MARCH 2018 # 40

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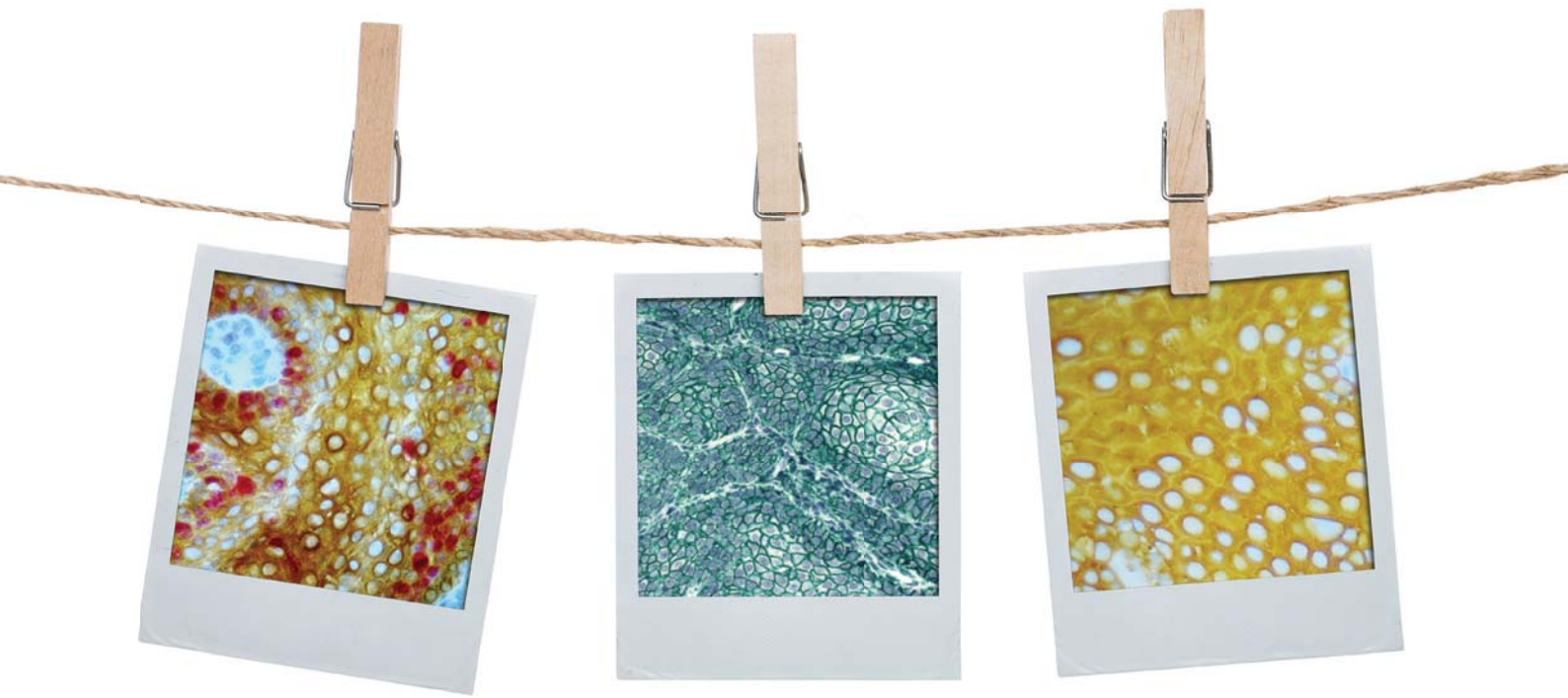

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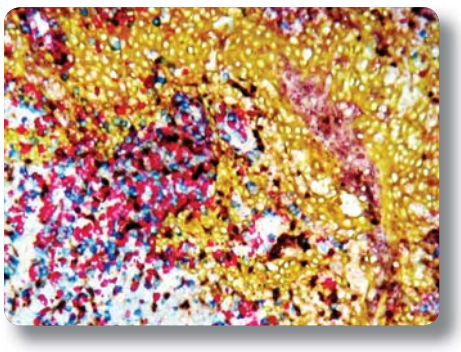

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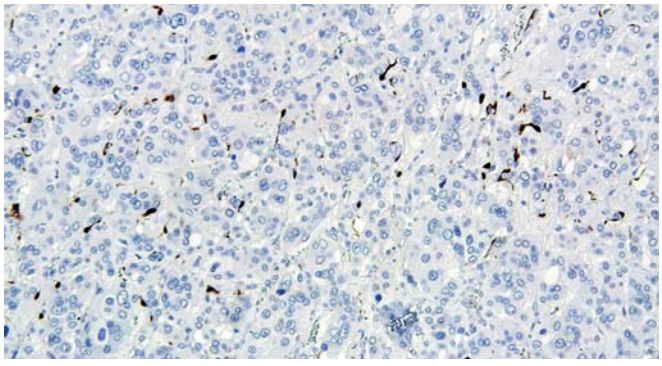
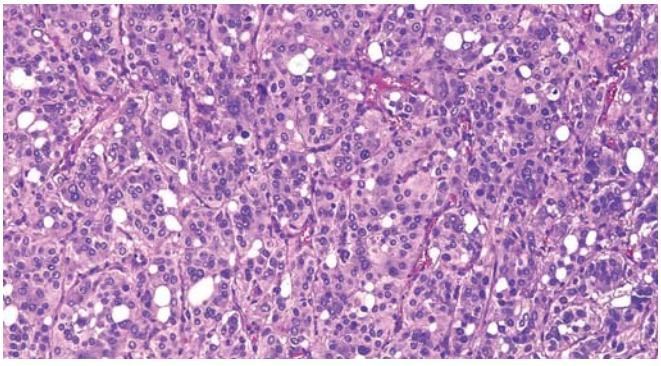
Case of the Month

This retroperitoneal tumor was removed from a 30-year-old man known to have a familial tumor syndrome. Another tumor of the same histologic type was found in the carotid body on the left side of the neck. Immunohistochemistry showed positive staining for synaptophysin, chromogranin and CD56. Antibody to S100 reacted with the slender support cells incompletely surrounding the groups of polygonal tumor cells (as shown in the photograph). Which germline gene is most often mutated in this familial tumor syndrome?

a. *Succinate dehydrogenase gene*
b. *Superoxide dismutase gene*
c. *Cytochrome oxidase gene*
d. *Tryptaminase gene*





Answer to last issue's Case of the Month…

B. Tuberculosis

On March 15, 1849, Thomas Addison made a presentation to the members of the South London Medical Society in which he described several men suffering from an unusual disease. Autopsies were performed on three of the patients; all were found to have a "diseased condition of the suprarenal capsules." Six years later, Addison published a short monograph entitled *On the Constitutional and Local Effects of Disease of the Supra-renal Capsules*. The figure presented in the previous Case of the Month was reproduced from that monograph; the drawing clearly shows the gross pathology of the enlarged and deformed adrenal glands. In Addison's time, adrenal insufficiency was almost invariably caused by tuberculosis.

Submitted by Ivan Damjanov, The University of Kansas School of Medicine, Kansas City, USA.

References

1. *PMF Bishop, "The history of the discovery of Addison's disease", Proc Roy Soc Med, 43, 35–42 (1950).*

To register your guess, please go to http://tp.txp.to/0318/case-of-the-month
We will reveal the answer in next month's issue!

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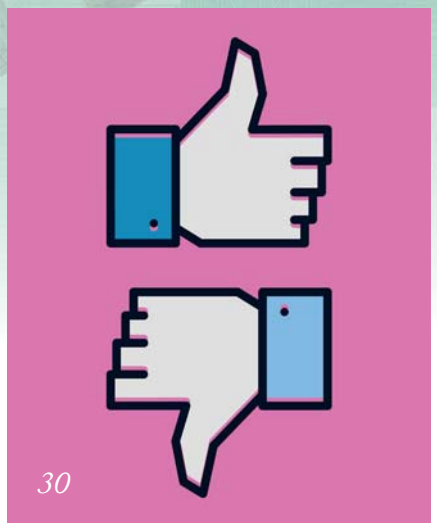
On The Cover

A game board symbolizing the hazardous path a sample must take from collection to laboratory testing.

Upfront

In My View



Feature

In Practice

NextGen

Profession

Sitting Down With

the **Pathologist**

ISSUE 40 – MARCH 2018

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+44 (0) 1565 745200
sales@texerepublishing.com

Distribution:
The Pathologist (ISSN 2514-4049), is published monthly by Texere Publishing, Haig House, Haig Road, Knutsford, Cheshire WA16 8DX, UK
Single copy sales £15 (plus postage, cost available on request info@texerepublishing.com)
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The Magic of Mystery

Why does a pathologist become a pathologist?

Recently, I was engaged in a rather hard-fought Nerf battle with the young son of a colleague. There were all sorts of rules – chief among them that I was never allowed to fire my gun, whereas it was apparently open season on me. At one point, his pneumatic weapon ran out of air pressure, and instead of launching themselves directly at my head, the darts made a half-hearted flop onto the floor between us.

"It didn't work," he said.

"No, because you ran out of pressure," I replied.

"Why does it do that?"

At six years old, I thought a full understanding of the physics involved might be a little beyond him – but for every piece of information I gave him, he had another question. "Why does the air make the dart fly?" "How does the pump make pressure?" "What's a molecule?" I was reminded that I knew from an early age that science was the right path for me – because there were always more questions than answers. Because the world was a mystery, and I wanted to solve it. Lofty goals for a small child – but, after all, isn't that what science and medicine are all about? And what greater mysteries are there to solve than those contained within our own flawed, fallible human bodies?

Shortly thereafter, I went on a tour of Great Ormond Street Hospital's diagnostic laboratories (prepare yourself for an epic cover feature next month). I had the privilege of meeting a group of professionals who are developing and refining a new approach to solving medical mysteries. Working on The Pathologist, I'm lucky enough to have the opportunity to find out about all kinds of new approaches – artificial intelligence, superpowered microscopes, integrated omics…

Even so, it's no less fascinating to explore tried and tested methods of investigation. Members of the Arkady M. Rywlin International Pathology Slide Seminar Club, for instance, conduct their investigations using exclusively glass slides, forgoing the ease of digital images. Last issue's "Case of the Month" focused on a medical mystery that dates all the way back to 1849. And with the rise of molecular diagnostics and other new technologies, it's more important than ever to remember the value of traditional histology and cytology in the laboratory.

We aim to fill each issue of The Pathologist with mysteries – both solved and unsolved; if you have a story (or slide) whose magic or enigmatic nature is worth sharing, please do get in touch (edit@thepathologist.com) – you never know what your colleagues might find interesting!

Michael Schubert
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?

Email: edit@thepathologist.com

Sub-Zero Substitute

MOFs could offer an alternative to freezing samples in low-income areas

In certain regions of the world, pathologists have limited (or no) ability to conduct analytical tests – but it's not always because of a lack of equipment or personnel; sometimes, it's down to the inability to implement a cold chain to transport samples. No matter how good the pathologist or technique, if a sample is not properly preserved, it may not be usable for testing – or worse, it may give false results (see our cover feature on page 18 to discover how worryingly widespread the problem is). A team of researchers from Washington University in St. Louis decided to tackle the gap in sample preservation, by enlisting the help of metal-organic frameworks (MOFs) (1).

"For the past few years, we have been working towards developing biodiagnostics for resource-limited settings," says Srikanth Singamaneni, Associate Professor in the university's School of Engineering. "As part of that effort, we have demonstrated the use of MOFs as protective encapsulants for preserving the functionality of antibodies conjugated to a biosensor surface. Following the successful completion of this work, we wondered if the technology could be used to protect protein biomarkers in the biospecimen, instead of antibodies on the sensor surface. And that led us to explore the use of MOFs for specimen preservation."

The team demonstrated their technique by encapsulating protein biomarkers in urine, blood, and plasma in a zeolitic imidazolate framework-8 (ZIF-8). When collected, samples need a MOF precursor added before being dried on filter paper. Recovering the protein for analysis simply involves dissociating ZIF-8 in a pH 6 buffer elution. Crucially, this final step doesn't affect protein analysis, meaning that workflows are minimally impacted.

The nanoporous MOF was able to preserve the proteins at both room temperature and 40°C in a comparable condition to samples frozen at -20°C. Singamaneni adds, "We have only explored proteins so far, but we would like to extend the technology to other biomarkers and test larger numbers of patient samples. We believe that the technique should be applicable to other biomarkers, such as nucleic acids and metabolites." He also notes that the reagents used are inexpensive and commercially available, meaning that the technique should be possible in even the most resource-limited areas.

