

# the Pathologist

**Upfront**  
The 11 faces of  
AML identified

12

**In My View**  
Thinking of introducing  
clinical mass spec?

16

**In Practice**  
Colorectal cancer screen  
could boost compliance

34 – 35

**Profession**  
Take action to avoid the  
“disappearing pathologist”

46 – 49

## We Need to Talk

When diagnostics goes wrong,  
how do we tell our patients there  
has been an error?

18 – 29



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guide the path  
her cancer takes



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# Image of the Month



This series of sculptures by artist Rogan Brown, laser cut from paper and hand mounted into three-dimensional sculptural compositions, is an attempt to depict the diversity and beauty of the vast colony of organisms that live in and on the human body: bacteria, fungi, pathogens, viruses, etc. “Our growing awareness of the complexity and importance of this colony is perhaps going to change the way we see ourselves as humans, no longer as a separate species but rather a symbiont one,” says Rogan.

Do you have an image you'd like to see featured in *The Pathologist*?  
Contact [fedra.pavlou@texerepublishing.com](mailto:fedra.pavlou@texerepublishing.com)



03 Image of The Month

07 Editorial  
Is It Good to Talk?  
by Fedra Pavlou

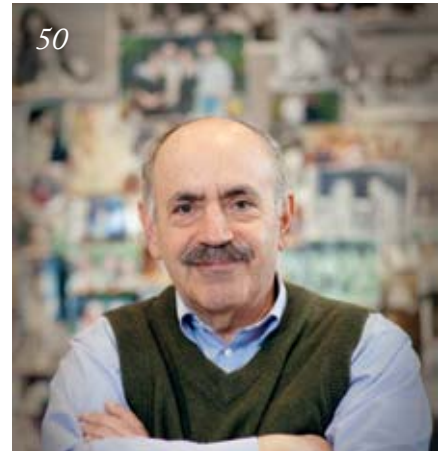
On The Cover



*Illustration of the pathologist being interrogated for diagnostic error; do most hold their hands up and disclose the truth?*

Upfront

- 08 An Electrifying Advance
- 09 Virus Vision
- 10 Simple Sample Sensing
- 11 Liquid License
- 12 The 11 Faces of AML
- 13 Fingerprint Fraud



In My View

- 14 Danielle Freedman stresses the importance of the involvement of laboratory professionals in the total testing process, not only to improve patient outcome, but to avoid harm.
- 16 Have you considered the financial and personnel implications of introducing mass spectrometry into the clinical laboratory? Y. Victoria Zhang guides on how you can make a successful transition.

Feature

18 We Need to Talk  
This is the first part of our two-part series, which takes an in-depth look into the controversial topic of diagnostic error. Should pathologists disclose error? If so, to whom, how, and what are the implications of doing so? We explore the intricacies and the challenges of this incredibly complex and multifaceted issue.

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## In Practice

### 34 A One-Two Punch for Colon Cancer

We report on a noninvasive multiple-marker test that combines DNA alterations with hemoglobin detection to diagnose colorectal cancer with high sensitivity and which could vastly improve screening success rates.

### 36 A Guide to IHC

Ping Tang shows how IHC analysis can help pathologists to make a correct breast cancer diagnosis and prognosis, and informs of the factors that might impact the quality of an IHC test.

## Next Gen

### 42 The Impact of COMET

Stephen Haggarty and Ralph Mazitschek describe a new approach to epigenetic control that could allow precise spatiotemporal targeting of disease-controlling mechanisms.

## Profession

### 46 Avoiding the Ripple Effect

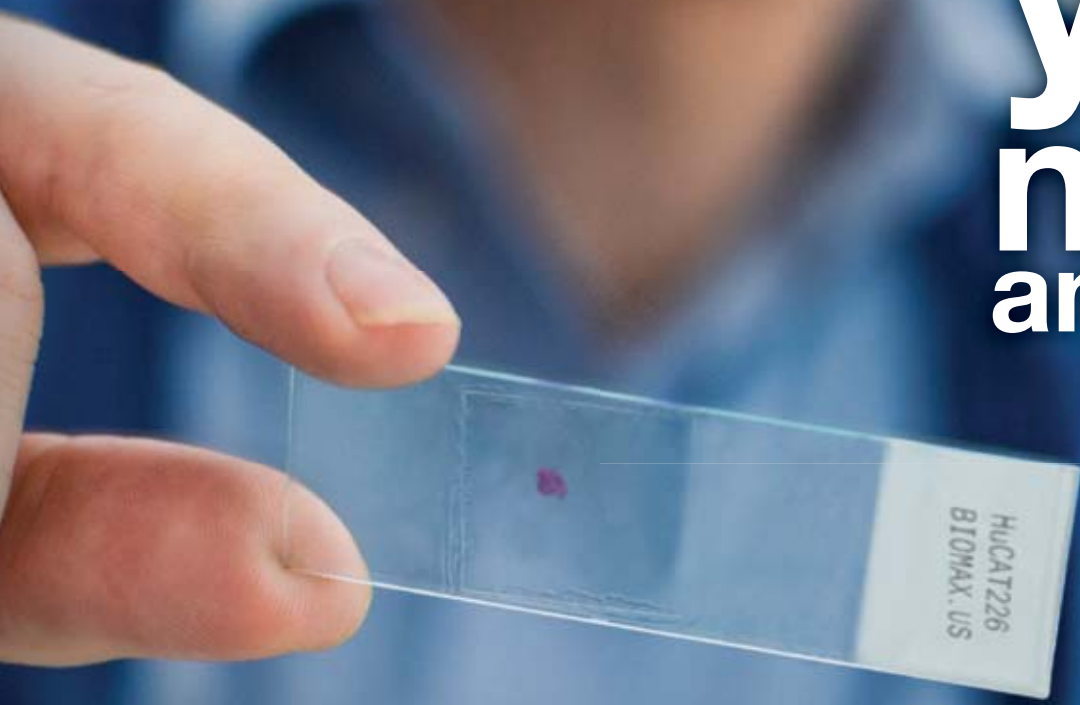
Charles Matthew Quick tells us how he is bucking the worrying trend that is seeing a continuous decline in the role of the pathologist as an educator. He has ignited interest in the field by successfully and creatively integrating pathology into medical students' first-year anatomy courses.

## Sitting Down With

50 **Robert A Weinberg**, founding member of the Whitehead Institute for Biomedical Research and Professor of Biology at the Massachusetts Institute of Technology, USA.

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## Is It Good to Talk?

*Pathologists are encouraged to speak up and be counted,  
but it's not always that easy...*

Editorial



**W**e speak a lot about public awareness of pathology (or lack thereof) in *The Pathologist*; how those in the lab need to stick their head above the parapet and talk more about the fantastic work they do. How they need to take every opportunity to educate medical students in this ever-evolving scientific discipline. How regular communication with other medical professions is imperative to optimizing patient outcomes. This might sound easy to most, but for those in the field, you know that it isn't. Not only is the profession challenged by changing medical school curricula that seem intent on deprioritizing pathology (check out this month's Profession article for some tips on how to address this, by the way), but in many, if not most, countries, pathologists are actively discouraged from communicating directly with others – in particular, patients. And this latter point is a highly contentious one.

I attended a meeting this week during which one of the presenters stated quite clearly that it is not a pathologist's job to communicate a diagnosis directly to a patient because "we're not equipped for it. We need to leave that job to our clinician colleagues." In my experience, this is not an uncommon view – while others that I've spoken with believe it to be an essential duty of the modern pathologist to be available to patients. This really does appear to have the community split and I'm on a mission to hear and to present both sides of the story.

Earlier this year, I had the pleasure of attending some fantastic presentations at the USCAP annual meeting in Seattle, which focused on patient communication and, an even scarier topic... diagnostic error disclosure. The sessions were standing room only and the audience highly engaged. It seems that the community wants some guidance on how to deal with the patient communication dilemma, and when it comes to error disclosure, the complexities that they face increase substantially. Lack of protocol, fear of litigation, reimbursement concerns, communication challenges... all cited as obstacles to effective disclosure of diagnostic errors. Naturally, I had to bring all of this to the attention of our readers. In this month's cover feature, we have the privilege of speaking with some key figures who are working hard to bring the issues around diagnostic errors, disclosure and communication into the spotlight. As I'm sure you'll agree, these topics are complex and multifaceted, which is why we're running our feature over two issues. Here we present the first part. And in the second, I plan to run some of your opinions too, so please do raise your head above that parapet and get in touch; I would love to hear your thoughts!

**Fedra Pavlou**  
*Editor*

# 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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## An Electrifying Advance

### Electrical fields can modulate sample flow through lab-on-a-chip devices for greater precision

Advances in pathology typically come from professionals in medicine or the life sciences who have dedicated their lives to unraveling the biology of human health and disease. But occasionally, such strides forward come from unusual sources – in this case, the Indian Institute of Technology's Advanced Technology Development Center, where a group of researchers have developed a way of improving control and flow rate in lab-on-a-chip devices. What makes this so exciting? The new method is easy to implement, improves device performance, and opens up a range of possibilities for future applications of microfluidics (1).

So how does it work? Usually, the tubes in microfluidic chip devices are made of polyvinyl chloride or Teflon. When placed in contact with aqueous electrolytes, these materials acquire a surface charge – which makes fluids behave as if they possess a net counter-charge, opposite in nature and magnitude to that of the tube's surface. The result? The ability to manipulate these types of devices by applying electrical fields.

Integrated devices that employ peristalsis – surface waves – are a hot topic in microelectromechanical systems (MEMS) research. Peristaltic micropumps can achieve a high surface oscillation frequency within a compact structure, but they aren't very flexible in terms of characteristics. For example, you have only a small range of actuation frequencies and surface wave amplitudes to choose from. By assisting or resisting the direction of peristaltic transport, auxiliary electric fields

address precisely this drawback. "It has been shown theoretically that one may achieve unprecedented control over the flow rates obtained by otherwise rigid devices," says Suman Chakraborty, senior author of the new research, "from completely reversed flow rates to twice the forward flow rate."

Electro-osmosis isn't an instant fix, though; there are still obstacles to be overcome. "The main challenges in implementing such a device lie in the microfabrication," says Chakraborty. "The concept of sputtering – depositing thin films of electrodes onto a surface – and the associated fabrication have been demonstrated by several researchers in the MEMS community. Obtaining the necessary actuators seems to be the restricting factor." There are also considerations with regard to utility. Electro-osmosis relies on transporting an ionic solution (one that can carry a current). To transport any other type of solution, Chakraborty explains, you'd need to set up a two-fluid system in which you use a fine-tuned ionic solution to control transport of the non-ionic one.

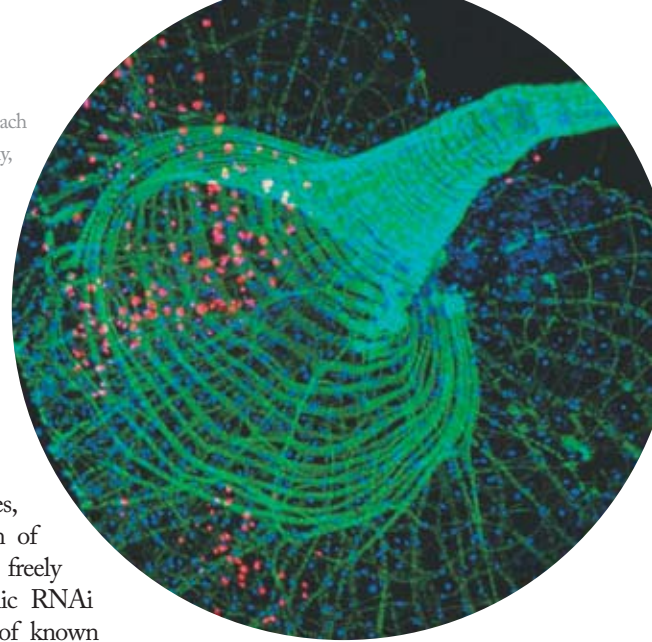
The next step for Chakraborty and his colleagues is to create initial prototypes of such devices using existing microfabrication facilities. "This could make way for integrated devices that require the slowing down or enhancement of flow rates without affecting any moving parts – thus maintaining high reliability." Although there's still plenty of research to be done into how charged particles move in electro-osmotically modified environments, the ultimate outcome may be tiny, finely tuned lab-on-a-chip devices with a wider range of applications than ever. *MS*

#### Reference

1. A Bandopadhyay et al., "Electroosmosis-modulated peristaltic transport in microfluidic channels", *Phys Fluids*, 28, 052002 (2016).



Immunofluorescence of the stomach of a Nora virus-infected fruit fly, showing muscle cells (green), uninfected cell nuclei (blue), and infected cell nuclei (red). Credit: Jens-Ola Ekström



## Virus Vision

### A new method uses fluorescent proteins to highlight virus-infected cells and shows which proteins are affected

Viruses are among a cell's tiniest predators – and for a long time, their size and biology have kept them a mystery to observing microbiologists. But what if we had an easy way to identify not only which cells were infected, but also which proteins were affected by the virus? That's precisely what Jens-Ola Ekström, Dan Hultmark, and their colleagues at Umeå University have developed with a new system called Munin (1). So far, they've used their new method to observe picornavirus infections in fruit flies – but the potential goes far beyond that.

What? Munin is a system for expression of any gene of interest in cells infected by a virus – and it can easily take advantage of the virus' specific proteases to determine whether or not a particular cell is infected. The system is based on a ubiquitously expressed target protein for a protease produced by the virus of interest. That target protein, Gal4, is a transcription factor connected to a membrane anchor (a transmembrane domain preventing the protein from reaching the nucleus) via a cleavage site for the viral protease. In infected cells, the virus-encoded protease releases Gal4 from its membrane anchor, allowing it to enter the nucleus and control transcription of any gene equipped with the yeast promoter *UAS*. If an infected cell contains a fluorescent protein gene controlled by *UAS*, virus-released Gal4 can activate that protein – but if no infection is present, Gal4 will never reach the nucleus, and the cell will never fluoresce.

The system isn't limited to detecting infected cells, though; it can be used to overexpress any gene of interest, including

those that researchers suspect may be affected by the virus. Even more importantly, it's also possible to knock down endogenous genes by expressing an RNA hairpin that feeds into the RNAi system. For fruit flies, the researchers' model organism of choice, the research community freely provides thousands of transgenic RNAi constructs covering a majority of known genes – which allows the testing of almost any gene of interest in a virus-host relation, and may even permit genetic screening if a simple enough readout is available.

Why? “The goal of our project is to understand the factors that control and limit persistent infections of RNA viruses,” say researchers Jens-Ola Ekström and Dan Hultmark. “With limited resources we may not be able to pursue this goal as far as we had hoped, but we are happy to share our plasmids and fly stocks with other researchers.”

Why choose *Drosophila* as a model system – and why picornavirus infections? “The very first step in viral immune defense in vertebrates is dependent on innate immunity,” explain the researchers, “and *Drosophila* is an excellent model for innate immunity.” Fruit flies have a record of discoveries with an impact on human innate immunity – for instance, the discoveries of toll-like receptors and defensins. The model organism also justifies the choice of virus; because some picorna-like viruses infect *Drosophila* species, Ekström and Hultmark thought they would be a good choice for detailed study of molecular virus-host interactions. “Specifically, we were curious about the phenomenon of persistent RNA virus infections such as those of the Nora virus, which infects the fruit fly and was discovered in our lab.”

Where next? “One goal of the project was to create a system for fast and specific detection of infection, as a tool for genetic

screening of host genes that affect viral infection. This goal was only partially achieved with the fluorescent readout,” say Ekström and Hultmark. “We can detect infection in living animals, but only when the Nora virus is injected – not when they are infected the natural way, via feeding. The normal gut infection can only be detected after dissection of the animal, which is not practical for screening purposes. We hope that the solution will be to use the Munin system to express a protein that gives rise to a phenotype that we can score from the outside. We have begun to work along those lines, but the problem is not solved yet.”

