precise identification of disease state and tissue type
Biomarkers for early-stage disease	Timely disease detection upstream of genetic changes
Detection from multiple sample types (blood, tissue, circulating plasma)	Minimally invasive, maximally informative diagnosis
Markers for therapeutic resistance	Identification of therapeutic resistance even in the absence of genetic alterations

Table 1. Key features of epigenetic biomarkers and their potential diagnostic or clinical benefits.

Why make the leap?

In a modern healthcare environment, where funding and resources are usually limited or restricted, cancer treatment and ongoing management represent a significant financial burden. Technologies that offer liquid biopsy sampling alongside automated epigenetic analyses may help to reduce healthcare costs on a number of levels. For instance, liquid biopsy sample collection does not require hospitalization or specialist involvement; support staff can take samples during routine visits, which allows cancer specialists to make more efficient use of their time by focusing on treatment, rather than on diagnostic sample collection. Additionally, disease identified early using epigenetic



biomarkers is likely to require less intensive therapeutic approaches, reducing patients' risk of treatment-related complications or toxicity issues. Finally, epigenetic biomarkers can guide or individualize appropriate treatment selection according to the individual patient's profile – supporting optimal therapy and cost-effective prescribing.

Liquid biopsy and epigenetic platforms offer a powerful combination of minimally invasive and maximally informative diagnostics, without sacrificing simplicity and practicality. Essential information concerning the nature and stage of disease, prognosis, risk, and drug resistance can be elucidated using potent epigenetic biomarkers identified from a simple blood test. This forward-thinking approach has the potential to shift the oncology treatment paradigm towards earlier and more effective treatment of disease for the benefit of patients, clinicians and healthcare systems.

Jason Mellad is former Chief Executive Officer at Cambridge Epigenetix. He has recently taken on a new venture and, as of November 15, 2018, Suman Shirodkar has taken over as Chief Executive Officer of Cambridge Epigenetix Ltd., Cambridge, UK.

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Twin Challenges: Collaboration and Communication

What must pathologists do to fulfill their roles on the health care team?

By Fred Bosman

We pathologists have two important – and often overlooked – roles to fill: collaborator and communicator. Those roles are often conflated; after all, it's difficult to have one without the other. Nevertheless, they are different aspects of our role as members of a health care team, and it's vital for us to understand and actively engage in both.

The pathologist as a collaborator

Modern health care teams include not only groups of professionals working closely together on one site, such as a ward team, but also teams with a variety of perspectives and skills at multiple locations. It is therefore essential for us to be able to collaborate effectively with a multidisciplinary, multi-site team of health care professionals for the provision of optimal care, education, and scholarship.

What does that collaboration look like? We must know how to effectively consult other physicians and health care professionals. We can start by synthesizing any relevant information we have that our colleagues may not and presenting written and verbal reports on our findings and our proposals concerning further investigation needed for a patient. Our speaking and writing skills matter – it's crucial to prepare and present reports in a concise and easily understandable form so that our non-pathologist colleagues can understand and make use of what

collaborator
kəˈlabəreɪtə/
noun
noun: **collaborator**; plural noun: **collaborators**
As a collaborator, the pathologist effectively works within a health care team to achieve optimal patient care.

At a Glance

- *Every pathologist must serve as both collaborator and communicator on the health care team*
- *As collaborators, pathologists must learn to work well with not only other pathologists, but also other professionals in the health care sphere*
- *As communicators, we must maximize the value of our spoken and written messages to other team members, patients, and the public*
- *In both, we must treat others with respect, act as an expert resource, and ensure that patients receive the care they need*

we're telling them. Each time we work on aspects of a case, we should record accurate, logical, comprehensive, and pertinent accounts – meaning that every examination, advanced analysis, diagnosis, and communication with clinicians should be filed, time- and date-stamped, and clearly attributable to us as the authors. And with medical records moving increasingly toward electronic models with patient access portals, we should make each note with the clear understanding that it may be read by the patient.