Reference

1. *C Wang et al., "Metal-organic framework encapsulation for biospecimen preservation", Chem Mater, Chem Mater, 30, 1291–1300 (2018).*

Genotyping Guidance

AMP releases guidelines for cytochrome P450 allele testing

The Association for Molecular Pathology (AMP) has recently published recommendations outlining cytochrome P450 2C19 (*CYP2C19*) genotyping (1). *CYP2C19* is involved in the metabolism of many common drugs, which is why AMP's Pharmacogenetics (PGx) Working Group wanted to define key points for clinical testing. "The purpose of this guideline is to aid clinical lab professionals when designing and validating clinical *CYP2C19* genotyping assays, and to promote the standardization of testing across different laboratories," says Victoria Pratt, first author of the recommendations, Associate Professor of Medical and Molecular Genetics at Indiana University School of Medical, and AMP PGx Working Group Chair. "As with all of our AMP guidelines, we base our recommendations on the evidence that has been published at the time, and what the evidence indicates is necessary to improve professional pharmacogenetics practice and patient care."

How will the recommendations affect existing assay workflows? They outline a two-tier system to improve validity of *CYP2C19* assays, using criteria such as function, population frequency, and reference material availability. Tier 1 includes a minimum set of alleles that are recommended for *CYP2C19* PGx tests; tier 2 expands the list to incorporate optional additional alleles.

If your laboratory already performs these tests, you're likely to be compliant with the recommendations already. "Based on reviewing the commonly available platforms, as well as proficiency data, we believe that most laboratories are already testing for *CYP2C19*2, CYP2C19*3*, and *CYP2C19*17*," Pratt says. "Given that, we do not expect these recommendations to have a large effect on pathologists' workflows."

The new document contains recommendations rather than legal requirements, but AMP nevertheless strongly encourages laboratories to follow them. Pharmacogenetics is a rapidly changing field and the complex nature of clinical PGx testing and interpretation mean that we need standardization to deliver the best possible patient care. Pratt assures us that AMP is already on the case. "The AMP PGx Working Group will continually assess new evidence as it accumulates, consider new testing technologies as they emerge and update these recommendations as needed," she says.

The Long View

Intratumoral heterogeneity of the ER appears to double risk of fatal breast cancer for up to 25 years

A recent study suggests that the estrogen-receptor (ER)-positive subset of breast cancer may have deeper ramifications than previously suspected (1). "Women who develop ER-positive breast cancer have a remaining long-term risk of fatal disease for more than 20 years," says study author Linda Lindström, Assistant Professor and Group Leader in the Department of Biosciences and Nutrition at the Karolinska Institutet, Sweden. "We and other researchers have shown that the ER can change when a breast cancer tumor spreads, which affects survival – patients with high intratumoral heterogeneity of the ER were twice as likely to die up to 25 years after diagnosis as patients with low heterogeneity."

The clinical trial includes patients with low-risk, node-negative, ER-positive breast cancer and is independent of other known tumor markers. Lindström adds, "Patients with luminal A breast cancer and high intratumoral heterogeneity of the ER were also twice as likely to die of their disease." This finding could help to identify patients at a high long-term risk within the luminal A breast cancer subtype for which patients usually have a good prognosis. Though there is currently no definitive explanation of the underlying mechanisms, the researchers suggest that, given that ER-positive disease is associated with an increased long-term risk of fatal disease, having dormant tumor cells with varying tumor characteristics, as compared with more homogeneous characteristics, may be beneficial for tumor progression and influence patient survival.

If validated, the researchers believe their findings could become actionable in the near future. Lindström says, "We are currently conducting several studies to further understand the long-term risk of fatal breast cancer as determined by patient and tumor characteristics, and we're also continuing our studies on intratumoral heterogeneity in breast cancer."

Reference

1. *LS Lindström et al., "Intratumor heterogeneity of the estrogen receptor and the long-term risk of fatal breast cancer", J Natl Cancer Inst, [Epub ahead of print] (2018). PMID: 29361175.*

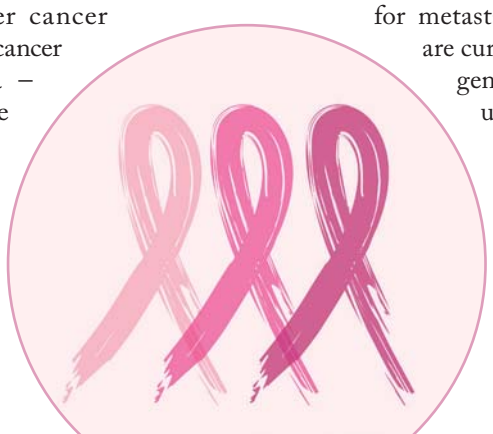
Delving into the DNA of TNBC

Researchers unveil more about the genetic alterations in triple-negative breast cancer

"Triple-negative breast cancer (TNBC) accounts for nearly 40 percent of all breast cancer deaths," says Daniel Stover, Assistant Professor of Internal Medicine in the Division of Oncology at Ohio State University Comprehensive Cancer Center. He adds that the disease is characterized by "relatively few mutations – many less than other cancer types like lung cancer or melanoma – but extensive copy number alterations."

Despite the significance of copy number variations to this challenging disease, relatively little is known about how somatic copy numbers affect metastatic TNBC, spurring Stover and a research team from his institution to further investigate the link (1). "Most TNBCs have over half their genome altered by somatic copy number alterations," Stover explains. "Understanding which alterations are more frequent in metastatic TNBCs relative to primary TNBCs may give us insight into the biology of aggressive TNBCs and potential therapeutic targets."

Their study investigated cell-free DNA (cfDNA) via liquid biopsy and found that specific somatic copy number alterations are enriched and offer prognostic value for metastatic TNBC. There are currently no prognostic genomic biomarkers in use for the disease, so the findings have important clinical implications for patients.



"We currently have two studies ongoing to validate these findings in a completely separate cohort, which is the first step," says Stover. "At the same time, we are working to transfer this assay into the clinical setting. Finally, we are designing a clinical trial for patients with metastatic TNBC through which we may direct therapy based on their cell-free DNA characteristics."

The researchers are also pursuing additional cfDNA sequencing techniques, including whole exome and whole genome sequencing in an effort to give greater insight into specific mutations that may provide evidence of specific resistance mechanisms or therapeutic targets. Stover adds, "We are also investigating cfDNA tumor fraction change as an early biomarker of response to therapy. We are determined to push the understanding of metastatic TNBC."

Reference

1. *DG Stover et al., "Association of cell-free DNA tumor fraction and somatic number alterations with survival in metastatic triple-negative breast cancer", J Clin Oncol, 36, 543-553 (2018). PMID: 29298117.*

Results You Can Count On

Is the number of metastatic lymph nodes in oral cancers a key prognostic indicator?

Allen Ho, Director of the Head and Neck Cancer Program at Cedars-Sinai, believes we have a staging problem when it comes to oral cancers: "Current staging does not distinguish between multiplicity of metastatic lymph nodes. In other words, staging did not distinguish between a patient having two or 20 metastatic lymph nodes." But, according to Ho, such knowledge is vital. After he and his colleagues noticed that patients with a higher number of metastatic lymph nodes seemed to have poorer outcomes, they applied statistical models to data from over 14,000 patients from cancer databases spanning almost a decade in the US. Those models showed that mortality risk increased with the number of metastatic lymph nodes – with no upper limit to risk (1). "We found that, in fact, there is a near stepwise escalation in mortality risk with each additional metastatic lymph node found," says Ho.

"This is a constantly evolving field with many factors that can impact a patient's prognosis. However, based on this nationwide data, the magnitude of lymph node burden is so high that it's difficult to ignore. It is an overlooked component of our staging system," says Ho. Notably, the research also found that many other features thought to be important in staging cancer are relatively insignificant when compared with the impact of including the number of metastatic lymph nodes – and that the association between metastatic lymph node burden and mortality remains largely intact even after adjusting for those other features.

In response to this discovery, Ho and his lab at Cedars-Sinai developed guidelines that may help predict more accurate survival odds for patients with oral cavity cancers. The researchers proposed a tumor staging template for oral cavity cancers that includes the number of metastatic lymph nodes, along with other factors. "We hope our results will help streamline the current staging system and better reflect the impact of quantitative metastatic lymph node burden."

The researchers are now further investigating the implications of their work. "There are numerous implications for metastatic lymph node burden," says Ho. "We are studying whether patients with high numbers of metastatic lymph nodes may benefit from treatment intensification – for instance, whether adding chemotherapy is appropriate and helps improve survival. Biologically, we also wonder why some patients metastasize quickly, whereas others grow large primary tumors without any metastatic lymph nodes."

Reference

1. *AS Ho at al., "Metastatic lymph node burden and survival in oral cavity cancer", J Clin Oncol, 35, 3601–3609 (2017). PMID: 28880746.*

Anticipating *NRAS* Resistance

How researchers are unraveling the mystery of melanoma drug resistance

Every medical treatment is a tradeoff between benefit and side effect – and part of any physician's job is to determine whether or not the downsides to a given treatment are a worthwhile price to pay for its potential health benefits. One factor in the therapeutic equation is resistance: what good is a treatment if the patient's disease can resist its effects?

Establishing whether or not a disease exhibits resistance mechanisms is especially important when it involves patients whose illnesses could be fatal if left untreated. A team at The University of Texas MD Anderson Cancer Center, led by Lawrence Kwong, decided to tackle this issue in patients with *NRAS* melanoma. To learn more, we spoke with Lawrence Kwong, Assistant Professor in the Department of Translational Molecular Pathology at MD Anderson.

Why focus on *NRAS* melanoma?

NRAS melanoma, which represents about one quarter of melanomas, currently has no approved therapies specifically for it, unlike *BRAF* melanomas. Even with the recent success of immune checkpoint blockade therapies such as anti-PD1 and anti-CTLA4, at least half of *NRAS* patients who receive such treatments will eventually relapse and require other therapeutic strategies.

My previous work indicated that MEK plus CDK4/6 inhibitors would be efficacious for *NRAS* melanoma, and early clinical trial results show promise. Our current study anticipates that some *NRAS* melanomas may exhibit resistance to this therapy, but also potentially informs other clinical trials of the same therapy in *RAS*-mutant lung, pancreatic, and colon cancer.

What does your research mean for patients?

Our results mean that, even before going in for therapy, a cancer patient might already harbor a small percentage of cells that will eventually cause drug resistance. However, current clinical tests might miss these – either because the test resolution is too low, or because they typically look at only one part of the tumor, meaning that they might miss resistant cells hiding in another part. Fortunately, we already know many of the common resistance-causing melanoma mutations for both targeted therapies (such *as BRAF* and MEK inhibitors) and immune therapies (such as anti-PD1 and anti-CTLA4), meaning that we at least know what to look for. If we can find out beforehand whether a patient already harbors resistant cells, we could possibly devise a course of therapy to pre-empt that resistance – when and if

we can prove that to be safe and effective. In the meantime, our study lets other researchers know how important it is to use high-resolution sequencing assays and to test multiple regions of a tumor to find such rare, pre-existing mutations. Building up a body of data on these will be critical to moving it into the clinic.

What's next for your work?

Our lab will continue to look at the large store of tumor samples available at MD Anderson across different cancer types and drug treatments to understand how resistance occurs over time. We are also creating a model in Petri dishes to figure out how cell populations evolve over long stretches of drug treatment, as a way to understand which cancer cell types are most relevant to the clinical setting. For example, other labs have shown that melanoma cells that express a protein called AXL show intrinsic tolerance to a wide array of drugs. However, it is unclear exactly how this cell type contributes to full-blown resistance, as many drug-resistant melanomas do not show high AXL expression. Some have suggested that these cells might provide temporary drug resistance before more permanent resistance mechanisms are acquired; others that they are relatively unimportant in generating permanent resistance compared with pre-existing drug-resistant cells. We are planning experiments to specifically test these different hypotheses; it's possible that both are correct in different contexts.

What more can be done?

I once took a tour of the pathology room at MD Anderson and was amazed by the volume, efficiency, and quality of the work being done. I also saw that large chunks of tumors were being discarded, which was clearly a practical necessity. Looking back on that, in light of the current study, it would be wonderful if researchers and clinicians communicated more often; that might lead to specific instances in which extra tumor pieces could be saved for multi-region analysis. I am personally striving to make those connections more often, and I would encourage anyone reading this on either side to reach across as well.

Reference

1. *G Romano et al., "A pre-existing rare PIK3CAE545K subpopulation confers clinical resistance to MEK plus CDK4/6 inhibition in NRAS melanoma and is dependent on S6K1 signaling", Cancer Discov, [Epub ahead of print] (2018). PMID: 29496665.*

In My View

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

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

Contact the editor at edit@thepathologist.com

An Alternative Image of Digital Pathology

The impact of digital pathology on immuno-oncology – and vice versa

By Mike Montalto, President of the Digital Pathology Association and Executive Director of Pathology & Clinical Biomarker Laboratories, Translational Medicine, Bristol-Myers Squibb, New York, USA.