Munin builds on transgenic technology, which means it's limited to model organisms and tissue cultures at the moment. That makes it useful for research, but not in clinical testing, because the need for models makes it less cost-effective than PCR- or sequencing-based analyses. But that doesn't mean there's no place for Munin in laboratory medicine. “We developed the system for research purposes, but it could probably have a role in broad analyses for certain groups of viruses, including clinically important ones,” say its creators, “as Munin is much less sensitive for mutations than primer- or probe-based methods.”

#### Reference

1. JO Ekström, D Hultmark, “A novel strategy for live detection of viral infection in *Drosophila melanogaster*”, *Sci Rep*, 6, 26250 (2016). PMID: 27189868.

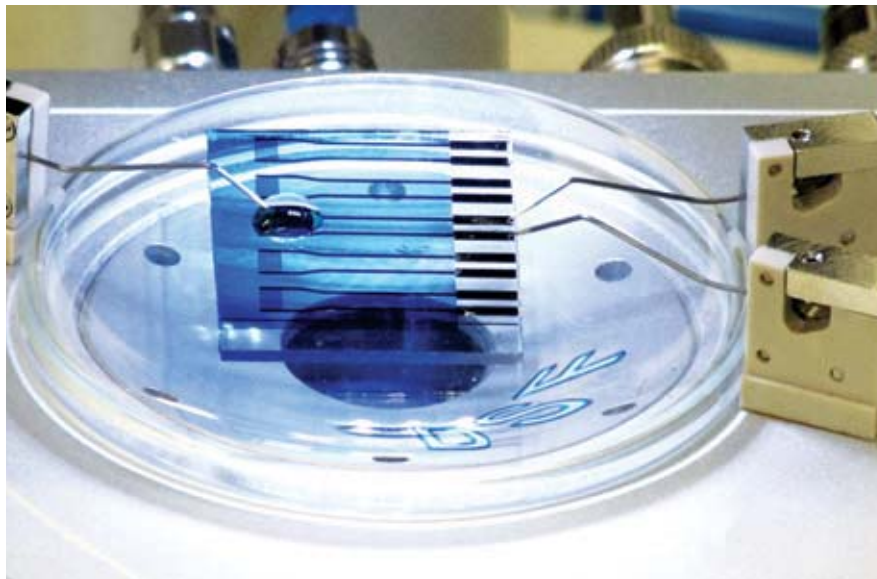
## Simple Sample Sensing

**Carlos Cesar Bof Bufon explains the first phthalocyanine-based water-gated transistor for disease diagnosis**

With a spotlight on everything from breath tests to dogs' noses, biosensors are an increasingly important aspect of diagnostic research. But not every sensor offers the same benefits – and it's important to find a balance between cost, ease-of-use, sensitivity and specificity. To meet this diverse set of needs, we propose refinements to an existing technology, the “water-gated organic transistor,” to improve its ability to assist in the diagnosis of numerous diseases including cancer, Alzheimer's and Parkinson's (1).

The device is an organic transistor that works on liquid samples. The sample forms a fundamental part of the device, resting atop a pair of electrodes coated with an organic semiconductor film. A metallic wire in the liquid (the “gate electrode”) allows users to control the capacitive coupling between the charges in the semiconductor film and in the sample simply by applying suitable voltages at the electrodes.

What makes our transistor attractive for biosensing is the biomolecule-friendly aqueous environment, the low ( $\leq 1$  V) operational voltages, and the ability to achieve high specificity by adding biorecognition elements at the gate electrode. The result is an electrical signal correlated to analyte concentration. It's not new technology – similar transistors are already used to detect molecules like dopamine, cytokines, and penicillin. The novelty in our work is that it's the first time such a device has been used to detect



The National Nanotechnology Laboratory's nanoscale biosensor for cancer and neurodegenerative disease.

glutathione S-transferase (GST), an enzyme related to a wide range of diseases. Despite its sensitivity, the test itself is straightforward: we simply attach a single layer of reduced glutathione (GSH) peptide to the gate surface and exploit the well-known specific interaction between GSH and GST, without needing any other chemicals or labeling molecules.

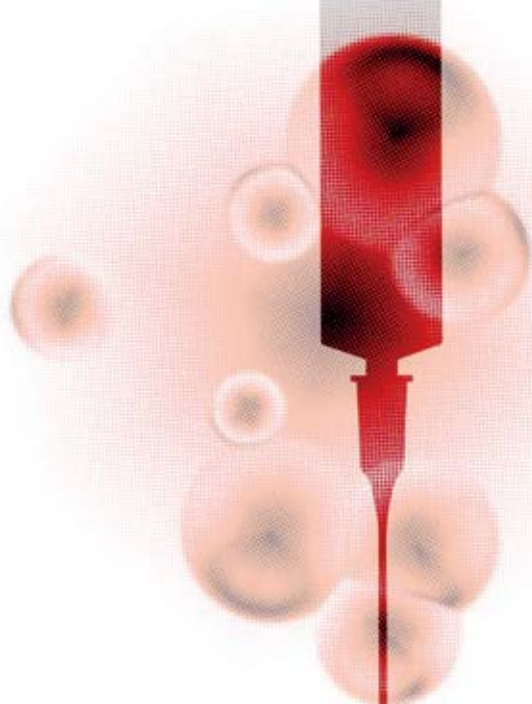
We believe that water-gated organic transistors can be used as biosensors for molecules related to a variety of pathologies. But to do so, we'll need thoughtful device design and engineering – not always an easy task. In our opinion, the possibilities – and the challenges – are numerous! To overcome the obstacles, we need interdisciplinary collaboration between physicists, chemists, physicians and health specialists to identify relevant analytes, determine safe concentrations, select sample types, and tackle technological trouble. In our laboratory, we work on device development, so we don't have much close contact with health professionals. We know, though, that there's currently no single test for diseases like cancer,

Alzheimer's or Parkinson's; instead, diagnosis is based on a combination of signs and symptoms, medical history, and imaging (which can be expensive and inconvenient). Our vision is that point-of-care biosensors will become an additional tool for the diagnosis of such pathologies, decentralizing examinations and reducing costs.

But before water-gated organic transistors can help pathologists with day-to-day diagnosis, we have some work to do: evaluating device stability (both in operation and storage), studying other organic semiconductors and determining the most appropriate materials, investigating the role played by interferents, and, most importantly, monitoring analytes in real samples. But that work is underway, and although the challenges ahead may sound intimidating, we believe that these devices represent a viable – if timid – step in the direction of clinical change. *CCBF*

### Reference

1. RF de Oliveira et al., “Water-gated phthalocyanine transistors: operation and transduction of the peptide–enzyme interaction”, *Org Electron*, 31, 217–226 (2016).



## Liquid License

### FDA issues first-ever approval for liquid biopsy companion diagnostic

Lung cancer is the leading cause of cancer deaths among both men and women, and with non-small cell lung cancer (NSCLC) being the most common type, it's no surprise that plenty of attention is focused on finding ways to treat it. In particular, tailoring treatment and diagnosis has been a key aim of most researchers active in the field and one major step forward in this regard has been in the development of the drug erlotinib, which inhibits the epidermal growth factor receptor (EGFR) and has proven to be maximally effective in NSCLC patients with *EGFR* gene mutations. As with many mutation-targeting therapeutics, the hunt for an effective accompanying diagnostic has been ongoing, and one such test has proven itself worthy of a Food and Drug Administration (FDA) seal of approval – the first diagnostic of its kind to get the nod from the US regulators.

Noninvasive, blood-based tests, or liquid biopsies as they are commonly known, have garnered a lot of attention recently, in particular because of their noninvasive nature. These tests involve

detecting DNA shed by tumors into patients' blood, allowing tumor DNA to be sequenced and examined without the need for an invasive and potentially risky tissue biopsy. Until recently, such tests were in their experimental stages, but as of June 1st, the FDA issued its first-ever approval for a liquid biopsy test (1). The cobas *EGFR* Mutation Test v2 is a companion diagnostic to erlotinib, which is FDA-approved for first-line treatment in patients with specific *EGFR* mutations (either an exon 19 deletion or an L858R substitution).

Although NSCLC accounts for over 1.5 million cancer diagnoses per year worldwide (2), this test approval is just the first step on a long road. If the trend continues, we may soon see liquid biopsies designed to detect mutations in a wide range of cancers, helping medical professionals personalize each patient's treatment based on their tumor's genetic profile – all without the need for tissue sampling. *MS*

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## The 11 Faces of AML

### Classifying AML by genetic abnormalities may yield more accurate diagnosis, prognosis and treatment

Acute myeloid leukemia (AML) is a disease of many faces. At the moment, the two main classification schemes are the French-American-British (FAB), which separates disease according to cell type and maturity, and the World Health Organization (WHO), which incorporates past disease history and specific chromosomal translocations (1). But the more we learn about AML, the more we recognize that genetic changes inform not only the patient's initial prognosis, but also disease evolution and response to treatment. A study published in the *New England Journal of Medicine* provides a detailed genetic analysis of patients in three prospective multicenter clinical trials, revealing that AML can be divided into a number of subgroups based on genomic changes and complex gene interactions (2).

The researchers conducted cytogenetic analysis and sequencing of 111 genes to try to understand the mutations driving AML. What they found was an extensive landscape of genetic changes – 5,234 driver mutations in total, spread across 76 genes. Of the patient samples analyzed, 96 percent exhibited at least one driver mutation and 86 percent had two or more. The size of the sample population even allowed the study's authors to examine correlations and clonal relationships between genes. For instance, they determined that mutations in epigenetic modifier genes (like *DNMT3A*, *ASXL1*, *IDH1/2* and *TET2*) were often among the earliest acquired, followed later by others like receptor tyrosine kinase or *NPM1* mutations. Tracing the acquisition of disease-causing mutations through time suggests that AML develops in

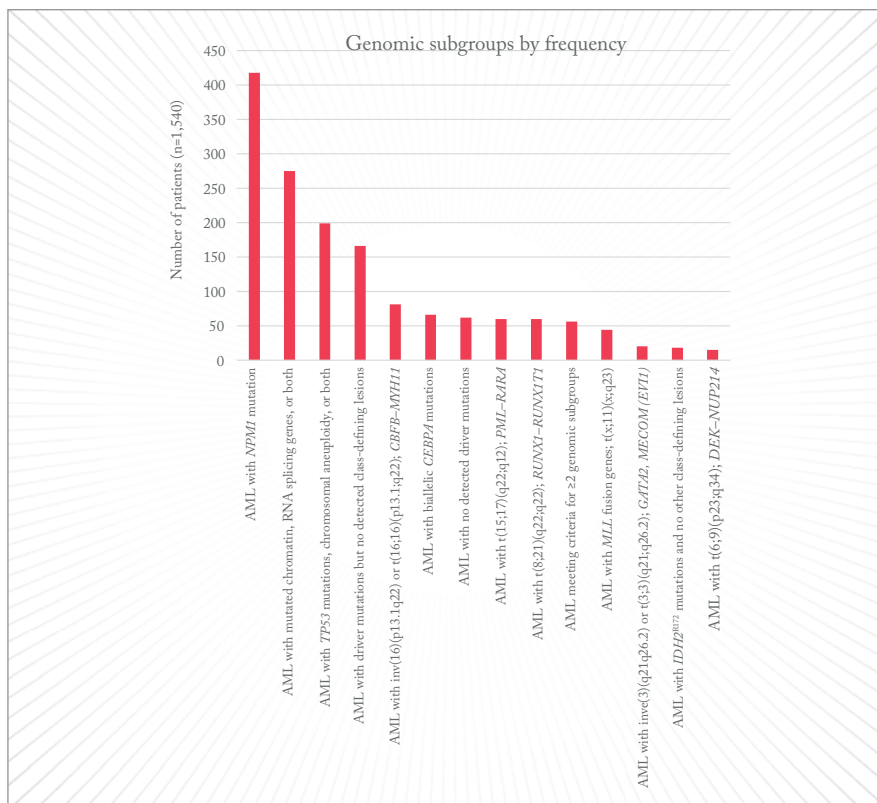


Figure 1. Proposed genomic classifications of AML in decreasing order of frequency in the study's sample population.

specific, ordered trajectories.

But what does this all mean for classification? The authors point out that nearly half of the patients in their cohort would not fall under WHO molecular classification criteria, despite having disease driver mutations. They suggest an alternative classification method based on Bayesian statistical analysis of their own results, leading to 11 new subtypes in order of frequency (see Figure 1). The different groups had different clinical implications, too; not only was overall survival linked to the number of driver mutations, but some specific groups (for instance, those with chromatin or spliceosome changes) had a poorer clinical outlook than expected based on their WHO classifications. Not all interactions are deleterious, though – whereas some complex gene interactions indicated an especially dismal prognosis, others conferred a survival advantage.

The AML genome is complicated and will need careful study to unpack, but one thing is clear: it's time to consider incorporating more genomic information into our disease classifications, especially where specific mutations are known to influence clinical outcomes. With the additional information provided by *TP53*, *SRSF2*, *ASXL1*, *DNMT3A*, *IDH2* and splicing-factor genes, patients in high-risk groups could be identified earlier, treated more aggressively, and provided with more accurate prognoses. *MS*

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## Fingerprick Fraud

**Founder Elizabeth Holmes admits that direct-to-consumer testing company Theranos' technology doesn't work – and wasn't often used**

Much like the rest of the world, pathologists have been watching with fascination as the story of direct-to-consumer testing company Theranos unfolds. From the debut of its revolutionary “Edison machine” technology – purportedly providing instant diagnoses from a single drop of blood – to the discovery that not all was as it seemed, Theranos has been making headlines ever since it first caught the public's eye in 2013, a decade after its launch. But all that glitters is not diagnostic gold, and in 2015, the Wall Street Journal broke a story announcing that the company's flagship technology wasn't being used for most of its tests – and that when it was used, the results might be inaccurate (1).

Since then, the Theranos rollercoaster has been heading steadily downward. Multiple organizations, including the US Food and Drug Administration and the Centers for Medicare and

Medicaid Services, found fault with the company's methods and technologies. Investors and corporate partners separated themselves from Theranos. But now, the saga is officially at an end – at least for company founder Elizabeth Holmes, who has admitted that the proprietary Edison technology doesn't work, that it was used for only 12 of the more than 200 test types offered to consumers, and that the results from those machines were thrown out. In the wake of this announcement, former partner Walgreens (an American drug retailer) has officially terminated its relationship with Theranos and shut down its on-site testing services (2).

Is this the last hurrah for the company? Officially, Theranos remains “fully committed to our mission to provide patients access to affordable health information and look forward to continuing to serve customers... (3)” But with no proprietary technology, no advantages over less expensive providers, and a criminal investigation underway by federal prosecutors and the Securities and Exchange Commission (4), the future looks bleak – not just for Theranos, but for the investors and patients who may have misplaced their trust. *MS*

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# In My View

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

*Submissions are welcome. Articles should be short, focused, personal and passionate, and may deal with any aspect of laboratory medicine. They can be up to 600 words in length and written in the first person.*

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## Let's Take Control

**Laboratory professionals need to be involved in the total testing process to improve patient outcomes and avoid harm**



*By Danielle Freedman, Consultant Chemical Pathologist and Associate Physician in Clinical Endocrinology, and Director of Pathology, Luton & Dunstable University Hospital NHS Foundation Trust, UK*

As we know, laboratory investigations are essential for diagnostic, preventative and therapeutic purposes and it is widely believed that the majority of clinical decisions are made based on data produced by the clinical laboratory. Nevertheless, I believe the real importance of laboratory medicine lies in the bridging of the knowledge gap at the clinician/laboratory interface.

According to a report by Research & Markets, the global in vitro diagnostic market was valued at US\$49 billion in 2012. It represents 3 to 5 percent of all healthcare costs and is expected to grow by 7 percent over the period of 2012 to 2017. However, it has been estimated that US\$6.8 billion of medical care in

the US involves unnecessary testing and procedures that do not improve patient care and may even harm the patient (1)! We need to help reverse this worrying trend.

Users of the clinical laboratory want information that enables them to make better decisions about their patients. They want assurance that the investigations they order will be quick, accurate, and inexpensive and that they'll get the right investigation on the right patient at the right time, with the results reaching the right clinician at the right time and in the right format. And the right interpretation is essential to ensure optimum patient outcome (2). So, we laboratory professionals need to be involved, not only in the analytical process, but also at the pre- and post-analytical points of laboratory utilization.