Once all of the information is on the table, we should enter discussions about diagnosis and treatment options with the other members of the team when appropriate. That means we must be able to recognize the limits of our personal and professional expertise and decide if and when other professionals' contributions are needed to arrive at a final diagnosis in a case. And whether or not one is reached, we still need to communicate effectively with every member of the team to ensure a shared understanding of our patients' problems and to foster continuity of care.

When we begin to see ourselves not only as individuals, but also as vital components of a larger healthcare system, our deep influence on patient care – at both a personal and a population level – becomes more obvious than ever. We don't just weigh in on individual cases, although that is a key part of our work. We should also be cooperating with other professionals to make efficient use of resources by coordinating care, and contributing to the management of our patients at the interface with other specialties. We should be working with

"When we begin to see ourselves [...] as vital components of a larger healthcare system, our deep influence on patient care becomes more obvious than ever."

laboratory staff to implement efficient, high-quality diagnostic laboratory services and appropriate quality control. And we should be ensuring that our colleagues and collaborators can finish what we start: by demonstrating good handover practices and ensuring continuity of care when we go off-duty; by accurately summarizing the main points of pending cases; by providing pertinent information to the appropriate staff; and being ready with urgent additional information when necessary.

Clearly, it's important for us to learn how best to work with our colleagues – both by expanding our collaborative skills and by exploring how we can invest in our roles on the multidisciplinary team. And the best way to keep tabs on how we're doing is to seek feedback from colleagues on the quality of care our patients are receiving. We need to cope well with both positive and negative feedback – and we must be seen to reflect on and use that feedback to improve our skills. Indeed, welcoming any kind of feedback is key to ensuring that other medical professionals are comfortable telling us what we need to hear.

Full marks for collaboration

The collaborative pathologist contributes effectively to other interdisciplinary team activities beyond the scope of patient diagnosis and treatment. The tools in our arsenal? Effective teamwork skills, an awareness of our personal roles and responsibilities on the team, and leadership skills – along with the discernment to use them when, and only when, appropriate. How do we know when that is? All members of the team should be able to define their own tasks and competencies in relation to those of the others – not just the doctors, but also laboratory staff and other health care professionals.

We must treat all members of the health care team with respect regardless of similarities or differences. We need to understand the clinical setting in which we work and the interactions that occur within it, and shape our work effectively in light of that insight. Any personal agenda must be secondary to the goals of the medical team – and, of course, our patients.

We cannot be good collaborators if we don't support our colleagues; therefore, we should assist laboratory staff with the design and implementation of new laboratory procedures, attend sign-out meetings, and accept directions from our expert colleagues. If we spot a potential problem in a complex case, it's our job to take pre-emptive action – and to assist our co-workers in doing the same.

The best collaborative pathologists work effectively with colleagues and management to help create a culture where the improvement of quality and safety becomes a part of daily practice. The best collaborators also never stand still – we always seek improvement elsewhere.

How can we improve our collaborative skills?