In the previous two issues of The Pathologist, we learned about the exciting promise of digital pathology – as well as some of the challenges that exist for use in mainstream clinical practice. I share this cautious excitement; I believe the combination of digital imaging and deep learning will transform pathology. However, many of us who have been working in the trenches of this technology for a decade or more can attest that change has been slow. Indeed, we have predicted a transformation more often than we have reported on its realization. But something is happening today that places digital pathology in a position of relevance more than ever before – and I am more optimistic than ever before!

As many of us know, the field of immuno-oncology (I-O) is exploding dramatically. Cancer immunotherapy's quick clinical success has led to more than 2,000 I-O agents in some stage of development, according to a recent study from the Cancer Research Institute. This success brought with it the realization that not all patients respond to immunotherapies in the same way, leading us to a new era of I-O treatment that goes beyond a one-size-fits-all approach.

The path to precision medicine for I-O requires tremendous insight into the tumor microenvironment, necessitating efficient and accurate ways to navigate complex information, so that we may see the complete biological story. Digital pathology enables us to interrogate the complex interplay between immune cells, tumor cells, and surrounding stromal components in situ. Artificial intelligence advancements and quantitative image analysis-based multiplexing give cancer researchers a composite, detailed view of tumor tissue, whereas deep learning generates the quantifiable data and specificity needed to precisely differentiate cell types in the tumor microenvironment and find predictive insights into treatment response. Whether applied to standard hematoxylin and eosin or immuno-stained samples, these rapidly developing technologies are crucial for I-O research, helping to guide our approach to rational combinations, patient selection, and clinical trial design.

"I believe the combination of digital imaging and deep learning will transform pathology."

Just as digital pathology has undoubtedly transformed the I-O research landscape by giving researchers unprecedented insight into cancer biology, I also think the field of I-O will radically change our view of the utility of digital pathology. The perspective that digital pathology contributes to enhanced workflows and more efficient and accurate diagnosis – although true – is limited in the scope of clinical impact. We need to expand the concept of digital pathology toward a means of selecting patients for appropriate I-O drugs that are only now emerging from drug company pipelines. The technology could follow an adoption paradigm similar to next generation sequencing, which is rapidly emerging as the next platform for clinically relevant biomarkers and companion diagnostics. Within the context of quantitation of complex interactions in the tumor microenvironment, digital pathology can – and should easily – follow a similar path to clinical utility.

As digital innovators, it is our shared responsibility to ensure a seamless transition to this inevitable future by training the next generation of pathologists, developing tools and protocols, and – most importantly – generating data that demonstrates the tremendous value digital pathology brings to cancer treatment for all involved… especially the patient. Armed with new perspectives from digital pathology, a more personalized approach to I-O is within sight.

The opinions expressed here are those of Mike Montalto in his personal capacity and not those of Bristol–Myers Squibb.

Managed Equipment Services: The Kenyan Story

Good intentions aren't all that's needed for a successful medical program

By Mercy Korir, Medical Doctor and Medical Journalist at KTN News, Kenya.

In 2015, the Kenyan government launched an ambitious program to upgrade hospitals by providing what they termed "specialized, modern, and state-of-the-art medical equipment" through a managed equipment services scheme. They claimed that the program's goal was to increase access to specialized health services countrywide, consequently improving the quality of health care as well as decongesting the main referral hospitals. The reality, though, was slightly different. Much of the equipment that arrived – surgical instruments, trolleys and radiology equipment – was pretty basic, and many medical professionals failed to recognize the promise of "state-of-the-art". With a seven-year lease period, many asked, "Are these tools really worth it?"

As it stands, this equipment is yet to be used as it was envisioned. Although some facilities earmarked for this equipment have made the effort to install and use it, many devices remain undelivered due to various challenges including the actual infrastructure to house them and the personnel to operate them. Getting information from the Kenyan Ministry of Health on the evaluated status of the equipment is akin to pulling teeth.

The government committed US$450 million of taxpayers' money to the project, touted as a flagship of the then-new administration. In addition to operating theater equipment, other tools on the scheme included sterilization stations, dialysis devices, intensive care machines, and radiology equipment – all under a public-private partnership model with various suppliers. Two hospitals in each of Kenya's 47 counties were selected to benefit from the program, as well as four of the country's national referral hospitals.

Theoretically, the project should have helped a developing country with an ailing healthcare system claw their way out of a possible collapse. In practice, though, the flaws in the scheme outweighed its benefits.

"The high cost and lack of planning make the scheme a poor investment of taxpayers' money."

At the time, Kenya had just started implementing a new constitution in which the 47 counties existed as devolved governments responsible for the bulk of Kenyan healthcare. Unfortunately, the counties were not consulted nor their needs analyzed prior to procuring the equipment. Instead, a one-size-fits-all approach was

used in designing and implementing the scheme. But even though the counties were not asked for opinions, the county governments were still expected to fund the scheme – to the tune of approximately US$1 million per county, per year. And the funding was expected to begin in the 2015/2016 financial year – before there was even any sign that the counties had the capacity to support the program!

Worse still, the criteria for selecting the facilities to be supplied with equipment were shrouded in mystery. One particularly egregious example is a county whose facility was only partially constructed when the scheme was proposed – and, although the facility was still incomplete at the end of 2017, taxpayers had already begun paying heavily for equipment that couldn't even be provided.

Kenya's physician-to-population ratio is about 1:4,000, and the nurse-to-population ratio is even worse. The introduction of the new scheme, without accounting for the personnel needed to support it, meant that the equipment was bound to lie idle in facilities. The government also hasn't factored in the time it takes to fully train healthcare personnel – we cannot rush through training in the timeframe the government has promised and then be expected to offer the quality our patients need and deserve.

Herein lies the third flaw: misinformation. Many Kenyans were led to believe that the equipment supplied is the answer to all their health problems, including cancer treatment. Cancer, to many Kenyans, is either a death sentence or a guarantee of perpetual poverty. It's understandable, then, that the new equipment scheme raised people's hopes – but the truth is that there was never any form of cancer treatment in the package. The closest thing included in the program is a mammogram (part of the radiology package) – but what use is a mammogram with no radiologists, pathologists, oncologists, breast surgeons, or support staff? Who's going to make the diagnosis or treat the patient?

Although the government's intentions in procuring such equipment to help ease the burden of care may have been right, my opinion is that the high cost and lack of planning make the scheme a poor investment of taxpayers' money.

This should be taken as a serious "what not to do" lesson in the healthcare sector for any country, particularly other developing countries struggling to provide essential services to their citizens. The Kenyan example should demonstrate the importance of planning and doing a needs assessment prior to any "mega-project," tenets that should not be rocket science for any serious government.

The Truth About Personal Genetic Tests

Is direct-to-consumer testing anywhere near as useful as it appears to the public – or to science?

By Suneel Deepak Kamath, Hematology/Oncology Fellow at Northwestern Memorial Hospital, Chicago, USA.

Direct-to-consumer genetic tests like 23andMe have evolved substantially in the last decade, faster than society's ability to comprehend their medical, scientific, and ethical implications.

The path for 23andMe has been a rocky, convoluted one as it initially struggled to balance its business interests with regulatory requirements. The company started with a much larger 250+ gene assay that, in addition to testing for genetic ancestry and lighthearted traits, such as eye color or the ability to smell asparagus, tested for *BRCA* genes and genes associated with alcoholism, obesity, Alzheimer's and Parkinson's. In November 2013, 23andMe was temporarily shut down by the FDA for failing to prove its assays were accurate and reliable despite numerous requests. It was a harsh but necessary move by the FDA. As any physician knows, the first questions about any assay are: how reliable it is? What are the positive and negative predictive values? Is the result clinically meaningful?

"My greatest concern is that a negative result on a home test might dissuade women from obtaining appropriate breast cancer screening."

The 23andMe health testing kits were reincarnated in October 2015 with a much smaller but better validated group of tests for personal genetic health risk and carrier status, with varying clinical utility. The fun trait tests for the alcohol flush reaction or sneezing with sunlight exposure are largely the same and remain good office water cooler talk but have limited health or practical value. The carrier tests include assays for sickle cell anemia, thalassemias, and cystic fibrosis, which could be useful depending on one's ethnicity and family history.

"The future of home personal genetic testing is filled with both peril and promise."

The personal genetic health risk tests are largely of questionable clinical value. Three conditions tested for, Alzheimer's disease (*APOε4*), Parksinon's disease (*LRRK2* and *GBA*), and age-related macular degeneration (*CFH* and *ARMS2*) are 100 percent nonpreventable in an asymptomatic person without these diseases. Though there are methods to slow their progression once the disease is established, these interventions do not prevent the disease from occurring in the first place. Thus, knowing your risk sooner won't help you prevent the disease and could be needlessly distressing. Despite common thinking, it can hurt to know more. Similarly, the hereditary thrombophilia tests (Factor V Leiden and prothrombin G20210A test) are of little value in someone with no personal or family history of thrombosis. The remaining three diseases tested for, celiac disease (*HLA-DQ A1* and *HLA-DQ B1*), alpha-1 antitrypsin deficiency (*SERPINA1*) and hereditary hemochromatosis (*HFE*) have potential to be clinically actionable results. How often these tests detect a disease that would otherwise have been missed or detected later remains to be seen. The pervasive assumption that early diagnosis is always better isn't always true. For example, many patients with hereditary hemochromatosis don't yet have iron overload and don't benefit from early detection.

Earlier this month, the FDA authorized 23andMe to report *BRCA* mutations to its consumers for the first time. Given the high profile of breast cancer and *BRCA* mutations in the media and among the general public, I expect 23andMe kits to fly off the figurative digital shelves as a result. However, a deeper look reveals that the two *BRCA1* and one *BRCA2* variants tested occur most commonly in the small Ashkenazi Jewish population and are otherwise uncommon. How useful will these tests be for the general population? My greatest concern is that a negative result on a home test might dissuade women from obtaining appropriate breast cancer screening. Additionally, will women with positive results have access to affordable genetic counseling to make sense of their results? It is clear that 23andMe will profit from more consumers buying their kits to get their *BRCA* results – but some (perhaps even most) consumers may not benefit from this small, three-gene panel. A broader panel for a larger number of *BRCA* variants, on the other hand, could be instrumental in breast cancer prevention.

The future of home personal genetic testing is filled with both peril and promise. The danger lies in how much genetic data companies store and sell access to other organizations. Indeed, 23andMe shares their data with several universities, including Harvard and Stanford, companies like Pfizer and Genentech, and several Parkinson's disease nonprofits. The reports shared with consumers are a mere fraction of genetic data generated and shared with these outside organizations. If health or life insurance companies obtained the same information, it could have catastrophic financial consequences for consumers with genetic predispositions for serious or costly illnesses. Employers could also discriminate against certain job applicants based on genetic data. Government regulations currently prevent these problems, but hacking or legal maneuvering around these regulations and the informed consent process could put powerful genetic data in the wrong hands. Conversely (and much more positively), the large repository of genetic data could lead researchers to some amazing discoveries. Traditional research involves identifying patients with a disease and retrospectively looking for genetic causes of that disease. A large, population-level genetic database could help us prospectively identify subpopulations at high genetic risk for serious diseases. Pharmacogenomic data could also help us individualize drug choices and dosing to maximize efficacy and minimize toxicity.

The All of Us research program through the NIH aims to compile a large genetic database similar to that of 23andMe, but as a nonprofit, academic endeavor. Will it protect participants' genetic privacy better than for-profit companies like 23andMe?

For now, personal genetic tests are mostly cute technological novelties with limited health value. Whether they will lead to medical breakthroughs, catastrophic breaches of privacy – or both – remains to be seen.

GARBAGE IN, GARBAGE OUT

The hidden reason laboratory test results may not be as reliable as they seem

By Carolyn Compton

Error. It's a subject no physician wants to think about, especially when it comes to their own practice. As professionals sworn to safeguard the lives and health of patients, we know that any incorrect or spurious result can impact our ability to do as we have promised. And yet errors still occur. Research is still irreproducible; clinical tests still show false positives and false negatives; results still sometimes make no sense at all. Why? In the medical laboratory, at least, the problems may not be integral to the test itself – rather, they may arise from the way a sample was treated before it ever underwent testing: the preanalytical phase.

WHAT IS PREANALYTICAL ERROR?

In our role as pathologists, we perform analytical tests on patient specimens to make diagnoses. The testing process is often separated into three familiar phases: preanalytical, analytical, and post-analytical (also known as the interpretative or consultative phase)

Preanalytical	*Analytical*	*Post-analytical*
The preanalytical phase includes any actions or factors involved in acquiring, handling, transporting, and processing a patient specimen prior to the actual analysis.	The analytical phase includes all factors related to the test platform and to the testing process itself.	The post-analytical phase refers to the interpretation of the test results in light of our expertise as physicians to formulate a diagnosis (or differential diagnosis) to guide patient management.