Not surprisingly, the use of laboratory diagnostics varies between countries and, according to Research & Markets, it was estimated to be five times greater in the USA than in the UK in 2006. Understandably, there are also large differences in laboratory utilization between individual practitioners. Indeed, many factors determine a physician's test-ordering practice. In literature surveys (3,4), physicians mostly cite fear of legal (malpractice) complaints as a primary driver of over-testing. Hoffman et al. (5) state the main driver of overdiagnosis and overtreatment is zero tolerance for error and uncertainty.

An analysis of 307 malpractice claims in the USA (6) found the primary cause of misdiagnosis in 55 percent of patients was the failure to order the appropriate diagnostic/laboratory test. And, there is growing recognition that errors in both test selection (inappropriate ordering) and result interpretation can have significant or adverse clinical consequences to patients and financial consequences to healthcare institutions (7).

Inappropriate test utilization is increasing the clinical burden for the health economy, as Moynihan et al. (8) wrote, “Medicine’s much heralded ability to heal the sick is fast being challenged by its propensity to harm the healthy. Too many people are being overdosed, overtreated and overdiagnosed.”

*“We, the laboratory professionals, play a crucial role in ensuring that laboratory utilization programs should not be exclusively based on reducing the number of tests, but also consider the clinical outcomes and change to patient management.”*

So what drives overutilization? Causes include patient pressure, duplicate ordering, lack of understanding of the diagnostic value of the test, ordering the wrong test, failure to understand the consequences of overutilization, defensive testing, perverse financial incentives and “availability creating demand.” In fact, resources wasted on unnecessary diagnosis and care can be much better spent treating and preventing genuine illness.

If we look at some of the statistics; Zhi et al. in their systematic review of the literature from 1997 to 2012 (9) found mean rates of testing overutilization to be 20.6 percent. Importantly, overutilization of low-volume tests was higher at 32.2 percent. Both Laposata and Plebani (7,10) showed the highest incidence of error in laboratory testing is in test selection by clinicians and interpretation of test results by clinicians. Others have concurred and recognize that not ordering an appropriate test is also an important cause of diagnostic error.

We, the laboratory professionals, play a crucial role in ensuring that laboratory utilization programs should not be exclusively based on reducing the number of tests, but also consider the clinical outcomes and change to patient management. Worryingly, Zhi et al. (9) found the mean rate of underutilization of testing to be almost 45 percent.

We play a key role in implementing strategies to support physicians in test ordering and providing guidelines, education, auditing, use of formularies, electronic order systems (CPOE), use of a minimum of resting intervals, and request vetting. Feedback to users with activity data and costs has also been shown to be important (2). The best approach to improving laboratory utilization therefore comprises multiple interventions.

I urge all laboratory professionals to refocus their efforts on the total testing process, rather than simply on the analytical aspects. This is fundamental, and requires input into appropriate test utilization, accurate results and interpretation. In addition, there is no point in ordering a test if no one looks at the results or acts on them. The impact of the failure to follow up and act on results also needs reviewing. We must be involved in implementation of policies to improve laboratory utilization, which will improve patient outcome and avoid patient harm.

Finally, we must not forget that “the target of requesting of the test and of the results should be the patient. It is the person who actually, in the end, is going to have to change their lives and start adopting new behaviors [...]” (11).

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## How to Succeed in MS

### Financial and personnel considerations for bringing mass spectrometry into the clinical laboratory



By Y. Victoria Zhang, Associate Professor, Department of Pathology and Laboratory Medicine; Director of the Clinical Mass Spectrometry and Toxicology Laboratory, University of Rochester Medical Center, New York, USA

The prospect of bringing mass spectrometry (MS) to the clinical laboratory is exciting. In my experience, implementing new technology and tests into a lab opens up tremendous opportunities. It is, however, not an easy undertaking and you will need to be aware of many potential pitfalls. Some examples of important things to consider are selecting the right test for the right patient population, justifying the cost, hiring the right personnel, and choosing the best instrument for your needs. All need careful consideration during the justification and preparation phase.

The first step is to choose the right test. Different from other platforms, such as immunoassays, liquid chromatography-tandem mass spectrometry (LC-MS/MS) systems are very specific and can provide definite answers, which is why many patients can benefit from results from MS analysis. Confirmation testing of drugs of abuse and screening for inborn errors of metabolism are good examples; specific compounds are identified or quantified using mass spectrometry to

provide definitive answers in drug testing and to elucidate complicated metabolism pathways. LC-MS/MS can also offer high sensitivity when examining testosterone and other steroid hormones. Therefore, the first step is to select well-documented tests that will suit your patient population at your institution.

A financial justification is most likely needed for implementing an MS system. The capital expenses have been a challenge for most institutions as LC-MS/MS systems are expensive. Fortunately, their operation costs are relatively low. The financial justification is usually based on savings from send-out costs. A great place to start is to evaluate the test volume and send-out costs for potential assays to be implemented on LC-MS/MS. If the sample volume is right, the cost per test can be significantly lower than sending them out. Typically, you can use a return on investment (ROI) calculation, as shown below:

$$\text{Return on investment (ROI)} = \frac{(\text{return} - \text{cost of investment})}{(\text{cost of investment})} \times 100 \text{ percent}$$

Although the savings gained by bringing tests in-house are easy to calculate using charge per test and the test volume, calculating the ROI can be more complicated, primarily because not all benefits are monetary. For example, reduced turnaround time, control over the sample process, and reduced transcription error by processing the samples in-house can have an enormous benefit to patients, but translating these into financial benefit for the institution is difficult. Typically these non-monetary benefits are listed as separate considerations.

In addition to this challenge, personnel needs and training are difficult factors to deal with when starting an MS lab, mainly because most clinical laboratory professionals won't be familiar with the technique. MS analyses are not as straightforward as

those performed using automated clinical chemistry analyzers. In particular, samples need to be processed manually before analysis, and the results need to be reviewed before reporting. This requires someone with extensive knowledge in clinical chemistry, sample preparation, LC, MS and a number of other areas of expertise.

You can broadly group clinical MS laboratory staff into three categories – day-to-day operators (the largest population), troubleshooting personnel (a smaller number) and a few experts. It is not necessary to enforce these categories in the lab and some overlapping will be very helpful for coverage and flexibility. However, the skills and experience should match these expectations. Finding the right people can be difficult, especially when starting a laboratory.

Typically, you can train a good medical technologist to run basic assays on an LC-MS/MS system within a few months. Also, being a member of an LC-MS/MS laboratory can advance staff careers, which proves useful for recruiting talented technologists. Troubleshooters will hopefully emerge and develop within the laboratory through continuous training and personal efforts. Experts provide method development and validation and are responsible for training others in the laboratory. The best “expert” candidates usually have extensive experience, creativity and attention to detail.

Overall, LC-MS/MS has become a powerful platform that has been proven to enhance clinical practice in many areas. It is not an easy undertaking to implement this technology. While it is appealing to bring it in-house, it is important to know that it may not be feasible to do so for all laboratories and sending out samples to a reference laboratory may be a better choice. When done correctly, though, the rewards and benefits far outweigh the costs and efforts. The ultimate goal is to have access to this advanced technology for the betterment of patient care.





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# We Need to Talk

## Pathologists, Patients and Diagnostic Errors

### – Part I

We can argue about how many diagnostic errors happen each year, but we can't deny that there are too many of them. And when one of those errors is ours, denial is especially inappropriate – but how many of us feel comfortable about a confessional with the patient? In the first installment of a two-part feature, we examine the difficulties around disclosure and communication of diagnostic errors.

*By Nick Miller*

**M**istakes happen, in medicine as in any other field. But medicine may be unique in the extent to which the scale of errors is contentious and perhaps unappreciated. A contributory factor, in many countries, is that deaths caused by medical errors can't be identified as such on the death certificate – there is simply no option to do so (1). And if you're not keeping records, you can't know the scale of the problem.

At present, therefore, national estimates of error-associated mortality rely on indirect methods. These only give approximate evaluations, but some tell a frightening tale. A recent calculation (1) suggests that medical errors result in ~250,000 deaths per year in the USA alone – which would mean “mistakes” are the third most common cause of death in America. This figure is not universally accepted, however; an alternative narrative (2) proposes a lower estimate of ~25,000 error-associated deaths per year in the country.

Whatever the exact number, diagnostic errors may represent an increasing proportion of the total (see Infographic on page 21). This may be partly due to the increasing complexity of healthcare; Ken Sands (Associate Professor of Medicine, Harvard Medical School) notes that the exponential growth in the number of branch points in a diagnostic investigation greatly increases the probability of a cognitive error. “It’s very hard to use standard safety systems, like checklists or forcing functions, to protect against cognitive errors, which is why we’ve found it difficult to reduce this source of patient harm,” he says. Thomas Gallagher (Professor and Associate Chair, Department of Medicine, University of Washington) agrees, commenting that diagnostic error is one of the main reasons why the medical profession has made disappointing progress on patient safety over the last 10–15 years. And Michael Laposata (Chairman, Pathology Department, University of Texas, Galveston) suggests that about a quarter of the US error-related deaths per year involve a diagnostic error (see Infographic on page 21). “Never mind deaths from terrorism,” he says, “this number is enormous – and it’s totally overlooked.”

The problem is by no means limited to the US. Speaking from a Netherlands perspective, Laura Zwaan (Assistant Professor, Institute for Medical Education Research, Rotterdam) says “Diagnostic errors are significant but underappreciated. Existing estimates of the incidence rates suggest that 10–15 percent of diagnoses are not entirely correct,” and Cordula Wagner (Executive Director, Professor of Patient Safety, Netherlands Institute for Health Services Research, Utrecht) reminds us that diagnostic errors have a severe impact – “There may be more implications for the patient than in other types of error,” she says.

But again, diagnostic errors are not consistently recorded, and therefore their precise frequency is unknown. As Ken Sands points out, however, there is little point in arguing over whether the figure is massive or gargantuan; whichever number you pick, it is too big. Thomas Gallagher adds that research in the field is not sufficiently mature to reliably quantify the frequency of diagnostic error. “I would prefer that people focussed their energies on understanding why diagnostic errors occur and how to reduce them,” he says.

Exact incidence aside, growing concerns about the frequency and consequences of diagnostic error in the USA persuaded the Institute of Medicine (IoM) to convene a committee to further investigate. The Committee, which included Mark Graber, a founding member of the Society to Improve Diagnosis in Medicine, and colleagues such as Gallagher and Laposata, oversaw the development of the report “Improving Diagnosis in Healthcare,” issued in 2015 (3). The publication makes occasionally uncomfortable reading for both the medical and the legal community (see Sidebar “Uncomfortable Truths?”),

albeit tempered with pragmatic suggestions for making our hospitals safer places. A key element of the report’s philosophy is full and prompt disclosure, not only to the healthcare institution where the error occurred, but also – critically – to the patients affected by the error. But how easy will this be for pathologists? Is it even possible?

**“Transparency is critical because it allows meaningful metrics – without knowing the denominator, we’re never going to get a handle on any of this.”** Suzy Dintzis

**“Diagnostic error has been called a blind spot – it’s an area of safety that has been, to some extent, ignored.”**

Thomas Gallagher

#### **Error disclosure: the silent treatment?**

To answer those questions, we first need to look at the status quo of error disclosure. How often do healthcare professionals talk about their mistakes – either to their colleagues or to their patients?

The data aren’t always completely clear-cut. Suzy Dintzis (Associate Professor, Anatomic Pathology, University of Washington) points to results from surveys and focus groups in North America (See Table 1 and Table 2). These indicate that although 96 percent of surgeons report that they would definitely disclose an ‘error’ to patients, “in fact only about 10 percent would use the word error and even fewer would apologize to the patient.” By contrast, although only 65 percent of internists agree that they would definitely disclose an error to patients, Dintzis relates that 71 percent of these would use the word “error” and 43 percent would apologize (4). Thus, there may not be a common understanding of what exactly is meant by the term “disclosure.”

Furthermore, it seems that action does not always follow intent. Other surveys (5) suggest that an overwhelming majority of anatomic pathologists and laboratory medicine directors (96

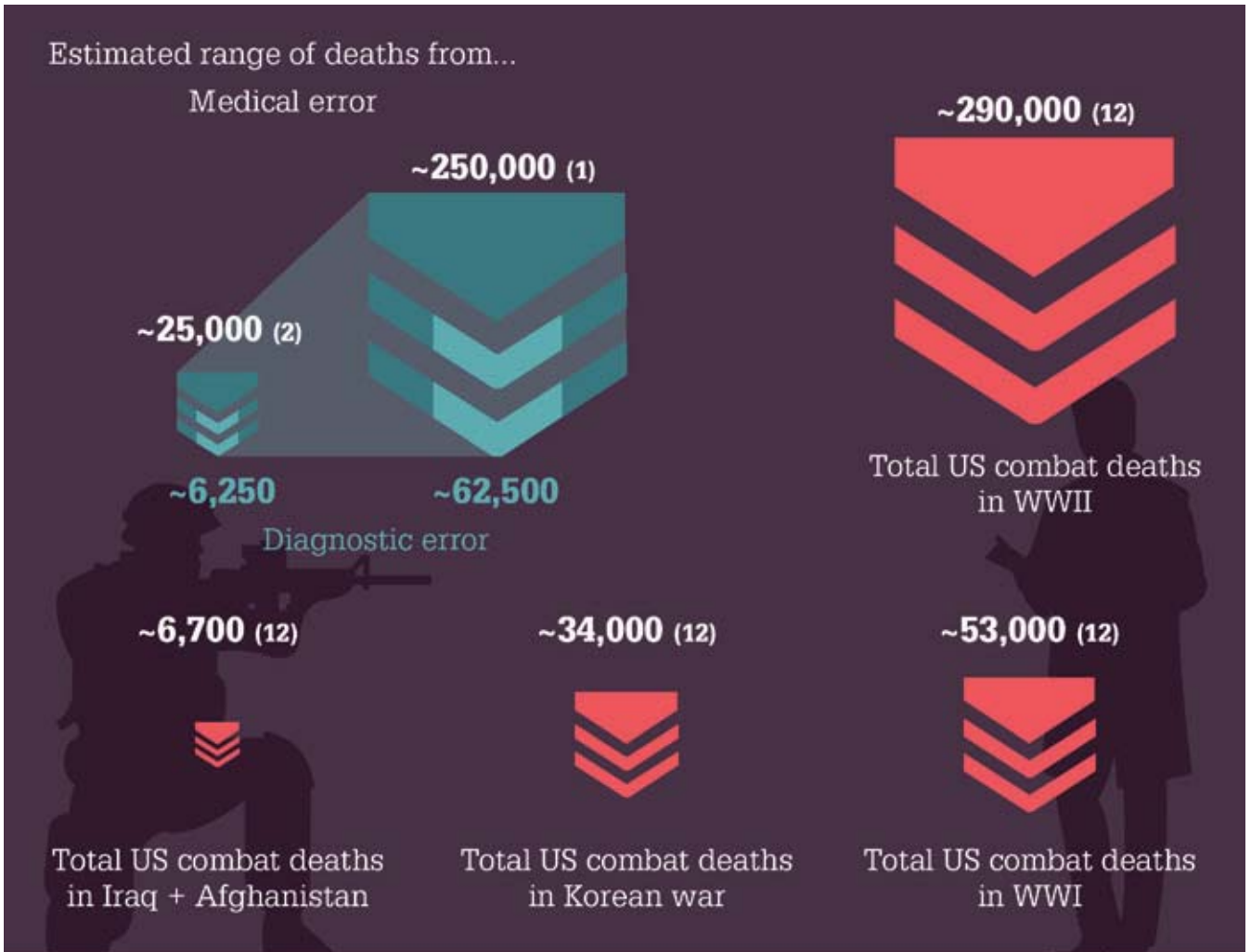




Table 1. Survey of 260 anatomic pathologists and 81 laboratory medical directors (response rate 51%).  
 Data from Dintzis and Gallagher (4, 5, 11).

percent and 99 percent, respectively) agree that serious errors should be disclosed to patients. Yet, while respectively 47 percent and 34 percent of these groups have been involved with a serious error, only 17 percent and 14 percent have actually disclosed a serious error to the patient. As Suzy Dintzis says, “There’s a gap between what they think they should do and what they’re actually able to do.” Clearly, the system prevents pathologists from being as transparent as they would wish. Why should this be?