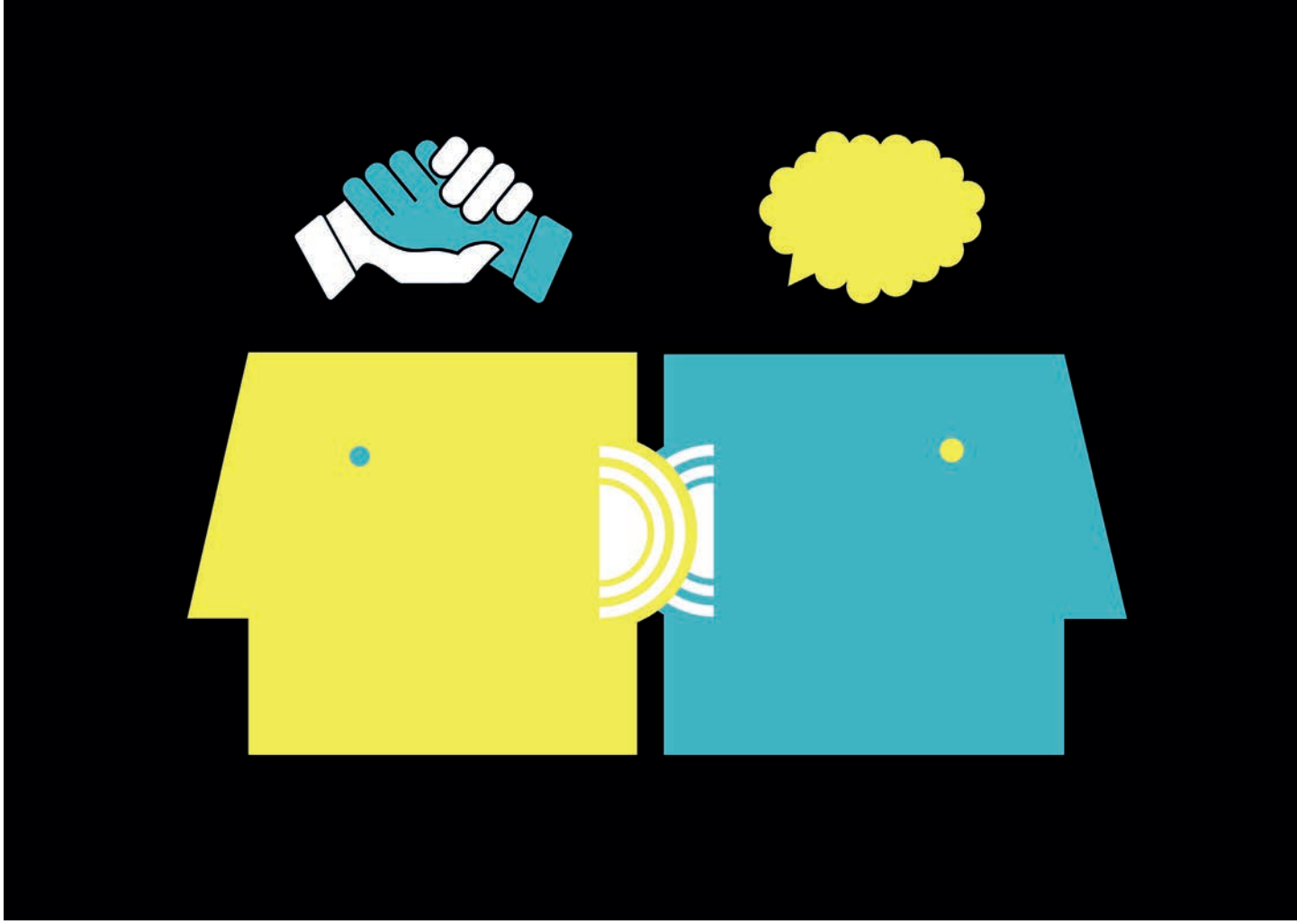
- Connect with others to assess, plan, provide, and review tasks such as administrative responsibilities and education.
- Encourage an atmosphere of open communication and appropriate directed communication within your team, so that those we work with feel they can come to us with feedback, whether good or bad.
- Seek out opportunities to learn in cooperation with other health professionals – pathologists, certainly, but also others within and outside your institution. It is surprising how much we can learn from other disciplines!

The pathologist as a communicator

Communication skills are essential for our day-to-day function, as well as necessary for exchanging information with requesting physicians and other health care professionals – not to mention individual patients or patient groups. Furthermore, these abilities are critical for assessing key factors that affect the quality of care we provide.