Much of our expertise as pathologists lies in performing and interpreting diagnostic tests – but that isn't all we do. We are also consultants – and the value of our consultative advice is dependent on the value and reliability of the test results we generate. We strive for precision and validity in all of our analyses so that the data we generate reflects the true biological state of the patient. It has been estimated that data from the pathology laboratory comprises as much as 80 percent of the objective, quantitative disease information that exists in a patient's medical record – and much of this data directly guides patient management. This leaves little room for error. Flawed results mean flawed medical decision-making. In short, an incorrect answer from even a single test can have serious consequences for a patient.

preanalytical error
ˌ ˈpriːænəˈlɪtɪkəl ˈɛrə
noun
Preanalytical errors are errors in test results that occur as a consequence of actions or events preceding the test or analysis itself.

Some preanalytical errors – specimen mislabeling, for example – are clerical; others are related to factors that compromise the quality of the specimen and may reduce or even destroy its suitability for certain types of testing. In other words, a particular test could be highly specific and sensitive, but would yield a spurious result if the analytes in the specimen of interest were artifactually altered or corrupted. For example, one research group has shown that a delay in time to stabilization (also known as "cold ischemia time") can artifactually render a HER2-positive breast cancer specimen negative on Herceptest® analysis (1–3). When the result of a companion diagnostic test such as Herceptest® functions as a gateway to targeted therapy, artifactually induced false negative test results could incorrectly rule out treatment with a potentially life-saving drug – a devastating consequence.

QUALITY BEGETS QUALITY

In this era of "precision medicine," diagnosis, prognosis, prediction, and treatment are often based on the molecular characteristics of the patient and on the molecular features of the disease. These characteristics are typically determined directly from the analysis of representative biospecimens – which means that, if we want to generate high-quality molecular analysis data, we need high-quality specimens. In fact, the increased power of modern molecular analysis technologies has raised the bar for the molecular quality of patient specimens; the better our testing methods get, the better our sampling methods must be to keep up. No matter how dazzling new analytical technologies may be, the "garbage in, garbage out" paradigm still applies to the data they produce. No technology can spin straw into gold!

Preanalytical issues are central to specimen integrity and molecular quality. The myriad steps involved in acquisition, handling, processing, transportation, and storage can have profound effects on both the composition and quality of different molecular species in patient biospecimens. Safeguarding their molecular integrity in the preanalytical period is an immediate challenge; it can't be delayed or disregarded. Once compromised, a specimen's molecular quality cannot be retrieved.

The molecular quality of a specimen at the time of fixation, when its biological activity is stopped, determines its fitness for testing. After that, if the specimen is well-preserved and carefully stored, its quality may remain essentially unchanged; otherwise, it will only further diminish as the specimen degrades over time. Therefore, preanalytical factors that directly impact a specimen's molecular integrity can unfortunately have an adverse effect on both real-time patient management and future decisions based on reanalysis of the same specimen.

"IF WE WANT TO GENERATE HIGH-QUALITY MOLECULAR ANALYSIS DATA, WE NEED HIGH-QUALITY SPECIMENS."

Additionally, if the patient enters a clinical trial and their specimens are used for correlative scientific studies or discovery research, the downstream consequences of bad data and irreproducible study results can be profound. We are just beginning to appreciate the fact that a huge amount – more than half, in fact (4) – of published biomedical data cannot be reproduced. No one has yet looked closely at the degree to which poor or unknown patient specimen quality may contribute to this problem. I suspect that, when we do, it will be significant.

A MATTER OF STANDARDS

Why are there currently no established or enforced standards around preanalytics? It's a difficult question – with a complicated, multifactorial answer.

First, I see a lack of awareness and a need for education about preanalytics throughout the medical community. Pathologists, surgeons, and every other professional who is part of the specimen chain of custody (radiologists, pathology assistants, nurses, phlebotomists, medical technologists and much more) need to be educated about preanalytics. It's vital that they all understand the role they play as links in an unbreakable quality chain.

Second, there is a dearth of biospecimen science data upon which to build evidence-based procedures for preanalytics that affect precision medicine. This kind of information is focused on the specimen itself and how it is affected by different preanalytical factors, alone or in combination. It's the data that everyone wants – but no one wants to pay for! We need much more biospecimen science to fully understand the impact of different preanalytical factors on different biomolecular specimens of different sample types. Furthermore, specific analytical platforms may have different requirements for analyte molecular quality – something else that I fear may often be overlooked. These data are foundational for precision medicine, and yet, at the moment, they are sadly lacking.

Third, old practice habits are hard to break. Legacy systems in pathology departments – and medical institutions in general – may be difficult to redesign to accommodate changes in preanalytical workflows. By and large, we are still handling patient specimens the same way we have for decades, with no sign of change on the way. In addition, patient specimen preanalytics cross many professional domains, and there are no cross-cutting standards to assure that key preanalytical steps are controlled and documented in an end-to-end fashion. In pathology, there are no enforced standards at all, with the possible exception of the ASCO-CAP guidelines for HER2 testing of breast cancer specimens (5) – one tiny candle in the dark. For all other specimens, there are no enforced requirements to either control or record preanalytical factors. Many authoritative guidelines exist, but they are voluntary; none are tied to accreditation or commendation and, unfortunately, that means they may often go unheeded.

Fourth, there is no specific reimbursement for the professional time, expertise and effort required to address preanalytics in real time – as they should be. This issue must be addressed to assure compliance with preanalytical standards across the board. People typically do what they are paid to do, even if they don't fully understand the scientific reasons behind the mandates.

Fifth and finally, there are still many who discount the importance of preanalytics, which I find very hard to comprehend. Worse still, they may discount the importance of specimen quality or reject the premise of "garbage in, garbage out" altogether! There are those who believe that, through the wonders of technology and data science, data quantity can overcome the challenges of poor data quality. In my opinion, this kind of thinking is unrealistic and unacceptable – even potentially dangerous – at the level of the individual patient. I would argue that it is misplaced at the population data level as well. If precision is truly the goal, there is no conceivable situation in which preanalytical variation is truly unimportant and can be confidently disregarded – and thinking so can only lead to disaster.

> **"IF PRECISION IS TRULY THE GOAL, THERE IS NO CONCEIVABLE SITUATION IN WHICH PREANALYTICAL VARIATION CAN BE CONFIDENTLY DISREGARDED."**

SOURCES OF ERROR

In a December 2014 think tank sponsored by the National Biomarker Development Alliance (NBDA), my private and public sector colleagues and I established a "top 10" list of key contributors to preanalytical error. It's actually the top five preanalytical steps that lead to nucleic acid or protein testing problems (the most common analyses in precision medicine) for tissue specimens and the top five for blood samples. The Pareto principle states that, for many events, about 80 percent of effects follow from only about 20 percent of causes. The College of American Pathologists' Preanalytics for Precision Medicine Project Team (PPMPT), which I lead, further refined and validated the concept by reviewing the published scientific literature. The team defined two "top five" lists – for molecular analysis of tissue and blood biospecimens, respectively – representing the 20 percent of all factors (inputs) that cause 80 percent of all of the problems on output.

For tissues, the top five sources of error are:

1. Cold ischemia time
2. Method of processing (section thickness, temperature, fixative volume to tissue mass ratio)
3. Type and quality of fixative
4. Total time in formalin
5. Storage conditions

For blood and serum specimens, the top five are:

1. Time to processing
2. Method of draw (draw order, tube type, tube fill volume)
3. Method of stabilization (tube inversions)
4. Method of processing (centrifugation speed, centrifugation time, temperature)
5. Storage conditions

Every one of these factors can have innumerable variations in routine practice in different practice settings, or even from day to day in the same practice setting. In other words, each is variably variable! And because there is no requirement to document any of these things on a specimen-by-specimen basis, these preanalytical factors are unknown for any given patient specimen. As a consequence, the molecular laboratory – and the person who actually performs molecular analyses – has no way of knowing whether or not a given specimen is fit for purpose and will yield reliable results. This, of course, means that the veracity of the readouts from the test platforms are also unknown – and yet, because they're all we have, we report them anyway.

"WE NEED TO CHANGE STANDARD OPERATING PROCEDURES IN EVERY LABORATORY SO THAT PREANALYTICAL DATA ARE A PART OF EACH SPECIMEN'S PERMANENT RECORD."

Our challenge for precision medicine is to decrease, as much as possible, the variation in the "top 10" factors by following recommendations founded on the current state of biospecimen science. In addition, the actual performance metrics related to the top 10 must be documented in daily practice – or, at the very least, every deviation from the recommended guidelines must be recorded. Otherwise, how can we know the provenance of a patient specimen? We need to change standard operating procedures in every laboratory so that preanalytical data are a part of each specimen's permanent record.

SMALL CHANGES, BIG RETURNS

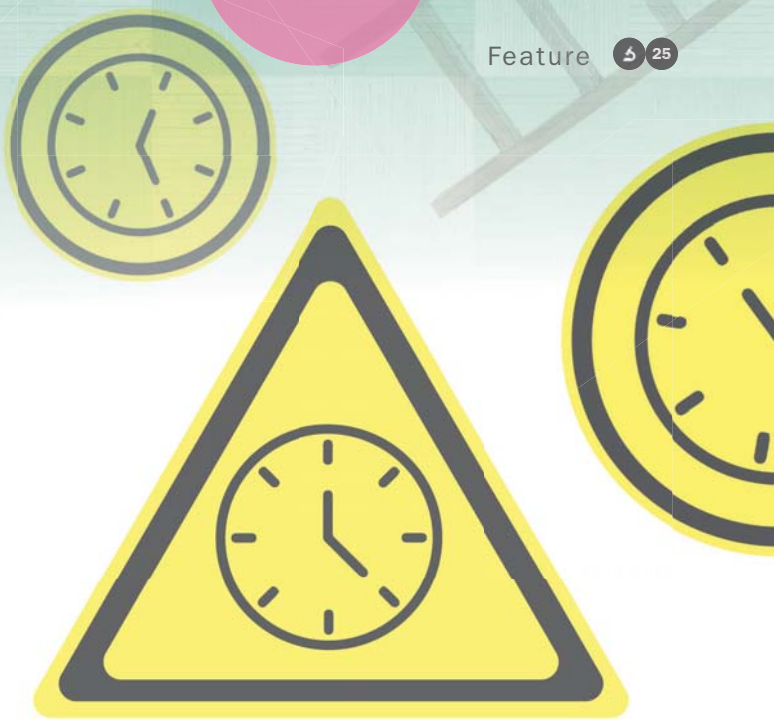
Based on the independent review the PPMPT has conducted over the past two years of the scientific literature related to tissue and blood preanalytics, the team has made five recommendations for each sample type.

For tissues, the areas where new approaches can deliver the greatest value are:

Area of concern	*Recommendation*
Time to stabilization	60 minutes or less
Method of processing	Section thickness ≤5 mm Volume/mass ratio ≥4:1 (optimal ≥10:1) Transport temperature: room temperature (20–25˚C)
Method of stabilization	Type of fixative: 10% neutral phosphate-buffered formalin (pH tested daily) Optimal time in fixative: 6–24 hours (includes time in formalin in processor); a maximum of 36 hours may be acceptable or even required for fatty tissues like breast
Tissue processor variables	Maintenance schedule: manufacturer's recommendation or a validated deviation Paraffin type: low melt <60˚C Total time in processor: 7.5–8 hours (forbid nonstandard practices such as "topping off" with nonstandard solutions)
Storage conditions	Room temperature (20–25˚C) Dry conditions

For blood, the areas of greatest value are:

Area of concern	*Recommendation*
Time to first processing step	60 minutes or less
Specimen acquisition	Tube type: • if processing time >2–3 hours, use acid-citrate-dextrose (ACD) tube • for proteomics studies, use EDTA • for coagulation studies, use sodium citrate • do not use lithium heparin for nucleic acid amplification studies Volume of tube fill: manufacturer's recommendation (if less than specified for tubes with additives, document variance) Draw order: • culture bottles • light blue (citrate) • gold (gel, serum) • red (no gel, serum) • green or tan (heparin) • lavender or tan (EDTA) • royal blue (EDTA) • grey (sodium fluoride) • tubes with other additives
Method of stabilization	Tube inversions: manufacturer's recommendation
Method of processing	Centrifugation speed and time: variable, depending on validated protocol and biomolecule of interest Temperature: room temperature, unless validated protocol dictates otherwise
Storage conditions	Freeze-thaw cycles: ≤ 1 for nucleic acids and proteins (use aliquots)



At the moment, quality assurance is close to completely absent from the preanalytical phase. Now that we've set out some recommendations and guidelines, our next step is to implement our generalized, five-point action plan to ameliorate preanalytical variability (see "Time to Act"). It's our hope that, by making recommendations and devising ways to achieve them, we can begin the process of establishing a quality assurance ecosystem.