It turns out that error disclosure may be hindered by a complex mixture of disincentives (6). These may vary in impact between countries, between individuals, and according to precise circumstances. To aid discussion, we outline disclosure difficulties under three categories: institutional disclosure, patient disclosure and general issues.

#### *Difficulties in disclosing to the institution*

It’s natural to be concerned about one’s reputation, and therefore, when things go wrong, instinct may tell us to keep quiet. As Laura Zwaan says, “The fear of what co-workers might think is an important factor.” Ken Sands elaborates further: “There has been a tradition of physicians feeling that they have to be perfect and that errors are not expected, or that they are a sign of professional incompetence.” Thomas Gallagher summarizes: “Physicians pride themselves on delivering very high quality medical care, and so when an error happens, it’s embarrassing.”

This attitude is not just motivated by ambition, if at all; the word that was consistently mentioned by experts in this context was “shame.” For example, Yael Heher (Anatomic Pathologist and the Director of Quality and Safety, Department of Pathology, the Beth Israel Deaconess Medical Center and Harvard Medical School, Boston) says, “As medical professionals, we’re very hard on ourselves; it’s very hard to accept that we’ve made a mistake – there’s a lot of shame around that.” And Cordula Wagner agrees: “People can be ashamed . . . and maybe also a little afraid of how it will look to their colleagues.”

But above and beyond reputation are fears for one’s actual livelihood. In cultures where discovery of medical errors tends to be followed by litigation, having one’s name associated with punitive patient compensation may not be a good career move. And in an age where an individual physician’s error record may be posted on the internet, error disclosure could result in a fall-off in patients for that physician – not good in cultures where physician payment is based on the fee-for-service system. “Error disclosure is potentially devastating for a professional career,” says Laposata. He draws unfavorable comparisons between the medical community and the airline industry: “The airline industry gives pilots a medal for admitting that they had a near miss, because it helps everyone to understand where the risks are. And there’s no risk to the pilot’s job because the



Table 2. Survey of 2,000 US physicians and 2,000 Canadian physicians (response rate 63%).  
Data from Dintzis and Gallagher (4, 5, 11).

“The exact incidence rates of diagnostic errors are still unclear – it’s very hard to determine the precise numbers”

Laura Zwaan

“All of us will likely experience a meaningful diagnostic error in our lifetime.”

IoM report, 2015.

“Pathologists believe that neither treating clinicians nor patients really understand the limitations of pathology.”

Suzy Dintzis

## Uncomfortable Truths?

Key findings from “Improving Diagnosis in Healthcare,” a report by the Institute of Medicine (IoM), Nov 2015 (3).

- ✔ The diagnostic process should involve collaborative teamwork between healthcare professionals and their patients/patients' families.
- ✔ Professional education and training relevant to the diagnostic process should be enhanced.
- ✔ Healthcare IT systems should support the diagnostic process.
- ✔ Diagnostic errors and near misses (see Note) should be identified, learned from and reduced.
- ✔ A culture that supports mechanisms for improving the diagnostic process should be developed.
- ✔ Introduce systems of error reporting and medical liability management that encourage identifying and learning from near misses.
- ✔ Establish environment of payment and care delivery that supports the diagnostic process.
- ✔ Funds for research on diagnostic process and diagnostic errors should be provided.

Note: “Near misses” are defined as “failures in the diagnostic process that do not lead to diagnostic errors.” “Diagnostic errors” are defined as “the failure to (a) establish an accurate and timely explanation of the patient’s health problem(s) or (b) communicate that explanation to the patient.” (IoM Report)



near misses aren’t publicized per person.”

But even if you remove reputational and employment concerns – even if the pathologist wasn’t responsible for the error – it can still be difficult for them to fully disclose errors that come to their attention. Why might this be? Laposata explains: “The pathologist is in a uniquely uncomfortable situation with regard to error reporting, because most of the mistakes we see are those made by our fellow physicians.” As Gallagher and colleagues point out (7), “Confronting the error of a colleague raises challenging questions about [ . . . ] which professionals carry what responsibilities, and how to talk with the patient about the event.” A significant part of the problem, Laposata asserts, is that the pathologist is unsure of how to disclose that a colleague has completely misdiagnosed a patient; for example, does the pathologist talk to the physician, or does he go to his institution’s risk management officer? And Laposata is clear: “People have told me that risk management is their absolute last choice.”

“Our profession has not been good at recognizing that errors are part of being human.” Ken Sands

“There has been a cultural attitude that medical professionals are perfect and don’t make any errors.”

Cordula Wagner

But this semi-clandestine error reporting can contribute to errors remaining hidden, because by cutting risk management personnel out of the equation, the choice of how to proceed with the error tends to remain with the physician, at least in the US. And unsurprisingly – given the disclosure disincentives outlined above – sometimes the error report goes no further.

The consequences of this failure to fully disclose can be massive; Laposata points to cases where children have gone into foster care – and the parents have gone to jail – because a physician mistakenly concluded that a child with a cerebral hemorrhage had been physically abused, when in fact they had a bleeding disorder. Says Laposata, “I’ve found it virtually



impossible to get people to admit that they overlooked a test in such cases, and that if they hadn't they would have reached a different diagnosis." He concludes, "Most people hold to their diagnosis in these circumstances because the admission of a mistake has huge consequences for them."

Even where circumstances are less extreme – where a misdiagnosis leads to a delay in appropriate treatment, for example – healthcare professionals may still be fearful of the consequences of bringing an error to light. This is said to be particularly the case when working in an organization with a punitive culture. Notably, the IoM report references data suggesting that more than half of healthcare professionals appeared to feel that their employer was punitive with regard to errors.

But a punitive attitude to mistakes may itself be a consequence of the fault-based medical liability system (3) (discussed below), and is certainly a symptom of organizational culture, which itself is a function of many other factors. It's starting to look like moving towards error transparency will require a large-scale, institutional or even supra-institutional effort.

#### *Difficulties in disclosing to the patient*

Directly disclosing errors to the patients themselves raises a new set of barriers. Obviously, there is the very human point that professionals don't like to make mistakes, and therefore admitting to them can be painful. But it's worse when you're talking to somebody who has suffered for your mistake. Laura Zwaan points out: "It's very difficult for healthcare professionals who not only have to bring bad news, but who are also partly responsible for it."

To that very human concern must be added to the fear of litigation. This is a consequence of the "deny and defend" status quo, in which the default reaction of healthcare organizations is to deny responsibility for errors or any harm therefrom, resulting in courtroom battles and sometimes large financial settlements. As Heher says, "Anxiety about repercussions is emotionally powerful, and in the US a big portion of anxiety relates to litigation."

Is "deny and defend" the best way of doing things? Perhaps not. From the patient perspective, it's been said to be slow, inequitable, and inefficient; from the physician perspective, expensive, stressful and inclined to incentivize "defensive medicine," i.e., the avoidance of higher-risk patients or procedures (3, 8).

According to this view, the current system sets up a tension between pathologists, who feel a moral obligation to disclose errors but are fearful of the consequences, and a system based on litigation and punishment. Fair enough; but does this

**"There's an enormous amount of shame and embarrassment that accompanies medical error."**

Thomas Gallagher

**"The pathologist is in a uniquely uncomfortable situation . . . most of the mistakes we see are made by our fellow physicians."**

Michael Laposata

**"Moving towards full transparency involves cultural change and is not easy, but it is the right thing to do."** Yael Heher

**"We need a broad cultural shift."** Suzy Dintzis

**"Healthcare professionals are afraid to talk about errors in case they say the wrong thing in terms of liability."**

Laura Zwaan

**"It's one thing to ask people to disclose within their institution – making it public is a step further."** Yael Heher

tension actually change behavior? In other words, does the fear of litigation prevent pathologists disclosing errors?

In fact, the results of surveys and focus group research suggest that fear of litigation may be less of an issue than many believe. Yael Heher asserts that most physicians have a very powerful moral compass and don't need fear of litigation to drive their behavior in the right direction. Suzy Dintzis quotes data that support this: "In our survey only about 10–20 percent of pathologists said that litigation fear might deter them from disclosing a serious error to a patient." She points to a survey of 2,000 doctors in the US and Canada; disclosure rates were identical in these two countries despite their very different litigation environments (see Table 2). "I think that's the strongest evidence that litigation is not really a big factor," she says. "It stresses you, but it doesn't stop you." Gallagher concurs: "The probability of being sued is about five times lower for a Canadian physician than for a US physician, so if fear of litigation were a significant factor, you would think that Canadian physicians would be much more willing to endorse open communication with patients than US physicians – but the two groups are virtually indistinguishable."

Perhaps then the litigation environment acts as a general stressor, which contributes to an environment of non-disclosure, but only drives non-disclosure in particular circumstances. What other factors may discourage transparency among pathologists in particular?

#### *General difficulties in error disclosure*

Dintzis is clear: "The two major reasons are the concerns around communicating technically complex results to patients and physicians, and challenges in pathologists' ability to communicate." She continues, "Pathologists are not confident in their communication skills. They're used to presenting their diagnoses in reports, but when they talk to individuals, they're very afraid of being misrepresented or misunderstood."

Dintzis quotes (9) one participant in a pathologist focus group as follows: "...my social skills are not such that I would ever want to (speak directly to the patient) – I'm not in pathology because I like meeting people." Gallagher agrees: "Uncertainty over what to say, and lack of confidence in communication skills, is an important barrier to communication." Cordula Wagner puts it like this: "They may not have the competence to explain the error to a patient – they don't know how to speak about it, how to mention it to supervisors or heads of department, or what they are allowed to say about insurance, for example."

Added to that, Dintzis says, is the lack of any pre-existing relationship between patient and pathologist; this can complicate initiation of any dialog. Hence another finding

from surveys and focus groups – that most pathologists believe disclosure should be at the discretion of the treating clinician. Heher agrees, "As pathologists, we don't have a pre-existing relationship with the patient, so we don't even have a ready forum for this kind of discussion – we'd have to bridge a major gap and set up a meeting, and how should we do that?" And Gallagher says that many of the pathologists he's spoken to in his research were very conflicted, feeling that they should be more involved in communicating with patients after pathology errors, but also that their fundamental relationship was with the treating clinician, not the patient. "They were very uncertain about how to proceed," says Gallagher.

**"Explaining complex results in a way that is not opaque, without going into a lot of technical detail, can be very challenging." Suzy Dintzis**

**"Many pathologists haven't spoken directly to a patient in decades." Yael Heher**

**"The image of a pathologist sitting in a room by themselves with a microscope . . . there's some truth in that." Suzy Dintzis**

On top of this communication skills issue is a feeling that the nuances of pathology, and its occasional ambiguities, are not always understood by physicians or patients (10). Indeed, some pathologists may feel that it would be more harmful for the patient to know about the error than to remain ignorant of it (10). Suzy Dintzis outlines data from focus groups suggesting that pathologists believe error disclosure is complicated by the difficulty in explaining pathology results. "Pathologic diagnosis can be very subtle and subjective, but many practitioners feel that diagnosis should be black or white – they don't understand that it's much more nuanced than that."



Furthermore, the complexity of diagnostic processes may make it difficult to actually determine if an “error” has actually occurred; Cordula Wagner points out that trouble in deciding whether a diagnostic error was truly preventable results in “grey areas” that can add to the disincentive to disclose.

For all these reasons, it seems that pathologists in general are ill-prepared for involving patients in the error disclosure process (10), and may even have concerns about communicating with the treating physicians.

Furthermore, there may be systemic issues that make it difficult for pathologists to justify spending time and resources on the error disclosure process. In the US, at least, it seems that the physician remuneration system itself may not support activities relating to identifying, reporting and learning from errors. In particular, the US fee-for-service payment system (FFS) – by which healthcare professionals are reimbursed for each service they provide – may discourage error disclosure activities, or even generate perverse incentives. Thus, FFS does not encourage activities such as time spent in communication

between clinicians, pathologists, and radiologists with regard to test ordering and interpretation, or time spent by pathologists or radiologists advising clinicians on the use and interpretation of specific diagnostic tests. Nor does it reward accurate over inaccurate diagnoses, nor encourage activities which result in a diagnosis that indicates that no treatment is necessary (3). Furthermore, healthcare professionals report that the system does not incentivize them to engage patients in the diagnostic process, often resulting in rushed communication (3).

As Michael Laposata points out, activities like running the lab and making sure that all the assays are accurate; advising a physician on the use, interpretation and significance of diagnostic tests; applying the various diagnostic tests that do not involve looking down a microscope; even running the normal array of blood and urine tests; these all pay little or nothing. He is clear on the issue: “The problem is that if you don’t incentivize people, if they can’t even make a living from providing advice about diagnostic tests, then you won’t retain the experts who can help patients.” Fortunately, reimbursement and payment

issues don't (at present) seem to be significant disincentivizing factors in Europe. "I've never heard of it having any influence on error disclosure here," confirms Wagner.

Finally, the paucity of accepted protocols and guidelines for error disclosure may be another important factor in the failure to disclose. Indeed, the fact that broadly accepted protocols or guidelines for error disclosure have only recently become available may reflect a deeper absence of rigor in this whole field. For example, even the definition of diagnostic error has been uncertain; the most recent is given in the IoM report (3) (see Sidebar "Uncomfortable Truths?"), but others have been used historically (3).

### To be continued...

Clearly, the status quo is unsatisfactory insofar as it embodies a number of human and system factors that disincentivize error disclosure. Something needs to change – but what, and how? The IoM report makes some recommendations for change, of which the key points are outlined by Michael Laposata (see Sidebar "It's Our Turn"). These and other points are discussed in more detail in Part II of this feature (July/August issue of *The Pathologist*). In the meantime, here are some concluding thoughts on how we might open up the discussion on error disclosure.

Firstly, pathologists want to be fully transparent, it seems; but in the absence of clear guidelines, what do they do – make it up as they go along? Indeed, what kind of guidelines exist, and what should the ideal disclosure protocol look like? Our next part of this feature examines progress in this field.

Whether or not litigation significantly changes disclosure rates among physicians as a whole, there seems no doubt that it is a significant contributor to stress and cultures of fear. Can this be changed, and if so how, and to what? Next month's issue hears views from experts on this point.

Then, significantly, there is the question of what constitutes current best practice in the field of communication after a diagnostic error; also, what impact has best practice had in the real world? Has it really improved outcomes? In the second section of this feature, we hear from proponents and developers of communication and resolution programmes (CRPs).

And finally, but perhaps most importantly, we look at diagnostic error disclosure from the patient point of view. How important is full disclosure to them? What do they really want? And how might pathologists be trained and supported in having difficult conversations with a patient who may have been significantly harmed by a diagnostic error?

Diagnostic errors are difficult to deal with, and perhaps even more difficult to discuss; we hope that by bringing together the views of several experts in the field, we are contributing to increased transparency in this hugely important and problematic area.

**"In the US, pathologists for the most part are paid only for looking through a microscope and making a diagnosis."**

Michael Laposata

**"Improving the diagnostic process is not only possible, but also represents a moral, professional and public health imperative." IoM report, 2015.**

*Nick Miller is Associate Editor of The Pathologist.*

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## It's Our Turn

### Michael Laposata, co-author of the IoM report, speaks out...

“Some of us – Mark Graber and associates – who appreciated the scale of the problem decided that it was essential to convene an Institute of Medicine committee to evaluate all the literature on diagnostic error and come to some sort of conclusion as to how to improve matters. And the fundamental take-home message for my fellow pathologists is this: it's our turn! The door has finally been opened – if you thought you'd been ignored, if you thought your information was unimportant to the treating physician, that changes now. But that also means that we have a responsibility to provide useful, important information. We must bring to bear the whole of clinical and anatomic pathology, not just what we see in microscope slides. We must learn about every individual laboratory test, be articulate about it, put together all the genetic and molecular pieces, and be valuable, indispensable members of the diagnostic team.