To develop a productive professional relationship with others on the health care team, we must first understand that good communication is a core clinical skill for pathologists, and that effective pathologist-physician communication can foster physician satisfaction and improve clinical outcomes. To that end, we should ensure that we are always courteous and considerate to technical and medical staff, treating them as we ourselves wish to be treated. Nonverbal

communicator
kəˈmjuːnɪkeɪtə/
noun
noun: **communicator**; plural noun: **communicators**
As a communicator, the pathologist facilitates the exchange of information and participative case management between medical experts in the context of the dynamic exchanges that form part of a patient-centered medical encounter.



cues can be valuable ways to discern how others feel about how we communicate with them. And, of course, we must respect our patients equally – including their confidentiality, privacy, autonomy, and right to know about and understand their own conditions and care.

The main purpose of communication is a two-way exchange of information. No one medical professional is an expert on the entire patient – including the pathologist – so we must seek out the perspectives of our colleagues to see the whole picture. If appropriate, we can also look for information from other sources, such as the patient's caregivers or the patient themselves. We will always take care to assess the potential impact of factors such as age, gender, and ethno-cultural background on our diagnostic reasoning. All of this should then help us explain to colleagues and other professionals our assessment of a case so that we can reach a common understanding on issues and problems and develop a shared plan of care. Because the other members of the care team are not pathologists, we should deliver information to them in a way that is understandable so that it encourages discussion and participation in decision-making. Above all, our goal should be to foster the highest quality of communication within the team, because it helps ensure that patients receive the best possible care.

That said, one can't always schedule a meeting to discuss every patient with every member of the team – so what else can be done? In such cases, written records are our friends. We will provide clear, concise, and complete reports to requesting physicians (and follow up with a verbal conversation whenever we feel it is necessary). We will routinely and comprehensively record personally attributable accounts of all examinations conducted for each case and the decisions based on the results; also record any verbal information we give to other members of the team. Whenever possible, we will support our written reports with published data, computer-based information, or both. Although our summary reports should be easy to read and refer back to, the existence of a thorough base of supporting information may be helpful in the future. This advice may sound familiar – why? Because many of the attributes of a good communicator are also those of a good collaborator.

Overall, the key points any pathologist must remember are to practice respect, encourage feedback, and model good communication and collaboration to other members of the health care team. In this way, not only are we acting as an excellent expert resource for our colleagues, but we are also providing our patients with the service and care they deserve.

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Genetics, GI Pathology, and Golden Retrievers

Sitting Down With… Wendy L. Frankel, Kurtz Chair and Distinguished Professor of Pathology and Chair of the Department of Pathology at The Ohio State University Wexner Medical Center, Columbus, USA

You come from a medical family…

My father was a prominent surgeon in our community and patients recognized him whenever we went out as a family. I was so impressed by their admiration, his passion for surgery, and his dedication to his patients that I wanted to be just like him. I used to go on patient rounds with my father on weekends when I was in high school and did a summer internship in surgery while in college. I never even considered entering a field of medicine other than general surgery. I planned to specialize in gastrointestinal (GI) surgery.

So where did you get your passion for pathology?

In accordance with my career plan, I spent five years in a general surgery residency at the Hospital of the University of Pennsylvania. In my program, most residents completed three clinical years, took two years for research, and then returned for two more clinical years. My research was in a surgical nutrition and GI surgical laboratory with John Rombeau, and it was that experience that inspired me to continue studying GI disease – but as a pathologist, rather than a surgeon. I found I was much more interested in studying disease pathogenesis, teaching, and research than in the technical aspects of operating.

I became interested in liver pathology while working with Linda Ferrell during my surgical pathology fellowship at the University of California, San Francisco. After that, I was fortunate enough to join The Ohio State University at a very exciting time: the start of our Columbus-area study on hereditary non-polyposis colorectal cancer (now known as Lynch syndrome). It's a surprisingly common condition; more than one in every 35 patients with colon cancer has Lynch syndrome. Our work, together with that of other centers, has demonstrated that all colorectal and endometrial cancer patients should be screened for Lynch syndrome, and that such screening is both feasible and essential. In the future, screening may be done using next generation sequencing, and I anticipate that other tumors will also be screened for microsatellite instability (MSI) given the new immunotherapeutic agents available to treat MSI cancers.