WHERE YOU COME IN

Individual pathologists are the key to success. If all politics are local, then all preanalytics are even more so. Pathologists can start by assessing what they themselves are currently doing in their own practice settings and what it would take to implement the "top 10" practice metrics. They will undoubtedly need to educate their administrators as to the importance of this upfront "investment in patient specimen quality" and how it will impact the quality of molecular testing data and – most importantly – the clinical decisions based on those data.

Individual pathologists can also educate and work with colleagues in their own and other departments to achieve total quality management from patient to lab test. They can educate their trainees and students and work toward making preanalytics education and training an integral part of residency and fellowship in pathology. Even industry partners can help – by filling in gaps in funding, or by developing tools and technologies that can automate or expedite this effort in application in everyday practice.

A BETTER BIOMARKER

The future of medicine depends on the development of molecular biomarkers. They can provide more precise diagnosis and patient stratification; detect early disease; elucidate risk of disease; predict disease outcome, response to therapy, and therapeutic toxicities; and permit monitoring of therapeutic management. Unfortunately, despite its importance, biomarker development has historically been fraught with failure. The majority of biomedical discovery research has proven irreproducible or invalid, and very few qualified biomarkers have been produced in the last decade. Failures in biomarker science have translated into failed clinical trials and, ultimately, the inability of biomedicine to deliver on the emerging promise of precision medicine.

Rigorous adherence to standards that are consistent, and consistently applied across the development process, is required to achieve the reproducibility we currently lack. Of primary importance, therefore, is the quality of the starting materials – the biospecimens used for analysis. Development of complex biomarker approaches represents an even higher bar. Preanalytical artifacts may abrogate any ability to define biological effects of

TIME TO ACT

The five objectives of our generalized action plan to ameliorate preanalytical variability are:

1. Verify the "Top 10" preanalytics from the published literature and translate these into practice metrics for pathologists – and then, of course, publish our findings.
2. Propose accreditation checklist questions to CAP's Laboratory Accreditation Program with the goal of enforcing the Top 10 through the College's laboratory accreditation process.
3. Educate pathologists about the Top 10 list, its scientific basis, and the practice metrics that need to be met to control and record them.
4. Educate other professional groups – such as surgeons, nurses, pathology assistants and other healthcare professionals – about patient specimen preanalytics. Assist them, individually as needed, in developing their own practice guidelines to assure specimen quality and in helping to orchestrate overall concordance among practice guidelines throughout the biospecimen chain of custody, from patient to analysis.
5. Seek financial support from payors and professional support from regulators and funders to implement and sustain the practices that control – and the infrastructure to document – patient specimen molecular quality for precision medicine and translational research.

interest or distinguish biological signatures of importance in patient samples. This problem is especially consequential when the biomarker assay is a companion diagnostic and the gateway to access to a therapy. Neither a false positive nor a false negative biomarker test is tolerable in that circumstance.

Regulatory approval of new biomarker assays is now also focused on specimen quality as it relates to the quality of the data on which approvals are based. The biomarker qualification programs of the US Food and Drug Association and the European Medicines Agency emphasize the need to document the biospecimen quality of diagnostic biomarkers used for either drug or device (assay) development. It is imperative that the entire biomedical community address the need for standardized processes and fit-for-purpose biospecimens to accelerate the delivery of accurate, reproducible, clinically relevant molecular diagnostics for precision medicine.

A RECIPE FOR FAILURE

The NBDA, a part of the Complex Adaptive Systems Institute at Arizona State University, for which I serve as Chief Medical Officer, has intensively studied the process by which biomarkers are currently developed and has identified the root causes of most biomarker development and validation failure. The most significant among these include the following issues:

- Discoveries often start with irrelevant clinical questions – that is, questions that may be biologically interesting, but are not useful in clinical practice.
- Biomarker discoveries are often based on "convenience samples" – biospecimens of unknown or poor quality.
- Rigorous, end-to-end, appropriately powered statistical design is often lacking.
- Technology standards are either lacking or disregarded if they exist.
- Data and metadata quality and provenance are often inadequate to poor.
- Analysis and analytics are often inappropriate or inadequate for the sophistication of the clinical question and/or design.

All of these issues would benefit from new approaches. In fact, all of them must be simultaneously addressed if the biomarker failure rate is to be reversed. We need cross-cutting standards that support biomarker development in an end-to-end fashion. At the moment, the development process is siloed and disjointed, adding to the likelihood of failure as we proceed from discovery through development to regulatory approval and clinical implementation. We need to collaborate across disciplines if we want to see biomarker development succeed.

LESSONS LEARNED

Over the past decades, breathtaking advances in technology have transformed the pathologist's power to analyze patient specimens. The amount of clinically meaningful and biologically significant data that we can now generate from biospecimens has increased by orders of magnitude. As our analytical methods and technologies have evolved, however, quality assurance concerns have been focused primarily on how we test specimens – with little or no attention paid to the specimens themselves.

Extraordinary efforts have been made in pathology to rigorously assure the quality of the test platforms, the standard operating procedures used to perform tests, the environment in which tests are performed, and the proficiency of the people performing the tests. However, little (if any) rigor has been applied to the control of factors that adversely affect biospecimen quality before molecular testing is performed. To repeat: no matter how sophisticated and technologically advanced our analytical platforms, the quality of the data can never be higher than the quality of the starting materials – the analytes.

We must make every effort to safeguard the molecular quality of patient specimens during the preanalytical period, if we want to generate valid analytical data on which to base valid diagnostic decisions. It is now possible to generate petabytes of bad data from bad specimens – and we can do it with unprecedented speed. The stakes are higher than ever. But regardless of how much effort is involved and how far we have to go to ensure full quality control, we need to remember that it's all worth it for one reason: our patients. They are counting on us.

Carolyn Compton is a Professor of Life Sciences, Arizona State University, and Adjunct Professor of Pathology, Johns Hopkins Medical Institutions, USA.

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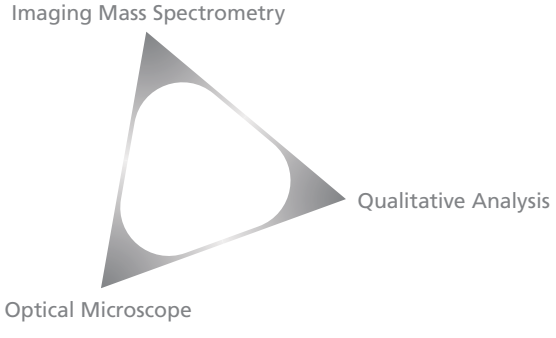

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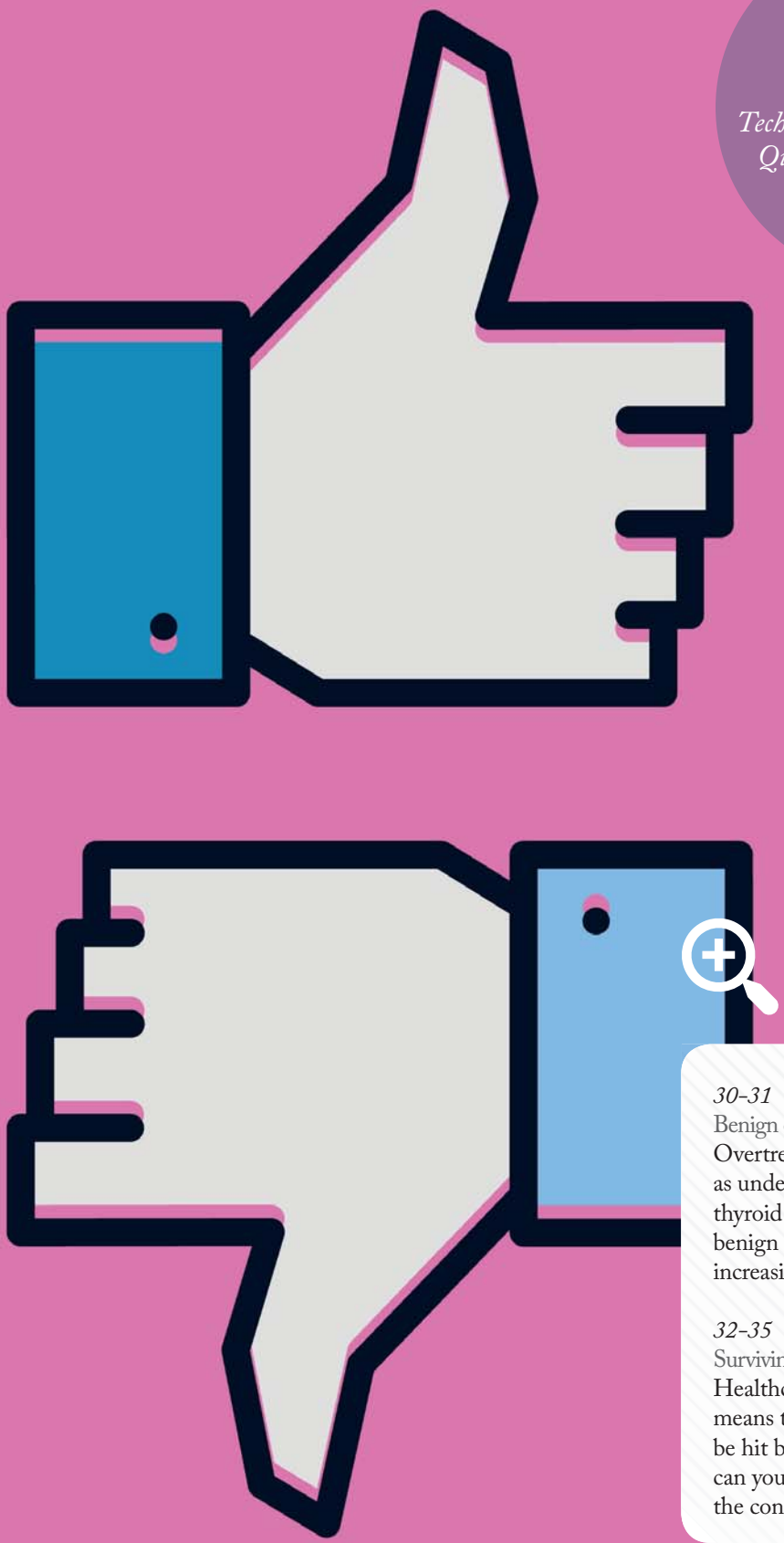
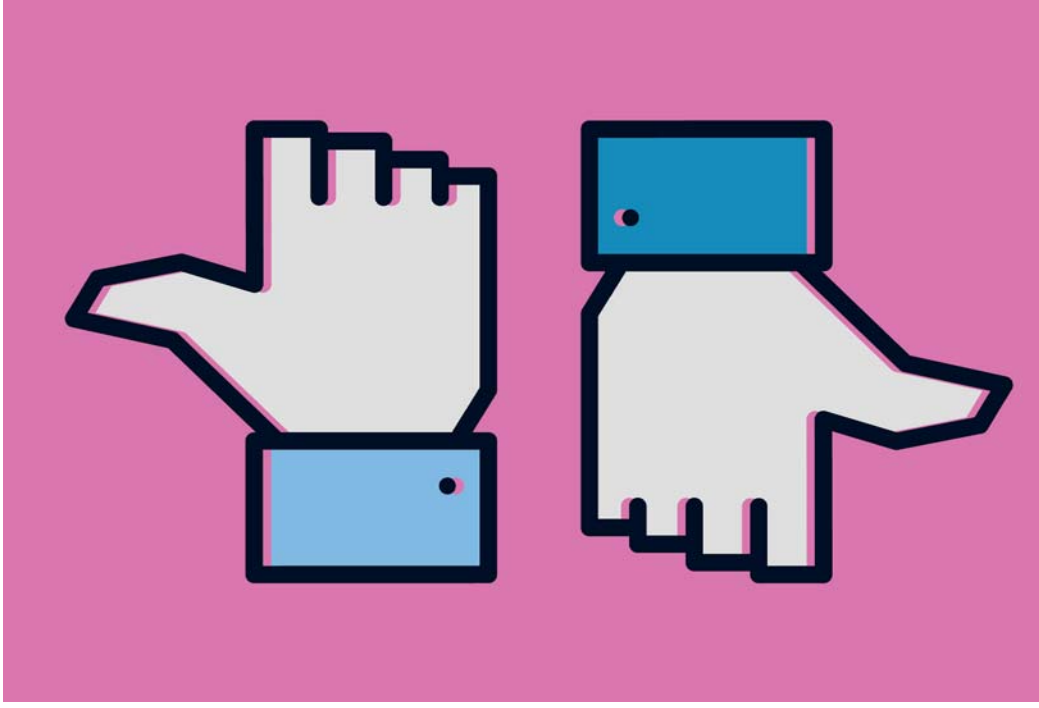




Benign or Malignant?