And that teamwork aspect is the second take-home message. In the IoM report, we concluded that the diagnostic process should involve collaboration. One important aspect of that is partnering with radiologists – I believe that pathologists have to expand what they do to include all diagnostic information, and that means connecting with the radiologists, because getting a diagnosis also involves imaging.

So taking responsibility for and learning the whole of pathology, having valuable conversations with treating physicians and partnering with our other diagnostic colleagues are the major points for the pathology community to consider. But my biggest fear is that pathologists won't walk through this open door. Because if we continue to practice the way we have – “Let me read my 500 slides and write a report on them, and don't bother me” – then we won't have the impact that we can and should.

It's unfortunate that not all pathologists are aware of the IoM report. The Institute's reports that cover quality of care issues are not always willingly accepted by people in medicine, partly because they uncover some uncomfortable truths. Our first report in the series on quality – ‘To Err is Human’ (1999) – appeared to indicate that there were approaching 100,000 deaths every year from surgical and pharmaceutical errors, and some people were up in arms about the whole thing. However, if you look at all the safety changes since that report – like the operating room ‘time out’ principle, where anybody in the OR

can say ‘stop’ if they think something's wrong – you have to conclude that the IoM's investment in these quality reports has paid off. But the reports aren't always immediately accepted, and in this case I think the biggest reason for resistance is that people are scared that error disclosure could be the end of their professional life, and so they think it's better to let the sleeping dog lie.

The report explicitly recognized that fear, of course. One of the changes we recommend is a complete rethink of the procedures for reporting medical error, to take it out of the courtroom and into Communication and Resolution Programmes (CRPs). We don't talk about negligence; we talk about standard of avoidability, which recognizes that some errors arise from very difficult diagnostic problems. The error disclosure procedure should include having somebody in the institution who is totally dedicated to patient safety, and also supportive of physicians and with a good understanding of diagnostic issues, such that all doctors would feel comfortable about approaching this person to establish if there has been harm and if so how to disclose it without destroying their careers.

Other recommended changes include alterations to the payment system, so that pathologists get paid for doing all the things that contribute to identifying, learning from and preventing errors. Because at present in the US, if all you do is help people with laboratory test selection and interpretation, you cannot make a living because those activities are not reimbursed. Therefore, pathologists can't provide that critical level of safety input regarding the most appropriate test. So the incentives for error prevention and disclosure are all misaligned at the moment, and that's what we hope to change with this report.

In conclusion, the IoM wants to change things, so that we can start talking about errors without threatening people's careers – that means dealing with errors not in a courtroom but in a different setting, such as via CRPs. I think that people will accept the easier recommendations of the report quite quickly, maybe within a couple of years – for example, internal systems such as the CRPs could be set up quite quickly, and this concept of diagnosis as a team sport is really just a matter of opening a dialog, and how hard is that? But the cultural shift away from reacting to errors with litigation will take longer – it's more aspirational at this stage, at least in the USA. Changing the court system in America is going to be hard!”



# NGS: Driving a Revolution in Cancer Diagnostics and Treatment Management

**The growing demand for multiple biomarker analysis is presenting diagnostics labs with many new challenges – do you outsource or adapt? And if you keep testing in-house, is the one test-one drug approach even sustainable? Possibly not...**

*Massimo Barberis, Director of the Clinic Unit of Histopathology and Molecular Diagnostics European Institute of Oncology (IEO), Milan, Italy, and Ian Cree, Molecular Pathologist at University Hospitals Coventry and Warwickshire, UK discuss their experiences with Next Generation Sequencing (NGS) and share their opinions about the technology's potential for more widespread adoption in clinical settings*

What numbers and types of cases does your laboratory handle?

Massimo Barberis: The Molecular Pathology lab in the IEO runs >2,500 mutational assays/year and 1,800 FISH assays. Most tests are for non-small cell lung cancer (NSCLC), colorectal cancer (CRC) and malignant melanoma. Others include gastrointestinal stromal tumors, soft tissue sarcomas and ovarian cancers.

Ian Cree: We handle about 6,000 bloods/day and 70,000 histopathology cases/year. These include ~500 molecular tests for cancer annually, mainly lung cancer, CRC and melanoma. We are sometimes asked to test other

tumor types – we did a Langerhans cell histiocytosis recently – and I think the range of tumors we assess will expand rapidly as targeted drugs become more widely used.

Is it advantageous to assay multiple biomarkers per patient sample?

MB: Yes. As more driver mutations are identified, it is increasingly necessary to assay multiple molecular markers on the same sample. For NSCLC patients, we must assess *EGFR*, *ALK* and *ROS1* status at a minimum, but this is expanding to include tests for *BRAF*, *HER2* and *MET*. For CRC patients, we routinely analyze *KRAS*, *NRAS* and *BRAF*, and in melanoma we evaluate *BRAF*, *NRAS* and *C-KIT*.

IC: I don't believe the one diagnostic to one biomarker approach is sustainable. To guide lung cancer treatment, we need to know the *EGFR* / *ALK* status. We also test *KRAS*, because it avoids unnecessary *ALK* testing, which can needlessly delay treatment. In CRC, we test *KRAS* and *NRAS* because they guide use of anti-*EGFR* drugs; we also check *BRAF* status. For melanoma we assay *BRAF*, and quite often *C-KIT* also, again because particular treatments are relevant to those mutations.

How does NGS address challenges associated with the multiple biomarker approach?

MB: The main challenge is that the small tissue samples typical of today's biopsies are not compatible with running multiple single tests. NGS, however, allows unbiased interrogation of multiple cancer-related genes from a very small tissue sample.

IC: Samples are reducing in size. For example, endobronchial ultrasound biopsies are commonly used in lung cancer, but are tiny – if you're doing multiple single-gene tests, you quickly use up the sample. But the OncoPrint™ Solid Tumor kit only requires 10 ng of DNA

– a huge advantage. Panel-based PCR methods are a halfway house between NGS and PCR, but when you get beyond two to three genes assayed per patient, the only solution will be to use NGS.

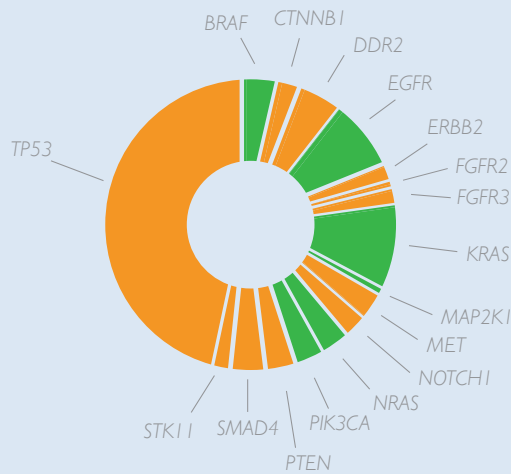
Another big plus of NGS is its coverage. The OncoPrint™ Solid Tumor kits include everything you need to assay in colorectal and lung cancer. This allows us to consolidate tests, and get a lot of information at once.

How easy is it to incorporate NGS into the laboratory workflow?

MB: We recently adopted the OncoPrint™ system. There were training costs, but NGS was readily accepted by all staff and provided economies of scale compared with single-gene testing. And although the turnaround time increases compared with other techniques, the time and costs of single gene evaluation are reduced.

One issue is that reimbursement is based on gene assays requested, not on the panel used; we still use PCR-based techniques if physicians only need a single-gene test for a specific targeted therapy. However, we find NGS is cost-effective when three or more gene assays are requested. Given the amount of NGS data output, interpretation and communication of the results to oncologists and patients represent additional challenges of the NGS technique. We have developed a three-tiered system for gene variant report based on the clinical relevance of a single gene variant identified.

IC: Integrating new systems is never easy. You have to make a business case for the introduction of a new technology, which can be difficult. NGS can be perceived as slow and expensive – it takes three days to do, which doesn't leave much time to interpret results if you want a seven-day turnaround – but it's a good technique, possibly replacing several single marker assays. Also, reimbursement affects NGS



There was 100% concordance between the reference methods (mass array, RT PCR and sanger sequencing) and NGS for markers which were analyzed: KRAS, NRAS, BRAF, PIK3A in colon cancer samples and EGFR in lung cancer samples.

The NGS detected further aberrations in another 12 cancer driver genes, providing additional information potentially relevant for patient management decisions now (inclusion in clinical trials) or in the future, once more treatments are available for routine use. Examples include TP53 Tumour Suppressor gene and KRAS, mutated in 25% of lung cancers (1) and considered to be mutually exclusive with ALK aberrations (2); PIK3CA and FGFR3 – multiple compounds are under clinical investigation in colon, lung and other cancer types (3).

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Spectrum of genes in which mutations were detected by NGS method (OncoPrint™ Solid Tumor kit CE IVD) in first 55 samples in IEO Milan.

uptake – molecular pathology procedures only recently began to get reimbursed in the UK. So we're introducing NGS very carefully, with extensive validation. It's important to ensure that NGS fits with laboratory workflow – the tissue preparations, the DNA extraction processes, and the libraries. More automation would help, and that's an advantage of Ion Torrent; but library preparation is still labor-intensive. It's evolving, however; template preparation and chip loading are automated, which improves data quality.

How challenging was validation?

MB: We adopted the OncoPrint™ Solid Tumor DNA kit, which is a CE-IVD-marked kit, so it needs only performance verification, not full validation. Our verification procedure was based on 90 NSCLC and CRC tumor samples, previously tested with Sanger sequencing, Cobas EGFR kits and Sequenom panels. We confirmed all known gene alterations in the samples, and also identified additional driver mutations.

IC: Our validation method involved testing 155 clinical samples for variants at

87 hotspots in 22 genes relevant to lung or colorectal cancer (1). The panel was tested by seven different labs in their own clinical settings. All previously identified mutations were confirmed, and other mutations were revealed. The study was very realistic – samples included difficult 'real-life' cases.

How do you see NGS being used in the future?

MB: In the future we will adopt more extensive NGS panels that not only include gene mutation but also gene translocation analysis in order to eliminate other time-consuming and expensive techniques, such as FISH. In the era of precision medicine, the implementation of NGS multiple biomarker tests offer a great opportunity to increase the number of therapeutic opportunities for every single patient, improving cancer patient's care. However, we need NGS to be endorsed as a routine diagnostic assay by the regulatory agencies.

IC: Initially, NGS will be an add-on to PCR, and will be used where it's critical to identify driver genes in order to direct drug choice. Ultimately, NGS will replace the 'single biomarker per diagnostic test' approach; we've already switched to a panel-based testing approach with UK-NEQAS (UK National External Quality Assurance Scheme), which has essentially become a panel scheme. Oncologists already require mutation information for some therapies today, and NGS-sourced information will become increasingly important in guiding therapy in the future.

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To learn more about OncoPrint™ Solid Tumor Kits CE IVD, go to [thermofisher.com/solidtumor](http://thermofisher.com/solidtumor)

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## In Practice

*Technologies and techniques  
Quality and compliance  
Workflow*



34–35

**A One-Two Punch for Colon Cancer**  
With patient compliance low and false negatives high, colorectal cancer screening faces challenges in the clinic. Can a new approach to testing alleviate them?

36–40

**A Guide to IHC**  
Breast cancer can be complicated; the more we learn about it, the more complexity we find. Immunohistochemistry can help untangle difficult classifications.

## A One-Two Punch for Colon Cancer

**A new approach to colorectal cancer screening may increase both accuracy and patient compliance**

By Michael Schubert

Colorectal cancer (CRC) is a disease full of challenges. It's awkward for patients to discuss, difficult to treat if not diagnosed early, and prone to high rates of screening noncompliance, thanks to challenges with stool collection and the necessary bowel preparation for colonoscopy. Even among patients who do submit to noninvasive screening, those who undergo fecal immunohistochemistry testing (FIT) alone run about a one-third risk of false negatives for cancer, almost all of which are early-stage, and a two-thirds risk of false negatives for significant precancerous lesions, especially sessile serrated adenomas (SSAs), which do not bleed. Taken together with noncompliance

### At a Glance

- Colorectal cancer screening poses many challenges—chief among them low patient compliance and high false positive rates in noninvasive single-marker tests
- Noninvasive testing, especially if it can be conducted privately at home, can increase patients' willingness to be screened
- Noninvasive multiple-marker testing that combines DNA alterations and hemoglobin detection can lead to a more sensitive test
- A new multi-target, stool-DNA-based test is currently showing success in the United States, with an eye to European and Asian expansion in the future

with annual screening, the outlook for FIT is pretty dismal for CRC prevention and early-stage treatment – but what can be done about it? Barry Berger is part of a team that has developed a noninvasive, in-home test that patients can perform at their leisure. The test uses a multi-target approach that looks for both fecal hemoglobin and DNA markers – and based on their results so far, it might just improve compliance, increase screening success rates, and contribute to decreasing CRC-related mortality and morbidity.

Let's make a test for that Stanley Lapidus – the inventor of the thin-prep Pap smear and a good friend of Berger's – arrived at his laboratory one day in 1995 with a box of equipment in need of a fume hood. He announced, "I know what we're doing next – we're going to do stool cytology for CRC screening." It wasn't the answer Berger was expecting! "I had two things to say," Berger recalls, "and the first was 'No.'" He didn't want to do stool cytology for two reasons: one, because he suspected that patients wouldn't participate, and two, because stool samples contain very few cells. "It's a great need, but not a good approach," he told Lapidus. "Go think of a different one." So Lapidus went on a quest and came back after having met Bert Vogelstein at Johns Hopkins. He reported, "Dr Vogelstein has found that colorectal cancer-associated DNA mutations can be found in stool DNA. Why don't we figure out how to make a test out of that?" And so, teaming up with additional collaborators like the Mayo Clinic's David Ahlquist, they did exactly that.

Originally, the researchers looked at mutations in genes known to be responsible for chromosomal instability. The trouble was that they needed so many of those mutations in order to get an informative panel that it was too technically challenging for a clinical lab. "We expanded our search to epigenetic changes," says Berger,

"and sure enough, aberrant methylation significantly overlapped with the carcinogenesis pathways. So just two markers allow us more coverage than 50 point mutations – though at the cost of a few more false positives. That was the genesis of Cologuard, the multi-target stool DNA test we have today."

For Cologuard, a combination assay of DNA markers and fecal hemoglobin analyzed algorithmically in a logistic regression equation, the investigators wanted only the best. "We were agnostic as to the markers we chose, but we were very particular about performance. So we incorporated 11 biomarkers into our assay – the minimal number for maximum detection." Why 11? Using additional markers resulted in larger decreases in specificity, but contributed only minimal increases in sensitivity – not worth the cost, or the burden on patients. The test included two methylation markers, *BMP3* and *NDRG4* (chosen because of their ability to discriminate between neoplasia and age-related methylation), as well as more standard markers like *KRAS* mutations. The test considers the markers as a group, using a regression algorithm to calculate a single score. "We found that optimizing individual marker results as in a 'marker panel' approach was less sensitive than looking at the composite logistic regression score," Berger explains. "The test is optimized based on the score and a cutoff threshold provides a qualitative positive or negative result. Overall, that yields increased sensitivity with only a small reduction in specificity compared with a panel of individually evaluated markers."

Promising performance

The Cologuard team performed a 10,000-patient study comparing their multi-target testing approach with FIT alone, with colonoscopy as the reference (1). The new test identified 92 percent of CRC, whereas FIT identified only 74 percent – and the difference was even greater when

limited to early-stage cancers. Hemoglobin alone tends to miss early disease because it doesn't ulcerate as reliably, so FIT identified only 70 percent of stage I and II cancers, compared with 94 percent using the multiple-marker test. That performance even held true for precancerous lesions like high-grade dysplasia (with 69 percent detected using Cologuard versus 46 percent using hemoglobin alone) or right-sided flat lesions (SSAs). "Those are particularly striking because they're so hard for gastroenterologists to find – they don't bleed, they're difficult to spot, and FIT often misses them," says Berger. The new test found 42 percent of SSAs  $\geq 1$  cm in size (compared with 5 percent from FIT) and 70 percent of those  $\geq 2$  cm (compared with FIT's 11 percent).