The start of my career at OSU was an amazing time of discovery in genetics and Lynch syndrome – and ever since, I have been fascinated by cancer genetics.

Why did you focus on becoming an active leader?

I was motivated to become a leader by the opportunity to change things, rather than just talk about problems. I was inspired by previous United States and Canadian Academy of Pathology leaders. I knew that I wanted to be involved at national and international levels, not just the local level, so I sought opportunities to get involved and have an impact on the future of our field. I've also considered getting more involved in leadership at OSU, or within the College of Medicine. However, I really enjoy working with residents and clinical colleagues at the moment. I don't think I'm quite ready to give that up in favor of taking on more leadership responsibilities yet.

How can others follow in your footsteps?

My advice to others with leadership goals? First and foremost: get involved. Consider formal leadership training opportunities. Get a mentor – and then, when you feel ready, become a mentor yourself. Be open to new opportunities and to modifying your plans.

Mentorship is key for pathologists and laboratory medicine professionals at every level, and I encourage all of my colleagues to be bold in going out and getting mentors even if none appear interested or available at their institutions. Teamwork, too, is very important. The work you do with others may be as important as what you accomplish on your own. Some of my highest-impact research has been as a member of a multidisciplinary team.

What's your view on social media's role in pathology?

Well, I have a presence on Twitter (@WendyFrankelMD), which helps me to stay in touch with other laboratory professionals at all ages and stages. Avid Twitter users such as Jerad Gardner and Christina and Michael Arnold have done their best to teach me the advantages of the platform! I have found that social media is a good way to keep up on literature while I am out and about. I have also recently been involved in Facebook live events and Twitter journal clubs and have been impressed by the wide impact and interest.

What advice do you have for your colleagues?

Our goals and interests change throughout our careers. I encourage others to consider new and exciting roles and not feel limited by preconceived notions or other people's opinions of what you should be.

Work/life integration is also important. Outside the laboratory, I have a wonderful husband, Brian Rubin (not the pathologist!), who puts up with my crazy schedule. I love golden retrievers; my first one was named Bili Rubin! My current dog, Cody, often appears in my talks as a very popular divider slide. I am an avid sports and outdoor enthusiast, so I try to get out of my office as much as possible. With a high-responsibility, high-stress job like ours, it's vital to have a personal life!

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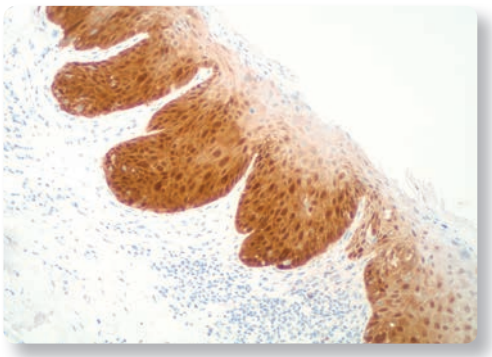
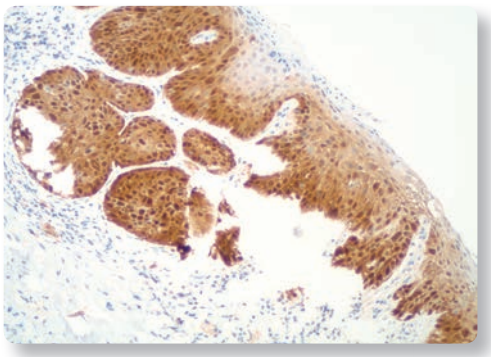


High-Grade Squamous Intraepithelial Lesion - Anal Tissue

Competitor p16 Antibody (mouse monoclonal)



High-Grade Squamous Intraepithelial Lesion - Anal Tissue

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