Thyroid nodules represent a diagnostic dilemma – but mistakes carry a serious risk of under- or overtreatment

By Saul Suster

Thyroid nodules are of clinical significance because of the ever-present danger that they may represent cancer. In most cases, it is impossible to distinguish clinically benign from malignant thyroid follicular nodules; no clinical test or imaging modality can provide an answer for the clinician. That's why tissue diagnosis has become the gold standard for the evaluation of thyroid nodules. The most commonly employed technique is fine needle aspiration (FNA) cytology. FNA is the least invasive technique, it's easy to perform in the doctor's office or in an outpatient setting, and it's both quick and cheap. Unfortunately, it's also only a screening procedure; in most instances, when it yields a suspicious or positive result for malignancy, it must be followed by biopsy or excision of the lesion.

At a Glance

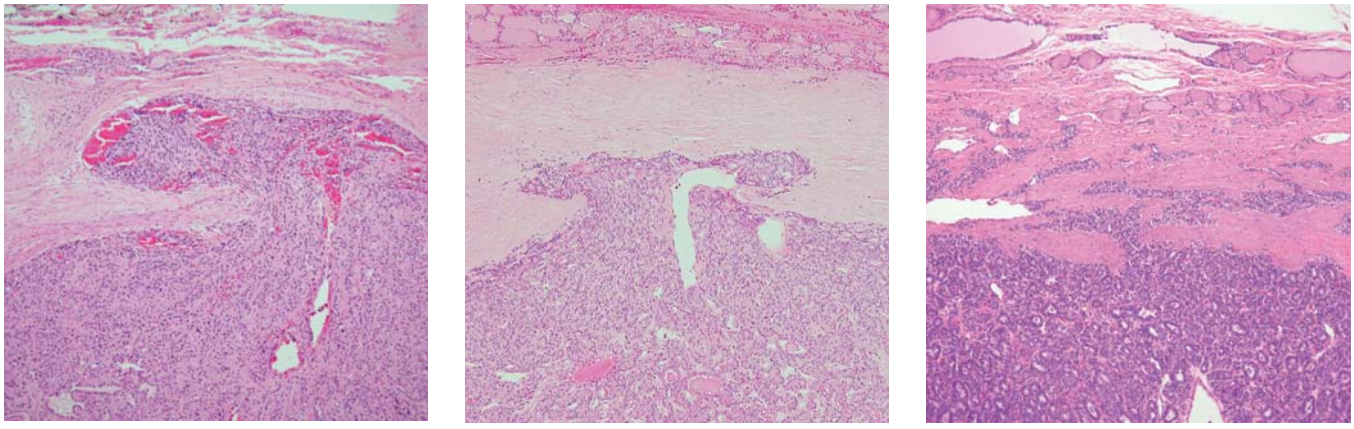
- *No clinical or imaging test allows the easy differentiation of benign from malignant thyroid follicular nodules*
- *Even with the application of cytology, positive results for malignancy require additional, invasive testing to confirm*
- *Some thyroid lesions that appear malignant at first glance may, in fact, be indolent and require little or no treatment*
- *Pathologists must proceed conservatively with thyroid nodules in the absence of clear-cut invasive features*

The more extensive procedures are needed because thyroid follicular cancers do not play by the same rules as other cancers. Unlike the majority of cancers in other organs, thyroid follicular cancers are not diagnosed based on their cytologic features, but on their architectural features instead. This means that proper evaluation requires examination of the entire lesion and its surrounding tissues, which can only be accomplished by surgical excision of the entire nodule – and then some. The principal criteria for the diagnosis of carcinoma in follicular lesions of the thyroid are capsular and/or vascular invasion, and this requires meticulous examination of the entire capsule of the tumor – a task not possible on FNA, core needle biopsy, or even a small partial biopsy. Papillary thyroid carcinoma, particularly the follicular variant, is more amenable to identification on FNA because the diagnosis is based on the nuclear features of the tumor cells (which can generally be appreciated on FNA). But even in these cases, a definitive diagnosis can pose difficulties, and small samples only exacerbate the issues – meaning that we often require complete resection of the lesion for certainty's sake.

Papillary pitfalls

The interpretation of thyroid follicular nodules can be tricky, and pathologists should be aware of several pitfalls. The most important one, of course, is separating benign from malignant lesions. Complete and thorough sampling is indispensable for accurate diagnosis – and it's something that may be overlooked, or may not happen without pathologist oversight. Another potential pitfall lies in establishing reliable and reproducible criteria for defining vascular and capsular invasion; there is still significant variability in the perceptions of, and criteria applied by, different pathologists. Finally, the subjective element involved in deciding on the nuclear features of papillary carcinoma is a factor that we can no longer ignore, given that none of the accepted features we use today are limited to papillary cancers; the degree of change required to declare a tumor malignant can vary from pathologist to pathologist.

Misdiagnosis of malignant thyroid nodules as benign can lead to undertreatment, creating the potential for spread and dissemination of the disease. On the other hand, interpreting a benign lesion as malignant may result in unnecessary overtreatment – along with its attendant morbidity, cost and potential decrease in the patient's quality of life.



Examples of follicular nodules showing infiltration of the capsule by tumor. In all three cases shown, the infiltration is not complete and does not break through the capsule into the surrounding tissues. In such cases, the pathologist must exercise caution not to overcall the lesion a "minimally invasive follicular carcinoma," and should expend extra effort in making sure that there is no evidence of vascular invasion on extensive and complete sampling of the capsule.

A diagnostic how-to

To diagnose a follicular neoplasm, the standard criteria involve demonstration of capsular or vascular invasion. In papillary neoplasms, the main diagnostic criterion for the past 30 years has been the nuclear features of the tumor cells; however, more recent studies have reintroduced architecture and circumscription as indispensable criteria for the diagnosis of papillary thyroid carcinoma, particularly the follicular variant. A recent JAMA Oncology study (1) by an international collaboration of pathologists indicated that tumors with the cytologic features of this variant, if well-circumscribed or encapsulated (i.e., noninvasive), are associated with indolent behavior and should be designated as "noninvasive follicular neoplasms with papillary-like features" (2) to avoid overtreatment; lobectomy alone is generally curative in those patients.

Immunohistochemistry (IHC) plays a limited role in the diagnosis of follicular neoplasms of the thyroid. IHC can be used in equivocal cases to tilt the scales in favor of benign versus malignant, but should be used sparingly and not regarded as definitive proof either way.

Instead, we now use molecular testing to further stratify tumors by assaying for various genetic alterations commonly associated with thyroid cancers (such as *BRAF, RAS*, or *PPAR*). The presence of such alterations points toward a clonal process operating in a given tumor and supports the neoplastic versus hyperplastic nature of the lesion. But unfortunately, these methods are far from foolproof. Certain genetic alterations, such as *BRAF* V600E, are a clear marker for papillary thyroid carcinomas, but they are not present in all cases – and that means their absence cannot be used as evidence for a benign process. Molecular testing holds great promise for the assessment of thyroid nodules and, as new studies are published and new technologies are implemented, I expect great progress over the next few years.

When a thyroid nodule is unequivocally benign or unequivocally malignant, diagnosis is straightforward and does not require ancillary tools – just the humble H&E-stained slides. The problem arises in borderline or equivocal cases in which the features are not clear-cut. Such cases can benefit from the restricted use of IHC and molecular testing – but, in a small percentage of cases, even these techniques won't resolve the issue. My recommendation in the absence of clear-cut invasion, unequivocal infiltration of surrounding tissues, or evidence of vascular invasion, is to exhibit great caution in calling any lesion – particularly a follicular neoplasm – malignant. In such instances, I prefer to use the terminology proposed by the Chernobyl Group of Pathologists in 2000 – "follicular neoplasm of undetermined malignant potential" – with a note recommending close clinical follow-up to avoid unnecessary, aggressive treatment (3).

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Surviving the PAMA Pinch

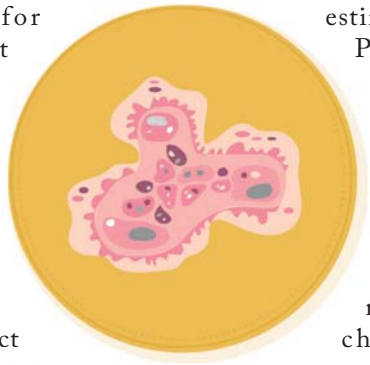
How the Protecting Access to Medicare Act (PAMA) will affect clinical laboratories – and what you can do about it

By Eitan Akirav and Dieter Schapfel

The PAMA problem

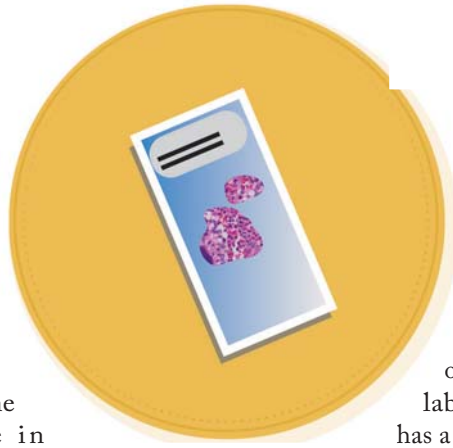
Healthcare costs are skyrocketing in the USA, surpassing US$3.4 trillion and totaling more than $10,000 per person in 2016 (1). With a growing number of aging Americans and an increase in life expectancy, these costs are expected to rise by approximately 5 percent per year over the next decade. If left unchecked, healthcare costs may top 25 percent of gross domestic product by 2025 – with a clear negative effect on the overall growth of the US economy.

Although the bulk of healthcare costs are attributed to hospital care and physician's office fees, it is estimated that clinical testing and laboratory services account for approximately 3 percent of total US spending on healthcare, or approximately $100 billion per year (2). These numbers prompted the US Congress to enact the Protecting Access to Medicare Act (PAMA) in an effort to revise the payment methodology for the majority of clinical diagnostic laboratory tests paid for by Medicare, the country's social insurance program for healthcare. The Centers for Medicare and Medicaid Services (CMS) predict that PAMA will save taxpayers as much as $670 million in 2018 alone, with additional savings estimated through 2020. PAMA is designed to affect Medicare Part B rates only; however, similar cuts in other government-based insurance plans and from private health insurance providers may follow. Such changes to Medicare payment rates, although phased over a period of three (or more) years, have raised major concerns among clinical laboratories. Despite ongoing lobbying efforts to the contrary, PAMA officially took effect on January 1, 2018 – a step that will have significant effects on the profitability and survivability of clinical laboratories nationwide.



At a Glance

- *Rising healthcare costs in the USA are negatively affecting the overall growth of the country's economy*
- *In response to this challenge, the US Congress enacted the Protecting Access to Medicare Act (PAMA), which revises Medicare's payment methodology for clinical laboratory tests*
- *Payment cutbacks will affect clinical laboratories' ability to maintain adequate profit margins*
- *There are strategies, including product focus, laboratory test development, referencing out tests, and partnering with other labs, that can minimize PAMA's effects*



Profit squeeze

Clinical laboratories, regardless of size and scope, are the backbone of healthcare in America. They provide timely and accurate diagnostic and prognostic information that influences a vast range of physician recommendations. With advancements in genetic testing and the discovery of new disease biomarkers, it is now possible to provide accurate diagnosis and prognosis for most common medical conditions. Moreover, public opinion supports more "personalized medicine" to enhance patient-focused treatment efficacy, and clinical tests are critical for public health emergencies, such as emerging diseases, acute infections, and pandemics (for example, the Zika and influenza virus outbreaks). It's clear that laboratory information has a significant and broad-ranging effect on patients' treatment options and overall care.

Clinical labs process hundreds of millions of patient samples each year. Despite the large volume, though, maintaining profitability is a constant challenge. A 2012 report by the Department of Health Policy at the George Washington University School of Public Health painted a grim picture of clinical laboratory profit margins, with nearly half of labs surveyed reporting profit margins in the range of 0 to 3 percent (3). A deeper look at the data shows that, as is the case with many high-volume "bread and butter" clinical tests, low fee limits and reimbursement rates are the main cause of these relatively small profit margins. Competition between large clinical laboratories and small- to mid-sized ones struggling with relatively smaller test volumes only adds pressure. And, of course, those who ultimately suffer most are the patients. The availability of key laboratory tests is crucial to patient welfare, but as small laboratories face the risk of losing profitability or even shutting down, patients' choice between labs will shrink, service availability will decrease, and turnover times will rise.