He explains, "False positives do happen – and with 11 biomarkers, you have more than you might with a single marker. We designed our test to have 10 percent false positive results to optimize early-stage CRC detection. This trade-off of sensitivity against specificity allows us to detect 94 percent of actual, curable-stage cancers. That's what we wanted to see. In the United States, where colonoscopy itself is a screening test, capacity is more than sufficient to accommodate the increase in colonoscopy numbers resulting from a somewhat higher single-application false positive rate. In turn, that increases the prevention and detection of disease – the primary purpose of screening."

#### A good day at the office

In terms of application, the new test addresses many of the patient preference factors that keep people from being screened. For those who won't have a colonoscopy, there's now a high-sensitivity alternative. "If everybody were screened – either by Cologuard or colonoscopy – we'd catch 19 out of 20 CRC cases in the early stages," says Berger. "Over a decade, we'd likely achieve significant decreases in CRC incidence and mortality. In addition, the

longer test interval of three years decreases the burden of screening on patients, physicians and healthcare systems. We designed the test with higher sensitivity so that we could extend that interval, because we know that patients are often unwilling to do annual fecal blood screening."

A recent study of 150,000 continuously insured patients over a 10-year period showed that, of those who used fecal occult blood/FIT for screening, only three of every 1,000 patients were compliant throughout the study period (2). "What we have seen is that people who want screening colonoscopies get them, but about half of the population won't. Those are the people who want everything in a test: safety, sensitivity, convenience, and noninvasiveness. Now, for the first time, we have a test for that population, and I think that's a big step forward."

A recent independent study (3) asked whether or not the test actually fulfilled its mission – namely, to appeal to people who were previously noncompliant. The researchers looked at 400 patients 65 and older who were defined as consistently noncompliant (meaning neither colonoscopy within the past decade, nor annual FIT testing). Those patients were offered Cologuard; 88 percent of them came in for screening, and nine out of 10 patients with positive screens went to colonoscopy for diagnosis. "We found four early-stage CRC cases (three stage I, one stage II), all surgically correctable, as well as 21 advanced adenomas and a high-grade dysplasia. All of those were patients who wouldn't otherwise have been screened, and who would eventually have presented with late-stage, difficult-to-treat disease. So that was a very good day at the office!"

#### The roll-out phase

"We want Cologuard to provide a standard of care for many patients," says Berger. "Right now, approximately 40,000 physicians use the test routinely, which means we have another 260,000 to go! It

takes time – about three years – to get a new test integrated into medical practice, but so far, the response has been good." In the new United States Preventive Services Task Force guidelines for colorectal cancer screening published in late June, Cologuard testing every three years was included, placing it on equal footing with all other included strategies – a major milestone for the test (4). In the next few years, Berger and his colleagues intend to focus on rolling the test out first in the United States, and then eventually in Europe (where Cologuard is already CE marked) and Asia. They also want to look at other cancers with an eye to prevention using similar technology.

"I know pathologists are curious about how Cologuard might change their day-to-day work. The good news is that it probably won't change a thing – at least at first. The test is only performed by Exact Sciences Laboratories, which functions as both a primary and reference lab. In the future, once the roll-out is complete and we've addressed any potential logistical issues, we'll consider the opportunity for centers of excellence to perform the test themselves using our kits. It's our hope that, at that point, we'll be able to defeat the great unmet screening need in colorectal cancer."

*Barry M. Berger, MD FCAP is an anatomic, clinical and cytopathologist, as well as Chief Medical Officer, Medical Affairs at Exact Sciences Corporation (Madison, USA).*

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## A Guide to IHC

### How immunohistochemical analysis can help navigate the complexities of breast pathology

By Ping Tang

The more we learn about breast cancer, the more we realize just what a broad classification it truly is. Despite what many patients still believe, there's no one "breast cancer" to rule them all – it's a heterogeneous group of tumors, and each type exhibits different characteristics, different behaviors and different clinical outcomes. As a result, we're parlaying this increase in understanding into ever more diverse tests to gain insight into the nature of each individual patient's disease – and although genomics is an up-and-coming field of study, the molecular tests aren't the only ones that play a role in tumor classification (1). Immunohistochemistry (IHC) plays an important part too.

#### At a Glance

- Our increasing knowledge of the diverse nature of breast cancer is giving rise to ever more diverse tests
- While molecular tests are increasing in importance, immunohistochemistry (IHC) analysis is just as useful in the differential diagnosis of breast lesions as well as for prognosis and prediction
- IHC can also be used as a tool in the discovery of new therapeutic targets, and in combination as a surrogate for molecular classifications and multigene prognostic panels
- Here, I provide a guide to how IHC analysis can help pathologists make a correct diagnosis and provide accurate prognostic and predictive information to clinicians, and to inform of the factors that might impact IHC test quality

Traditionally, IHC analysis has been used for diagnosis in difficult cases, but more recently it has served as a prognostic or predictive marker, as a tool in the search for new therapeutic targets, and even in combination as a surrogate for molecular classifications and multigene prognostic panels. But with a wide range of biomarkers available for IHC testing, it's important for pathologists to understand what's out there, how it can be used, and where things might go wrong.

#### Invasive or in situ?

Myoepithelial (MEP) markers are vital for distinguishing between in situ and invasive tumors; they are present in in situ lesions and absent from invasive lesions (2). Basal-type cytokeratin, smooth muscle myosin heavy chain (SMMHC), smooth muscle actin (SMA), calponin and p63 are frequently used MEP markers – but because they have varying specificity, they're often used in combination, or alongside the less common collagen IV and laminin markers. For microinvasive carcinoma ( $\leq 1$  mm), adding keratin staining can highlight the presence of malignant cells.

However, not every invasive tumor follows the rules for MEP markers. Some invasive lesions, like adenoid cystic carcinoma and metaplastic carcinoma, consist of cells that are positive for MEP markers. These cases present a tricky diagnostic challenge, so it's critical to carefully evaluate the location of MEP marker-positive cells for a correct diagnosis. Also, there's one benign lesion with unusual MEP marker status: microglandular adenosis, a benign breast lesion that also lacks marker-positive cells (such as p63) and mimics the more aggressive tubular carcinoma. To distinguish the two, check for ER, PR, and S100 status; you'll find that the benign lesion is negative for the former two and positive for the latter, whereas the carcinoma is the opposite.

#### Pondering papillary lesions

Papillary lesions are a broad group that includes benign, atypical, in situ and even invasive tumors. Because of their diversity, they're one of the most problematic areas in diagnostic breast pathology – so we routinely use IHC analysis to assist with diagnosis (3). The two most commonly used markers are CK5 for the presence of usual ductal hyperplasia (UDH) and p63 for the presence of MEP cells. What can we diagnose with those two biomarkers?

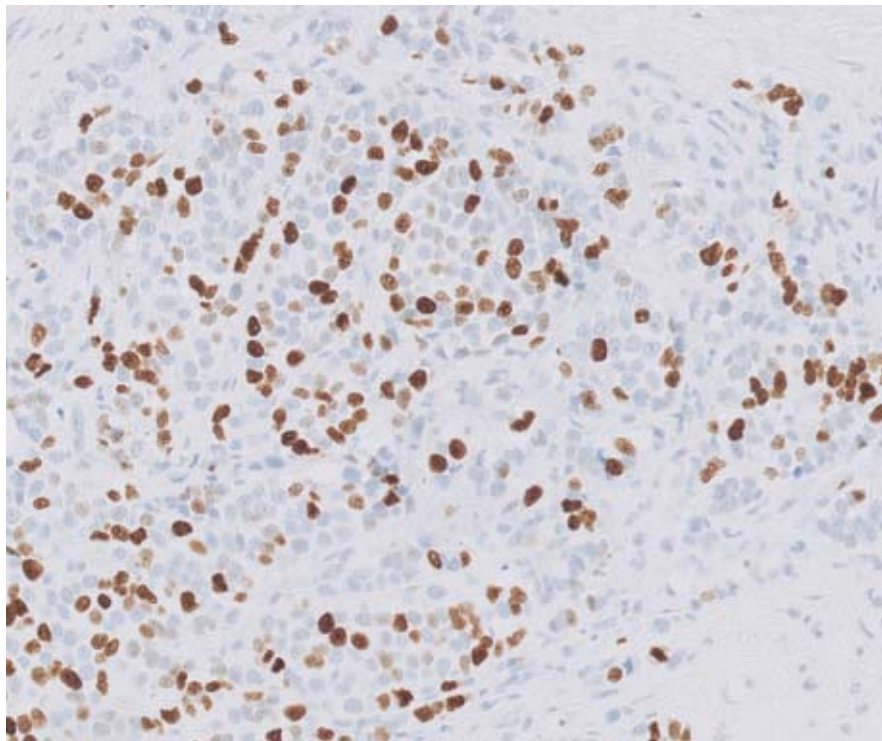
- Intraductal papilloma (IP): a benign papillary lesion characterized by fibrovascular cores lined with MEP and epithelial layers. IHC analysis for MEP markers highlights the presence of MEP cells along the fibrovascular cores and at the periphery of the lesions.
- IP with UDH or atypical ductal hyperplasia (ADH)/ductal carcinoma in situ (DCIS): the presence of CK5-positive cells can be helpful for diagnosis of florid UDH within an IP; the lack of those cells, along with uniform ER expression, is helpful for diagnosis of IP with ADH/DCIS.
- Intraductal papillary carcinoma (IPC): consists of slender fibrovascular cores covered by a single layer of monotonous neoplastic cells; MEP cells are retained only at the periphery of the lesion.
- Encapsulated papillary carcinoma: a variant of IPC, characterized by fine fibrovascular cores covered by low to intermediate nuclear grade neoplastic cells and surrounded by a fibrous capsule. It lacks MEP cells both at the periphery of the lesion and within the fibrovascular cores and is staged as in situ.
- Solid papillary carcinoma: another variant of IPC that shows a solid growth

pattern and inconspicuous fibrovascular cores; it is strongly ER/PR-positive. About two-thirds of these lesions express one or more neuroendocrine markers (4). Smooth nodular borders stage this tumor as in situ regardless of the presence or absence of MEP markers.

Perhaps a better question is: what can't we diagnose with CK5 and p63? That list is much shorter – only two types of tumor. One is IP with diffuse florid UDH and sclerosis, which can be difficult to differentiate from invasive carcinoma. Benign cytology, strong CK5 positivity, and ER negativity are helpful diagnostic clues. The other exception to the rule is adenomyoepithelial lesion with predominant papillary patterns. This lesion closely resembles IP with MEP hyperplasia, but IHC analysis can highlight its dual ER/PR/HER2-negative epithelial and MEP marker-positive cell populations.

**Intraductal hyperplasia: UDH or ADH?**  
The two intraductal epithelial proliferations – UDH and ADH/low-grade DCIS – are not only biologically distinct, but have very different clinical implications. UDH consists of a heterogeneous proliferation of mixed cells (including luminal, MEP, and even apocrine metaplastic cells), and carries minimal subsequent risk of cancer development (5). A significantly higher risk (three to five times that of the general population) comes from ADH/LG-DCIS, a low-grade monotonous population of luminal cells (6). To distinguish between the two, pathologists often call on IHC analysis – especially in difficult cases involving necrosis or mitosis, where differential cytokeratin staining can be the key to diagnosis.

What do they look like under a microscope? UDH tends to show a



Lobular breast carcinoma (FFPE) stained with anti-Ki-67 antigen.

mixed phenotype for low (luminal, such as CK7, CK8, or CK18) and high (basal, such as CK5, CK14, or CK17) molecular weight keratins, along with a heterogeneous or mosaic staining pattern for ER. ADH/LG-DCIS, on the other hand, typically shows restricted luminal cytokeratin expression along with high levels of ER expression. The one exception to this rule is basal-like DCIS, which can sometimes mimic the appearance of UDH. So how can you spot the difference? Neoplastic cells in the more aggressive tumor are usually high nuclear grade, with abundant mitoses and necrosis, and can be negative for ER and PR.

#### Markers for mysteries

We often use IHC markers to answer specific questions about breast tumors. They can be used to identify lobular lesions, spindle cell involvement, and even trace the origins of metastatic tumors

with unknown primaries. These are often clinically important questions – lobular lesions, for instance, behave differently to ductal carcinomas, so it's impossible to provide a prognosis or select a treatment plan without identifying which of the two you're facing.

Fortunately, lobular lesions are easily defined by examining two markers: E-cadherin and P120 catenin. Loss of E-cadherin expression is the most consistent molecular change in lobular lesions. IHC analysis of this particular marker is especially helpful when distinguishing between two variants of lobular carcinoma in situ (LCIS): pleomorphic lobular carcinoma in situ (7) and solid LCIS with central necrosis (8). P120 catenin is particularly helpful for clarifying invasive lobular carcinoma lesions with sparse single tumor cells (9), as it demonstrates diffuse cytoplasmic staining, while E-cadherin will only give a negative stain.

ER	Up to four-fifths of breast cancers are ER-positive; strong ER expression indicates a breast primary, but lack thereof doesn't necessarily exclude breast origin.
GCDFP-15	This marker has 98 percent specificity and 58 percent sensitivity for breast lesions. It tends to be strongly expressed in lobular and apocrine lesions.
Mammaglobin	More sensitive, but less specific than GCDFP-15. In particular, it's more sensitive than GCDFP-15 for non-triple-negative breast cancers, but less for triple-negative.
<i>GATA3</i>	Over 90 percent of breast cancers express this marker, which is 100 percent positive for non-triple-negative cancers, 60 percent positive for triple-negative, and 0 percent for metaplastic. Other <i>GATA3</i> positive tumors include urothelial carcinoma, germ cell tumors, cutaneous basal cell carcinoma and benign skin adnexal tumors.

Table 1. Markers with known breast tumor specialty that can assist in identifying a lesion's origin.

The diagnosis of spindle cell lesions, in particular the common metaplastic carcinomas (MC) and phyllodes tumors (PT), often relies on IHC analysis with a panel of IHC markers because the spindle cell component can be deceptively benign or overtly malignant in appearance. Distinguishing between the spindle cells in these two lesions requires a wide spectrum of cytokeratin markers and p63 for MC, as it's present in over 90 percent of these tumors (10), as well as CD34 for PT, although the amount of staining is inversely associated with PT grade. To confirm or reinforce a diagnosis, look for a biphasic fibroepithelial component (for PT) and the presence of invasive carcinoma of no specific type (for MC), as this is also very helpful.

Comparing the morphology of a metastatic tumor with the original lesion is the single most important step in determining the primary lesion's origin – but when the primary lesion isn't available, IHC analysis with markers known with breast specialty can help (see Table 1).

#### Prognosis and prediction

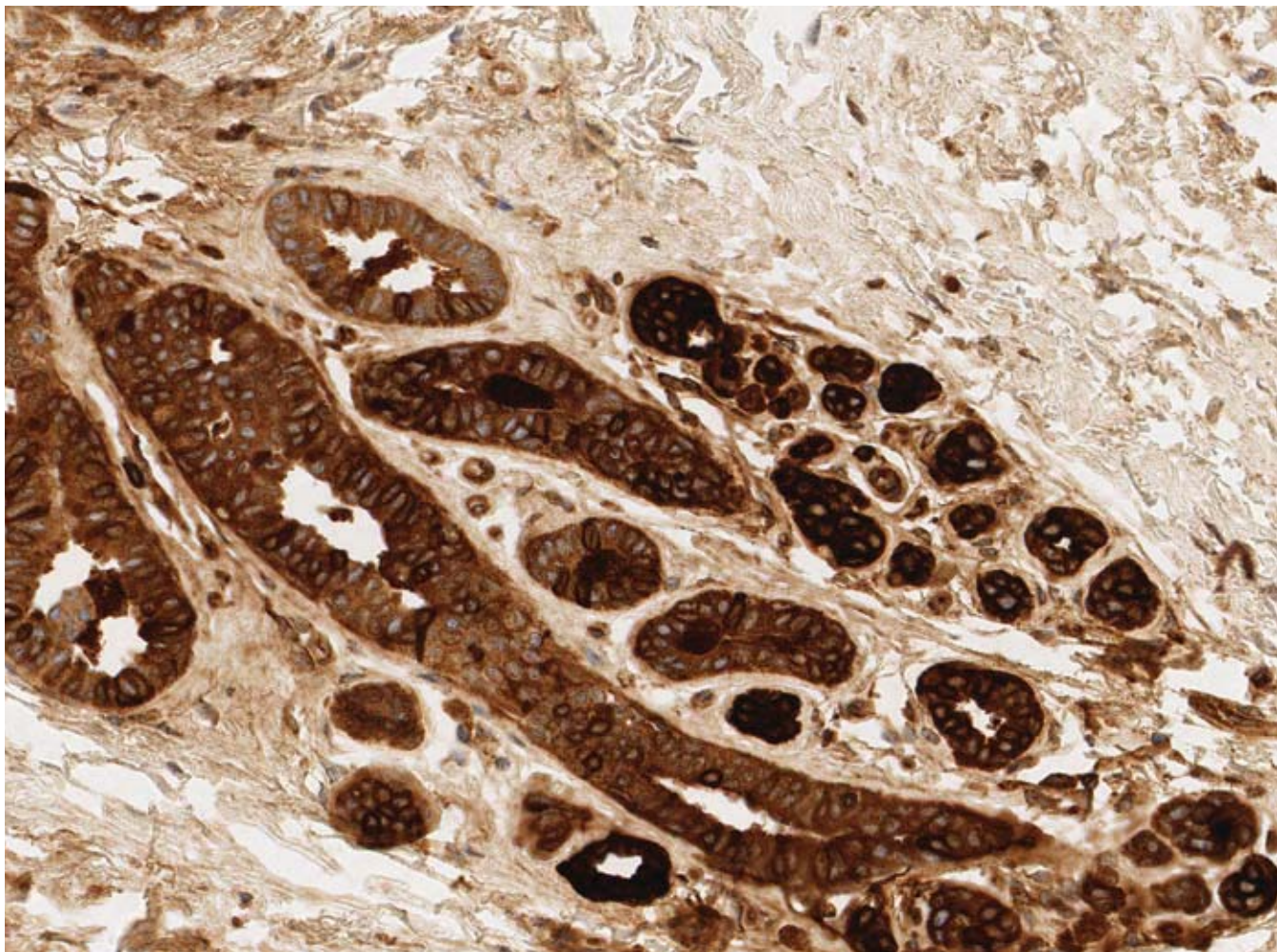
The use of IHC analyses as predictive tests is fundamentally different from diagnostic classification, because clinicians will use the results from these test directly to guide their management decision; this means that quality specifications and standards are more important than ever. Prognosis and prediction can be conducted using single biomarkers (like ER, PR or HER2) or combinations. Each approach carries its own benefits – and corresponding challenges.

ER and PR, for instance, are expressed in about 75–80 percent and 65 percent of all breast tumors, respectively. ER drives disease progression for positive tumors (those with over 1 percent of tumor cells showing nuclear staining of any intensity), leading to a direct correlation between the level of ER expression and the likelihood of response to hormonal therapies (11). The loss of PR, a transcription factor largely regulated by ER, in ER-positive tumors is associated with decreased response to tamoxifen therapy and a worse overall prognosis

(12). Up to one-fifth of invasive breast cancers are also HER2-positive, a trait associated with an aggressive clinical course and poor outcome. But ER, PR and HER2 testing by IHC shouldn't occur in a vacuum – with an eye to quality, the evaluation should always include normal breast tissue as an internal positive control, and take into account clinical and morphologic features as well.

Hormones aren't the only biomarkers that can be used for prognosis. Ki-67 is a proliferation marker associated with both poor prognosis and better response to chemotherapy. It can be used to classify breast carcinoma, separating luminal A and B subtypes, and has been included in a number of multiple-gene assays that evaluate the potential for recurrence (13, 14). Why isn't it more commonly used in the clinic? At the moment, we lack standardized methods for its evaluation, so it hasn't been recommended for routine breast cancer evaluation.

Instead, pathologists can consider the wider environment in which the cancer exists – for instance, the context of the patient's immune system. The tumor microenvironment plays a critical role in the immune-editing process (elimination, equilibrium, and escape). Stromal tumor-infiltrating lymphocytes (sTIL) are a reflex of that environment and can be a powerful prognostic and predictive marker for breast cancer patients, especially in HER2-positive or triple-negative tumors. sTIL should be viewed as a continuous variable; for every 10 percent sTIL increase, there's a significant reduction in disease-free and overall survival (15). Other immune markers include the current "hot topic," PD1/PD-L1. Binding between PD1 (expressed on activated T cells) and PD-L1 (expressed on antigen-presenting and tumor cells) results in immune suppression and tumor progression,



Breast hyperplasia (FFPE) stained with anti-GCDFP-15.

and the proteins' upregulation has been associated with poor prognosis in some cancers. Targeted monoclonal antibodies, recently developed, have shown promising and durable responses in many human malignancies – including in a few Phase I studies on breast cancer, with positive response rates between 12–42 percent (16).

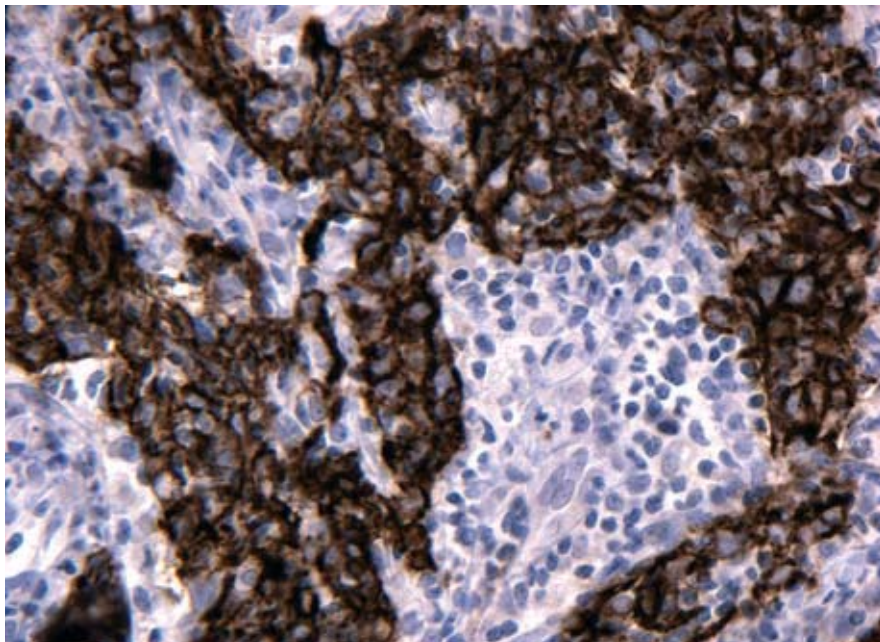
#### Making use of multiples

Although we've used individual breast cancer molecular markers for many years, the concept of using several together was raised after the introduction of molecular classification by gene expression profiling.

Many large studies on molecular classification have been performed with IHC surrogates – most commonly ER, PR, and HER2, dividing breast tumors into luminal, HER2-positive and triple-negative. Adding Ki-67 separates luminal A and B subtypes, whereas adding CK5 and epidermal growth factor receptor (EGFR) separates basal-like from non-classified triple-negative breast cancer. More recently, biomarkers such as androgen receptor and p53 have been shown to further stratify these molecular subtypes (17).

The IHC4 score is an assay developed using a retrospective cohort of 1,125

ER-positive breast cancer patients (13). The semi-quantitative ER, PR, Ki-67 and HER2 results of IHC analysis were used to calculate a risk score, known as the IHC4 score, using weighting factors and an algorithm. The score was found to provide prognostic information independent of traditional histopathologic variables and demonstrated prognostic utility similar to the 21-gene recurrence score (RS) assay. In a subsequent study, the IHC4+C score (which also incorporates clinicopathologic parameters) reclassified more than half of patients stratified as intermediate-risk into the low-risk category.



Breast ductal carcinoma (FFPE) stained with anti-E-cadherin.

Another option for multiple-biomarker evaluation is the Magee equations. Using several key pathologic parameters like tumor grade, tumor size, and status of ER, PR, HER2 and Ki-67, Klein et al. developed three equations that produced estimated recurrence scores (ERS) highly concordant with the RS assay and useful in breast cancer (14).

Is it really that easy?

Despite its helpfulness and the variety of available markers, IHC analysis for breast cancer is not entirely cut-and-dried. There's still some concern about the lack of reliability and reproducibility of IHC analysis in routine clinical settings as a result of poor assay standardization. A wide range of factors can impact the quality of tissue samples for IHC analysis, including tissue handling, fixation, antibody reagents, staining protocols and the pathologists' interpretation of the assay results. The American Society of Clinical Oncology and the College of American Pathologists published guidelines for HER2 and ER/PR testing

(18, 19) in an effort to improve test quality, inter-laboratory agreement and test reliability for breast cancer patients – so those guidelines now serve as a reference for laboratories undertaking molecular tests. There's also a need for caution when performing IHC analysis on decalcified or alcohol-fixed specimens, because most such tests aren't validated in those types of specimens.

Traditionally, IHC analysis has been used only to aid in diagnosis. But with the development of targeted therapies for ER and HER2, testing for expression of these biomarkers has become an assay with treatment implications – and the possibilities for new personalized treatments are increasing every day, so it's not unreasonable to expect other tests to go the same way. The use of combination IHC biomarkers as surrogates for molecular classification and other multi-gene prognostic panels has also intensified the focus on immunohistochemical analysis, and means that – if the proper precautions are taken for quality control and

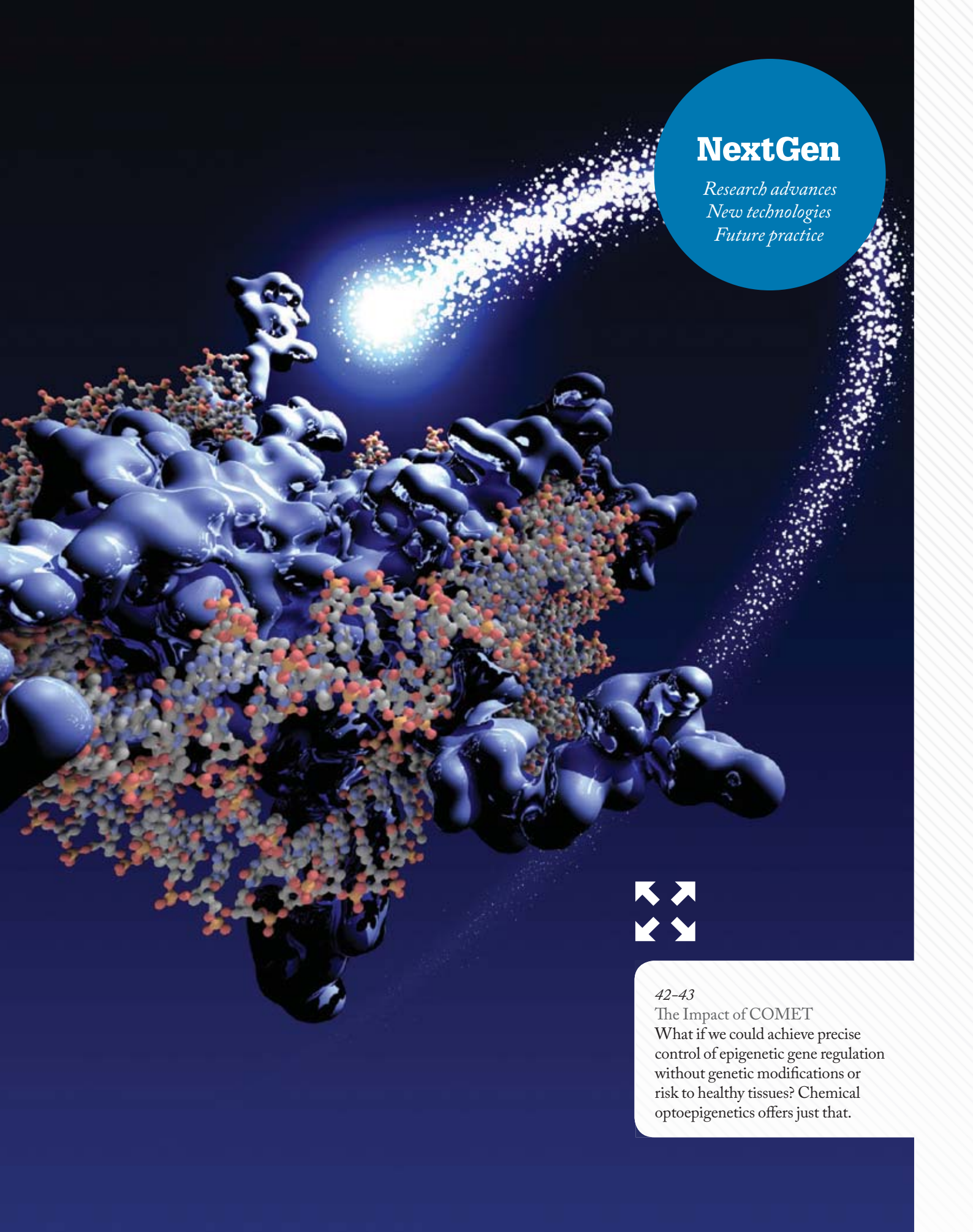
independent verification – IHC can continue to become an ever more prominent tool in the quest to defeat breast cancer.

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## NextGen

*Research advances  
New technologies  
Future practice*



42-43

The Impact of COMET

What if we could achieve precise control of epigenetic gene regulation without genetic modifications or risk to healthy tissues? Chemical optoepigenetics offers just that.

## The Impact of COMET

### A new approach to epigenetic control may allow precise spatiotemporal targeting of disease-controlling mechanisms

By Stephen J. Haggarty and  
Ralph Mazitschek

The more we learn about epigenetics, the more we see its involvement in a plethora of human diseases. Gene dysregulation plays a role in cancers, neurodegenerative diseases, developmental disorders, and more. But the epigenome presents a challenge – how do we address its failings in diseased tissues without disrupting the careful balance of regulation in healthy ones? Our current methods aren't sufficient; the few epigenetic drugs that have received approval treat the whole body, risking epigenetic changes in undamaged tissues. Even targeted approaches are difficult, as most require the introduction of a genetic modification before they can be applied. But what if we

#### At a Glance

- *Epigenetics is a factor in a wide range of diseases, but we lack effective ways of examining or treating epigenetic dysregulation*
- *Current approaches require either administering treatment to the whole body or making genetic modifications to the cells you want to target*
- *Chemical optoepigenetics – the use of light-controlled small molecules to target the epigenome – could provide an alternative*
- *There's still work to be done before the method hits the clinic, but ultimately, it may open up new avenues for research and treatment*

had a way to precisely control epigenetic gene regulation using nothing but small molecules and visible light? We propose a new approach known as COMET that might provide exactly that.

#### Purposeful precision

Epigenetic regulation, a critical means of controlling DNA in the human genome, is integral to health and disease. What exactly does it entail? It's a collection of highly dynamic processes involving multiple chromatin modifying and remodeling enzymes. Those enzymes control access to genes and their expression through the covalent modification of DNA and its associated histone proteins. Developing precise tools to control the epigenome has been of great interest in basic research for some time – and it's gaining increasing attention from the clinical side of the equation as we begin to grasp the widespread potential application of epigenetic therapies for disorders ranging from cancer to Alzheimer's disease.

We refer to the general technique we've developed as “chemical optoepigenetics.” What does that mean? *Chemical* refers to our use of small molecules to modulate the epigenome, and *opto-* means that we use light (photons) to control the inhibitory activity of those small molecules. As you can probably guess, the technique relies on optically controllable small molecules that target a class of epigenetic regulatory enzymes called histone deacetylases (HDACs). We refer to these small molecule inhibitors as COMET (Chemo-Optical Modulation of Epigenetically regulated Transcription) probes and use them to provide high-resolution control of epigenetic mechanisms (1).