PAMA's implementation is poised to make the picture bleaker still. It impacts the calculation of Medicare payment

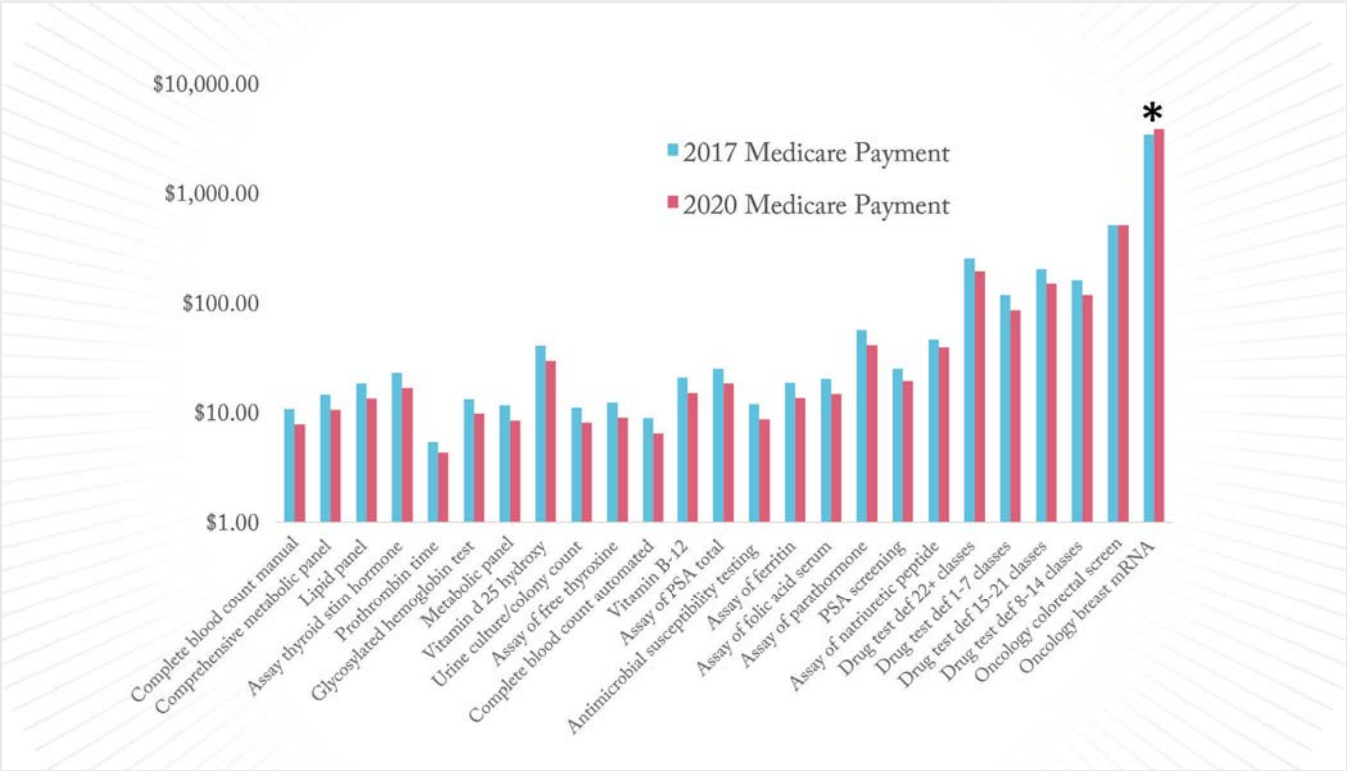


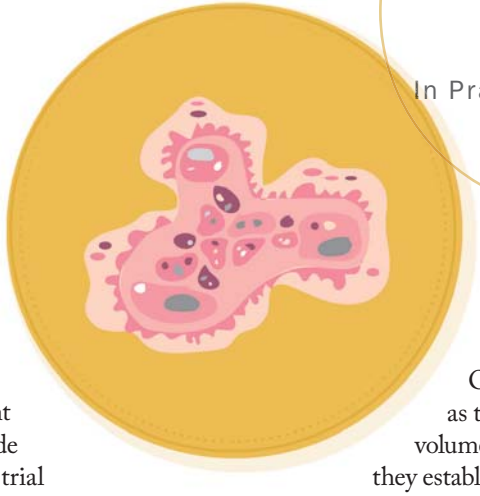
Figure 1. PAMA-based changes to reimbursement rates for the 25 most common clinical tests.

rates for more than 1,250 Current Procedural Terminology codes and decreases Medicare reimbursement for certain tests by as much as 10 percent per year from 2018 to 2020, and up to 15 percent per year from 2021 to 2023. Examination of the top 25 tests by volume and costs reported by CMS shows that all but one will suffer a decrease in payment (compared with 2017 rates) of anywhere from 0.7 percent to a staggering 27.1 percent by January 2020 (see Figure 1). In contrast, more than 340 tests will remain at the same or higher rates throughout that time. Nevertheless, with labor costs increasing, decreases in Medicare reimbursement spell bad news for labs performing the common tests affected, and for those already struggling with small profit margins.

Controlling the effects of PAMA

For laboratories to remain competitive in an era of reimbursement cutbacks, managers and medical directors will need to be creative. Business operation considerations in realms including sample acquisition, delivery, logging, and processing can all improve the bottom line and increase profit margins. For example, focusing on specific geographic regions, increasing employee productivity, and introducing automation can all reduce overall costs. But if we want to have an immediate impact on profit margins, we can take some additional steps to reduce expenses and negate some of the effects of PAMA:

- *Scale-up:* The power of numbers applies to many markets; clinical testing is no exception. Increasing the volume of identical or closely related laboratory tests should allow clinical laboratories to do more with less. Focusing on specific tests that use common equipment can minimize the need for large capital investments and additional personnel training, providing a cost benefit. For example, laboratories focusing on tissue histology services may benefit from simply increasing the scope of biomarker tests provided. Autostainers and imaging hardware and software are flexible in nature and offer the opportunity to incorporate new stains, antibodies, and other ways to detect disease biomarkers. Such an approach requires little capital investment and training over the short term, providing some relief from the PAMA changes. Moreover,



pre-existing networks of physician offices, hospitals, and other patient care centers may be more receptive to expanding their relationship with clinical laboratory partners already offering histology services. Similar approaches can be employed in laboratories focusing on other lucrative test areas, such as molecular or genetic testing.

"For laboratories to remain competitive in an era of reimbursement cutbacks, managers and medical directors will need to be creative."

- *From IVD to LDT:* FDA-approved in vitro diagnostic (IVD) tests are used in most Clinical Laboratory Improvement Amendments (CLIA)-approved settings without the significant burden of assay validation. IVDs are appealing not only because of their FDA stamp of approval, but also because they offer a highly standardized testing platform that can be used anywhere in the US. In contrast, laboratory-developed tests, or LDTs, describe diagnostic tests developed and performed by a single laboratory entity. A test offered as an LDT cannot be "exported" from one laboratory to another and remains under enforcement discretion. Nevertheless, they still have significant value. LDTs provide the opportunity to trial new or alternative laboratory tests as part of the path to an IVD. LDT approval is state-dependent and, in most cases, regulated by CLIA, making approvals more efficient and less expensive. This approach translates to a lower cost per test in the long run and will yield a clearer route to IVD certification.
- *Reference out:* Higher volumes and LDT development are not viable gateways for every laboratory. In some small- to mid-sized labs, it may make sense to reference out some tests instead. Larger clinical labs can offer discounted prices to smaller labs due to their high sample volume and lower reagent costs. Although referencing out may seem negative at first glance, such an approach may provide some financial relief. For example, referenced tests require minimal or even no capital investment and, with good shipping logistics, may bypass the need to maintain costly courier contracts. Furthermore, by establishing strong relationships with other clinical labs for reference services, smaller laboratories can expand their menu of tests to match those of larger labs, enabling them to find new customers or capture additional business from existing ones. Finally, reference laboratories often offer in-house LDTs and assume the cost and time associated with their development. Small- to mid-sized laboratories can partner with reference laboratories with unique LDTs, increasing the diversity of their portfolios without the cost of developing such tests themselves – at least in the first instance. Of course, as the lab's test volume grows and they establish a market by offering a particular test, they can later invest in an LDT of their own, thanks to the initial boost from their partnership.

Despite PAMA's anticipated negative effects on profit, all is not lost when planning for the future. Clinical laboratories are not only a vital component in providing optimal patient care, but also experts in cost control, automation, and process efficiency. By focusing on and accelerating our efforts in these areas and simultaneously working to reduce operational expenses, it is possible to maintain profit margins while still fulfilling the clinical laboratory mandate to improve patient care.

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The Esophageal Balloon

The story behind an inflatable pill and a sensitive biomarker that pair up to improve screening for Barrett's esophagus – and help prevent a highly aggressive cancer

By Sanford Markowitz

Esophageal adenocarcinoma (EAC) is the fastest-growing cause of solid tumor-related deaths among adult Americans, and, with a five-year survival rate of only 20 percent, it now accounts for more deaths than ovarian cancer. The best chance of reducing EAC deaths is to detect individuals who harbor Barrett's esophagus (BE), a condition characterized by abnormal cell growth in esophageal epithelia that can be a precursor of EAC. BE can easily be treated when progression toward dysplasia is detected. The challenge? BE is frequently asymptomatic. Endoscopy can detect the condition, but such procedures are expensive and require the patient to commit a day of their time and to undergo sedation. And that's why most individuals with symptoms of gastrointestinal reflux – a cause of BE – treat their symptoms with over-the-counter medication and don't undergo endoscopy. Consequently, most BE cases remain undetected, leading to 90 percent of EACs being diagnosed in patients who were unaware they even had BE. Our group at Case Western Reserve University has a long commitment to finding a better solution, which we believe we achieved with our recent publication (1). The impetus for our project was to develop a biomarker panel and to invent a simple, patient-friendly sampling device that could replace the need for endoscopy.

At a Glance

- *The preventable indicator of esophageal adenocarcinoma – Barrett's esophagus – goes undetected in many patients*
- *The current gold standard for testing Barrett's esophagus is endoscopy, which is expensive and time-consuming for patients*
- *A biomarker panel and a novel swallowable balloon may combine to offer an easier way to detect the condition*
- *Endoscopy could be a procedure primarily for identifying and treating patients with dysplasia.*

The search for the right biomarker – and the right sampling tool

My group previously pioneered methylated vimentin exon 1 as a DNA biomarker for detecting gastrointestinal cancers, starting with colon cancer – a concept that became the Exact Science/LabCorp ColoSure stool DNA test. In 2012, we also published findings showing that methylated vimentin DNA is an even more sensitive and specific biomarker for detecting BE and EAC than it is for detecting colon cancers. We wanted a detection technique that could

"We want to be able to identify all individuals who harbor BE in the most cost-effective way."

offer even greater sensitivity, leading us to identify cytosine methylation of *CCNA1* DNA as a second marker of BE. Paired with the established methylated vimentin assay, the two-marker test provided over 90 percent sensitivity and specificity for BE detection. Having identified a robust analytical method, the next step was to work on replacing the endoscopy.

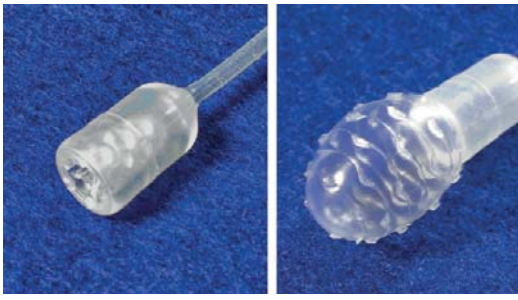
Our team of inventors – Amitabh Chak, Joseph Willis, and I – met with a team of industrial designers and brainstormed about how we could create a swallowable device to sample the esophagus. We considered dozens of different approaches, but as soon as the idea of a pill-sized balloon came up, we all jumped on it as the answer. We immediately realized that a balloon offered the opportunity to create a device that would be small and comfortable for the patient, but effective in sampling the esophagus. We then realized that if we encapsulated the balloon, we could gain additional benefits by being able to target sampling to just the lower esophagus, where BE develops – plus, we'd be able to protect the sample from dilution and contamination (by avoiding dragging the exposed balloon through the entire esophagus, throat, and mouth). Next, we had to optimize the surface texture of the balloon for collecting cells, which we explored first by using chicken skin and afterwards by testing pig esophagus explants. It took over a year to engineer something that worked, but once we had it in hand, the simplicity of the approach was truly alluring.

The technique's high sensitivity and specificity led us to think it could be a first line of defense for screening individuals who have symptoms of gastric reflux, instead of undergoing endoscopy. Endoscopy could be reserved for individuals whose symptoms progress despite medical treatment for reflux. Moreover, we hope that the accuracy and cost-effectiveness of the test could allow it to be used to screen asymptomatic individuals, who would not be considered for endoscopy. Endoscopy would also be needed to follow up on a positive balloon test so as to distinguish standard BE from BE with dysplasia, which is closer to being cancer and is treated endoscopically by ablation or removal.

More work to do...