Having precise spatial and temporal control of epigenetic mechanisms – without the need for genetic modification – opens up new avenues to dissect the dynamic process of HDAC-mediated genome control. Optogenetic methods using light-responsive ion channels have

transformed neuroscience, and we now also have genetically encoded, light-inducible transcriptional effectors. But both of those methodologies require prior delivery of genes into the target cells. Our “all-pharmacological” approach to controlling gene expression is a major advance for the field. That's not to say that there were no pharmacological options before COMET – but current chemicals in epigenetics, for instance those that reversibly modify the N-terminal tails of histone proteins, are limited in terms of their selectivity and our ability to control them. COMET probes will allow more precise targeting of dynamic chromatin modifications, potentially at subcellular resolution. We hope that will eventually lead to a better understanding of the direct and so-called “off-target” effects of small molecules.

#### COMET in the clinic

There's still optimization work to be done before we can start looking at COMET for clinical applications. One day, though, our probes may translate into novel therapeutic strategies that make use of conditional and selective epigenome modulation. So far, it's oncology that has seen the greatest advances in epigenetic therapies. Even now, we have epigenetic drugs like vorinostat and romidepsin on the market and many others in clinical development – but none of them can be precisely controlled. Outside oncology, an area of growing interest is the application of epigenetic therapies to a range of brain disorders, including neurodegeneration found in Alzheimer's disease and affective disorders like depression or bipolar disorder. Exciting preclinical proof-of-concept studies with non-optically controllable HDAC inhibitors already exist, and those provide us with an impetus for exploring the use of COMET probes in the same context. The added precision we can bring to the table in such a complex tissue as the brain may confer additional advantages. Other, more accessible tissues like blood, the retina and

The LED array assembly used in the Mazitschek laboratory to conduct a COMET assay. The instrument can be seen in action at <http://go.nature.com/1rrsKDe>



the ear might also see significant benefits from that kind of precise control. And of course, not having to genetically modify the targeted tissue to obtain optical control of a biological process has a number of distinct advantages.

Ultimately, we'd like to see a wide range of disorders benefit from COMET, including cancer and nervous system disorders that involve focal dysregulation of epigenetic mechanisms in specific regions or tissues. Being able to control where and when an epigenetic therapy is applied could conceivably overcome many of our existing approaches' shortcomings – for instance, the fact that current epigenetic treatments expose the entire body to an active drug, whereas we would provide a system for careful targeting.

COMET technology allows researchers to devise and implement new types of epigenetic studies that could not be contemplated previously. They can control

the epigenome of a single neuron within a complex circuit without having to first genetically modify the cell. They can create a dose-response gradient within a single tissue or tumor sample.

And we encourage them to come up with new ideas! The Arduino-based, microprocessor-controlled LED array platform we developed is inexpensive and readily available, which we hope will increase the rate at which investigators discover novel photochromic probes and optimize their use.

#### An optoepigenetic future

All this is only the tip of the optoepigenetic iceberg. Our COMET concept can be applied to a wide range of epigenetic mechanisms beyond HDACs, including other epigenetic “writers” and “erasers,” like the proteins involved in histone methylation and demethylation, or “readers” like those that bind to sites of chromatin modification. Each strategy will require the development of novel chemical probes with appropriate binding kinetics and selectivity profiles. As part of this, we're exploring other photochromic scaffolds with improved optical properties for use in vivo in tissues where photon scattering

(deflection when a particle hits molecules larger in size) and absorption can be minimized through the use of longer wavelengths of light.

Current versions of our COMET probes can be turned on with light, and we're investigating the alternative possibility of optical control that allows them to be turned off instead. Of course, while we explore these new concepts, we're also advancing our HDAC COMET probes toward clinical translation. It's our hope that a step up in simplicity and precision control could lead to a new day for epigenetic research – and, eventually, patient care.

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*\*To enable commercialization of this technology, the inventors have filed for a patent (WO 2014160221) on the novel compositions and methods of treatment, and are currently seeking licensing arrangements for application for therapeutic development.*

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## Profession

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46-49

**Avoiding the Ripple Effect**  
There is a very real danger of pathologists being phased out of medical student education. It's time to think creatively and take control.

## Avoiding the Ripple Effect

### How can pathologists stay relevant? By taking an integrative approach to pathology education...

By C M Quick

The classic role of the pathologist in medical school education was quite diverse not so long ago. We often served as course directors, responsible for crafting learning objectives, creating content and designing test questions. We regularly found ourselves teaching lectures to eager medical students, or spending afternoons in gross or microscopic pathology labs, explaining disease processes. Clinical presentations, gross pathologic findings and histology were often combined and explained alongside one another, creating

#### At a Glance

- *The role of the pathologist as an educator is changing, in some cases to the detriment of the profession*
- *Rising volumes of information are forcing us to distill our knowledge and, in certain instances, phase out some forms of pathology training entirely*
- *At our institute, we have integrated pathology into medical students' first-year gross anatomy course; during the class, anatomy is taught alongside radiology, histology and disease process*
- *Though student workloads are already high, developing creative, interactive course content has successfully ignited interest and enthusiasm for pathology among students, and this model should be used as a blueprint for other pathology educators*

a seamless picture of the pathobiology of a disease. This fairly simplistic model worked pretty well back then. But the great strides that have been made in disease research in recent years means that our knowledge has soared, and as a result, so too has the mountain of information that could potentially be taught to medical trainees. So today, we're often left with the job of distilling that knowledge, and deciding what is important for the undifferentiated future physician to learn and what will fail to make the cut – not an enviable task.

*“Is there a danger that pathologists will be phased out of undergraduate medical student education? Sadly, unless something is done, I think the answer to that is, quite possibly, yes.”*

Change is happening. Recent trends in medical education have moved toward (or, in some cases, gone back to) the concept of integrated, or “modular,” curricula – i.e., instead of discipline-based courses (such as pathology or microbiology), they are integrated and organ-based. This model has the benefit of presenting a coherent picture of basic structure and function working alongside

pathophysiology and treatment. This way, students are exposed to one organ system at a time, covering everything from histology to pharmacotherapy and microbiology specific to the system. And it seems to have worked; migration to this circular structure has been shown to increase performance on standardized examinations (1) – exams that seem to dominate the psyche of medical students more with each passing year. In the United States, a major checkpoint of this distillation and passage of information is the USMLE Step 1 exam. This single test has become the focal point of the preclinical medical school years. If information is not commonly tested on this exam, it is often deemed superfluous. While these changes in curriculum have led to more efficient medical education with an emphasis on high-yield topics, they have also required a change in the role of the pathologist as a medical educator.

Importantly, this transition has meant that much of the control of course content has shifted from specific (such as pathology or microbiology) departments within colleges of medicine to whole committees of the colleges of medicine. The result? Standardized education, more staff support and an infrastructure that likely substantially exceeds that which a department could provide alone. How can pathologists ensure that they have significant input into this new teaching model? Ideally, by serving on curriculum committees and giving input into what pathology should be taught, and how. But is this happening in the real world? Or is there a danger that pathologists will be phased out of undergraduate medical student education? Sadly, unless something is done, I think the answer to that is, quite possibly, yes.

The “disappearing pathologist”  
Overall, this loss of discipline-based courses has the net effect of decreasing the pathologist’s role in designing, creating

## The Survey Says...

Percentage of first-year medical students responding "Agree" or "Strongly agree" to questions following the completion of the revised gross anatomy course, incorporating pathology.

**95.4%**

My interaction(s) with pathologists in the laboratory improved my understanding of the impact of disease on a specific organ or multiple organs

**57.3%**

Based on my interaction(s) with pathologists in the dissection laboratory, I am more likely to consider pathology as a potential career

**96.2%**

The consultation, which my dissection team had with a pathologist to review the histopathology of our biopsied specimens, was an excellent learning experience

**96.9%**

I would recommend that the course/module continue this event next year

**99.2%**

I enjoyed the experience of trying to determine the cause of death of our donor

and, sometimes, delivering educational instruction to preclinical trainees. There's also a very real risk that traditional material taught by pathologists (i.e., histology) could be culled in the interests of efficiency. If pathologists are not involved at the earliest levels of planning, we run the risk of losing the opportunity to teach what we love. In fact, many of us have already witnessed in some capacity the presentation of a pathology lecture by a clinical colleague – for example, an oncologist or internist.

The "disappearing pathologist" may become a reality as the material we deliver and the contact hours we have with students are eroded. This phenomenon has the potential to cause ripples that may loom over the entire field of pathology in the coming years. As many

pathologist educators have experienced, the pathophysiology course at a medical school is a (if not the) predominant driver of interest in pathology as a career. Many medical students have their first interaction with pathologists via these courses, and inevitably some choose pathology based on this interaction (as was certainly the case for me). And these courses also provide students with a faculty contact, which is very important, in particular for those who are potentially interested in pathology as a career. Worryingly, loss of these courses, if uncompensated, could lead to a decrease in interest in the field.

Bucking the trend

Here at the University of Arkansas for Medical Sciences, we are working hard to buck this damaging trend. We have

reinstated our pathology interest group (with support from extramural grants from the Intersociety Council for Pathology Information and our department of pathology), and we've established a summer preceptorship, both of which have increased student awareness and interest in pathology as a career. But we haven't stopped there... We've partnered with other disciplines to integrate pathology into medical student education from the very start of their undergraduate training.

It's important to recognize that the opportunity to teach pathology is everywhere, and pathologists must explore new avenues to continue to be at the forefront of preclinical medical education. One, mostly underutilized, resource for pathology education, which we have taken advantage of, is the

collaborative integration of pathology into other courses. This, of course, does not have to be limited to pathology, but may involve any interested subspecialty. At the University of Arkansas for Medical Sciences we have an expansive collaboration occurring within the first-year gross anatomy course between anatomy (David Davies), radiology (Sharp Malak) and pathology (me). This collaboration has led to a greatly enriched learning experience for medical students in their first weeks of medical school. What could be better than starting the journey of medical education working with scientists and clinicians teaching anatomy, radiology, histology and disease process from day one?

Our approach explained

The way it works at our institution is as follows: pathology attendings and residents are present during selected gross dissection lab sessions; these sessions usually include dissection of the chest, abdomen and pelvic organs. The pathologists, usually five to six in number, rotate among the 35 dissection teams, which are composed of five students. The role of the pathologist is to answer questions, identify and explain grossly evident disease and to assist the teams in taking of biopsies; each team is allowed to take up to seven. Discernment of which areas to sample is left to the students, which plays an important role in emphasizing self-directed learning. Additionally, it stresses resource utilization and clinical decision-making development. Clinical decision-making is further enriched by allowing the dissection group time to research their differential diagnosis before taking samples.

As the students obtain biopsies, they are processed by the histology department, and slides are returned to the students so that they may begin evaluating them. Obviously, medical

students in their first month of school may lack the foundation to critically evaluate slides, or, in some cases, describe expected normal histology, so a network of educational resources has been put into place. Students have access to pathologists and microscopes and are encouraged to ask questions during laboratory sessions. Additionally, they are referred to online resources for help with histologic and pathologic features.

*“I would urge any interested pathology educator to use this article as a blueprint, follow a similar model and take the earliest possible opportunity to ensure that pathology is taught to first-year medical students.”*

These resources allow them to explore the slides as a team and develop and refine their differential diagnosis. The ultimate resource they are allowed to utilize is a 15-minute pathology consult with an attending pathologist. At this consult the students review their slides at a conference microscope, and the attending pathologist identifies and describes the disease processes present in the biopsy material. In general, the

pathologist lists “take-home points” that could be ascertained from the biopsies, and gives possible causes of death for the students to consider and research as they move into the next phase of the project, the abstract and presentation.

Thinking creatively

In developing this curriculum, it was determined that “meaningful” documentation of the findings was necessary to reinforce learning. Clearly, when working with pathology slides, photography is an ideal way of presenting and archiving findings; however, the manpower needed to help medical students photograph hundreds of slides would easily overwhelm most departments. To counter this, cell phone camera mounts were purchased for the microscopes and students were given a brief tutorial on how to use a microscope and slide photography. The photographs that the students obtained could be easily shared with group members and inserted into abstracts and group presentations. Use of the students’ own phones helped to generate interest and buy-in to the process of pathologic evaluation of biopsies.

Following the pathology consult and group evaluation of their material, the students are required to produce a scientific abstract, composed of an introduction, methods, findings and conclusion. They are urged to focus on the suspected cause of death, a disease process the group found interesting, or, in the case of a normal cadaver, the histology and physiology of an organ system of their choosing. This abstract acts as an outline for a 15-minute oral presentation that each group delivers to their peers and faculty judges at the end of the course. The presentations are graded for content and group professionalism and provide an opportunity for the faculty to provide narrative assessment of the group’s progress. Truly, the entire





process, from the lab to the presentation, helps to anchor gross anatomy, histology, radiology and pathology in clinical context and provide a deep learning experience for each group.

#### What do the students think?

Of course, the success of a major change in curriculum depends heavily on the attitudes of those who take part in it, both teachers and students. This project was deployed in addition to an already packed schedule, which many new students find intimidating. In its pilot year, significant concern existed as to whether students would be able to tolerate the increased workload associated with the course. To evaluate the impact of the project and student

attitudes, a detailed survey was given to the students. Impressively, there was strong and universal praise for all facets of the experience, despite the resulting large increase in workload (see Infographic). While the pathologists were not formally evaluated on their perception of the project, their attitude was enthusiastic and many faculty and residents signed up for additional time slots after their first visit to the lab. Many pathologists expressed enjoyment because they were teaching in a lab and at a microscope, as opposed to in a lecture hall. In addition, resident pathologists appreciated the additional opportunity to gain formal experience in teaching medical students, an experience that has become sparse at

our institution in the last few years.

I would urge any interested pathology educator to use this article as a blueprint, follow a similar model and take the earliest possible opportunity to ensure that pathology is taught to first-year medical students, impressing upon them the true awesomeness of pathology.

*Charles Matthew Quick is Associate Professor of Pathology at the University of Arkansas for Medical Sciences, Little Rock, USA.*

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# Oncogenetic Pioneer

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



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

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

What has been the overarching theme of your work?

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

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

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

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

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

I am not one of those people who plans out their lives; I just put one foot in front of the next. Working in cancer research was really just a series of fortuitous accidents. I was interested in studying mRNA, and tumor

viruses were a tool to do that. I ended up sharing a lab with David Baltimore, who had just discovered reverse transcriptase, and began to work on RNA tumor viruses that could infect and transform cells. Over time, my interests evolved and I ended up studying the cellular genes that control cancer. My main ambition is simply to do interesting things.

What led to your discovery of the first human oncogene?

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

What projects are going on in your lab today?

In 2003 we started to work with genes involved in the cell biological program termed the epithelial-mesenchymal transition (EMT) and found that in primary carcinoma

cells, such genes could impart the ability of these cells to physically disseminate and seed metastasis. That discovery governs our research agenda to this day. We're interested in how activating the EMT program in a poorly invasive and poorly metastatic epithelial cancer cell can transform it into a powerful cancer-initiating cell.

What are the main roadblocks in the field right now?

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

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

What about President Obama's "Cancer MoonShot?"

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

Where are the most exciting advances?

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

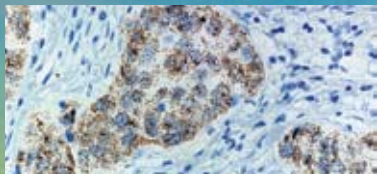
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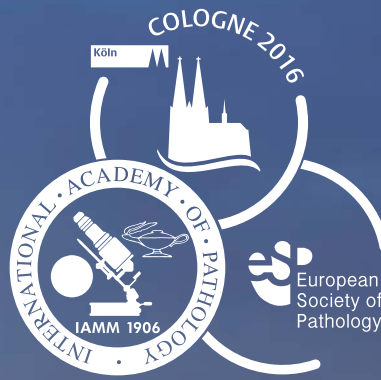
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