We want to be able to identify all individuals who harbor BE in the most cost-effective way so as to facilitate surveillance and treatment as soon as it starts to progress. Ultimately, we want to stop anyone from ever developing EAC. To that end, we are currently working on the second generation of the balloon to make it even easier to swallow and even more effective in collecting cells. We're also identifying biomarkers to distinguish between BE with versus without dysplasia.

In our current study, we tested many individuals who were already known to have BE – that is a standard way to investigate any new diagnostic test. Our next study will be designed to identify individuals with BE out of a general population studied in a national clinical trial at multiple centers.



Carla Schaffer/Moinova et al./AAAS

It takes a multidisciplinary team to make such discoveries, and especially to translate them into practical advances for use in patients. We are lucky that at Case Western Reserve University and at University Hospitals Cleveland Medical Center we have such a team. Our work is truly the result of a team effort, involving gastroenterologist Amitabh Chak, pathologist Joseph Willis, molecular biologist Helen Moinova – and a medical oncologist and cancer geneticist (me).

We have also been fortunate to have tremendous support from the NIH, which has awarded us a Barrett's Esophagus Translational Research Network (BETRNet) center, led by Dr. Chak and a GI Cancers Specialized Program of Research Excellence – GI SPORE that I lead.

Sanford Markowitz is the Markowitz-Ingalls Professor of Cancer Genetics, and Medical Oncologist and Colon Cancer Researcher at the Case Western Reserve University School of Medicine and University Hospitals Seidman Cancer Center, Cleveland, USA.

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Variant Database Collaborations – for Cancer and Beyond

Is there a better way to sift through the vast quantities of information found in the scientific literature – and still find what's relevant?

The already complicated task of organizing and updating scientific literature becomes increasingly more so as information continues to amass at a frightening pace – a challenge that's especially relevant to cancer genomics. Not only is it important to collate relevant literature for each subject, but also to ensure that any stored information is updated with the latest knowledge. Such a gargantuan task is too much for one group alone to tackle. Here, Obi Griffith and Heidi Rehm discuss how the scientific community at large could offer a solution.

At a Glance

- *Keeping genomic data updated with the latest research and clinical findings is valuable to ensure that your patient information is always accurate*
- *To keep up with the volume of genomic information being produced is too big a task for a single institution, so collaboration is key*
- *The CIViC, ClinGen and ClinVar databases allow a curated collaborative approach to keeping genomic information up to date*
- *If widely used, this approach could be integrated with healthcare information to inform clinicians of new indications in old patient data*

How did you become involved with cancer genomics?

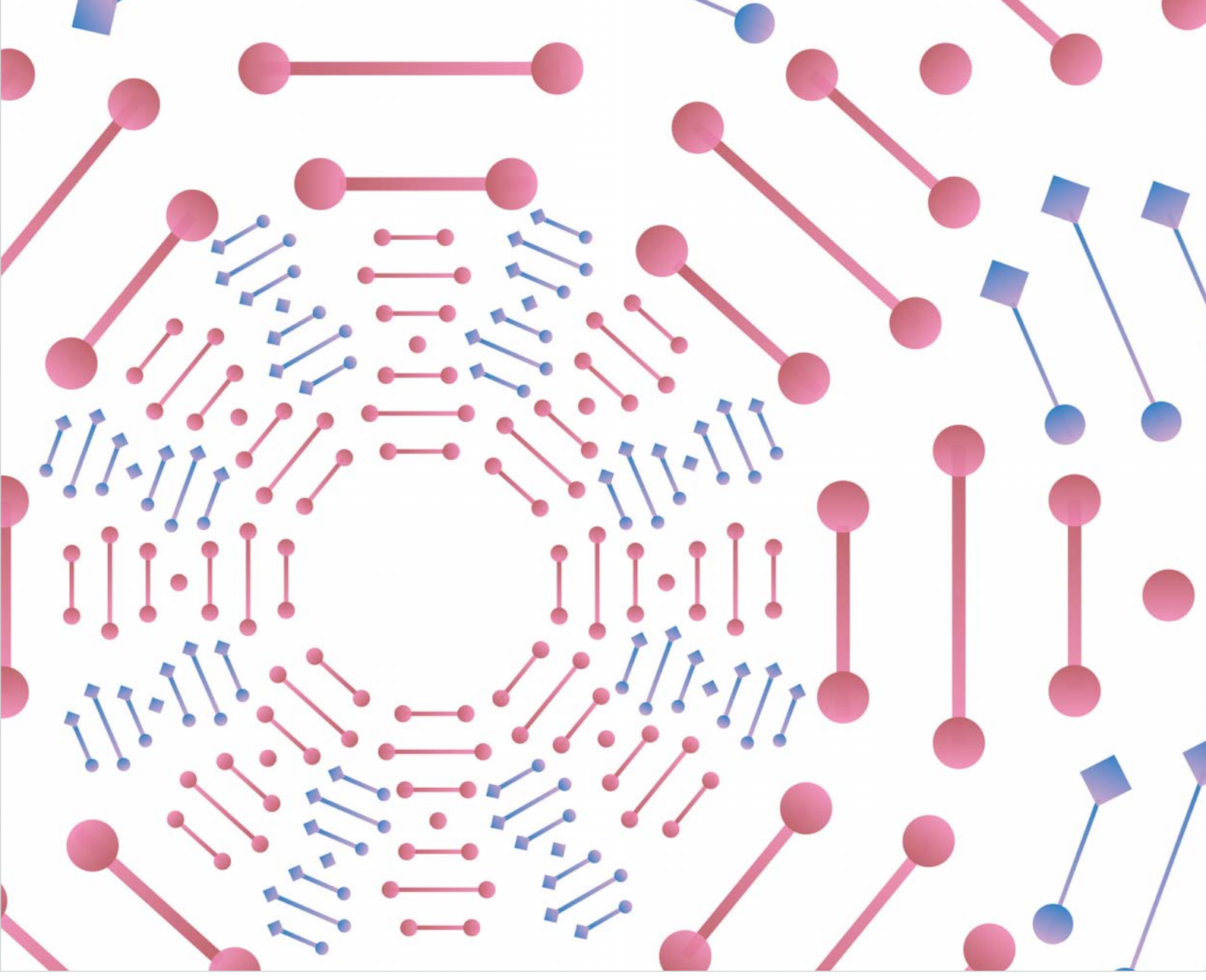
Obi Griffith: I started in bioinformatics. Shortly after college, I worked at Canada's Michael Smith Genome Sciences Center, which was one of the first big genome centers in Canada – and it was at the forefront of next generation sequencing. We had sequenced genes from humans and many other species, with a strong focus on cancer because of the support from the BC Cancer Agency. Next, I did a post-doc in cancer genomics at Lawrence Berkeley National Laboratory, before moving to the Washington University McDonnell Genome Institute six years ago – again, with a focus on cancer genomics. I work with cancer genome, sequence, and expression data, but I'm also active in bioinformatics, developing software, tools, and databases, so I tend to work on a variety of cancer types.

Heidi Rehm: I've been interested in genetics since high school. I majored in molecular genetics and biochemistry at Middlebury College, then went to Harvard for my graduate studies and became more interested in the human disease aspect. I ended up studying genetic hearing loss in my graduate and post-doctoral studies but, soon after, I was hired to start up a new clinical lab, Partners Healthcare Laboratory for Molecular Medicine. Over the course of the last 15 years, it has become apparent that the current model of how we understand and interpret genetic variation is insufficient for the quantity of information being amassed. Clinical research labs cannot maintain the level of deep expertise in all disease areas needed to provide proper, high-quality interpretation for all indications. That's why I started thinking about a way for us to all work together as a community.

"Even if, at one point in time, all the experts in the world review a variant interpretation as one thing, the next day new information could invalidate it."

What is the solution?

HR: There were people who said, "We need to build a clinical grade database." At the time, there was the Human Gene Mutation Database, which was based on people going through the literature and finding variants that had been reported in patients. But it had a serious shortfall: there was never really any scrutiny of the evidence base for any of the information. It;s a good place to find data in literature reports, but many of the interpretations are incorrect. That was the status quo we were dealing with five years ago, and we weren't really happy with it. Knowledge constantly changes; even if, at one point in time, all the experts in the world review a variant interpretation as one thing, the next day new information could invalidate it. From that perspective, it almost seems like an impossible scenario to create a curated database able to encompass all the relevant information… Almost! The solution was to let the scientific



community become part of the curation process, which is how ClinVar and ClinGen came into being.

ClinVar is a database managed by the National Center for Biotechnology Information, within the National Library of Medicine and National Institutes of Health (NIH). The aim is for people to submit interpretations of variants with supporting evidence, both published and unpublished, to spread the workload of curation across the community and have a means of sharing unpublished data on variants. A star rating system accompanies ClinVar to help users understand the level of review of variant interpretations; ratings go from zero stars (little to no documented methodology) up to four stars (Expert Panel-reviewed).

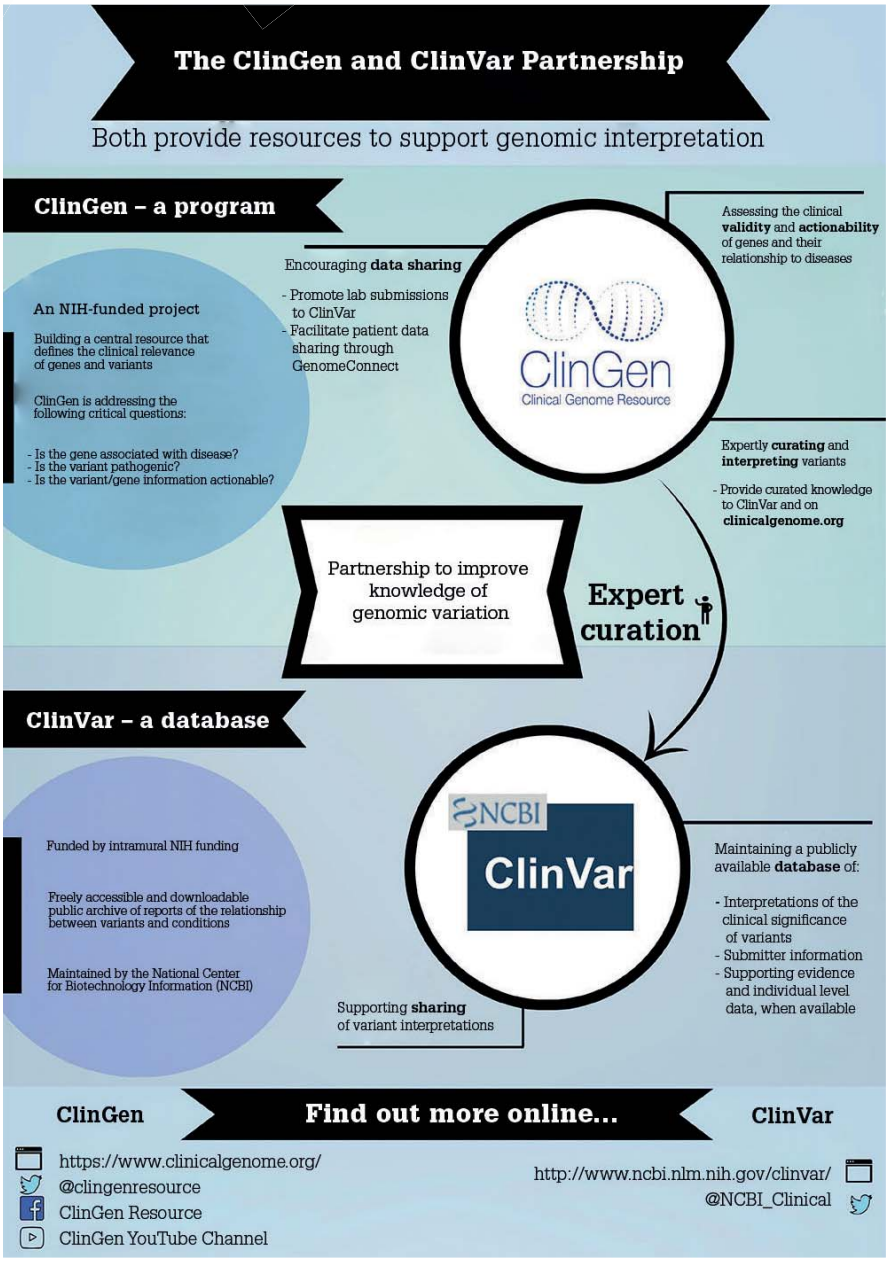
ClinGen, on the other hand, is a NIH-funded program that aims to develop authoritative resources to define the clinical relevance of genes and variants for use in medicine and research. The ClinGen program has over 570 members – spanning 230 different institutions across the world – participating in working groups. ClinGen forms and approves Expert Panels that review variants submitted to ClinVar from single labs.

OG: Clinical Interpretations of Variants in Cancer (CIViC; civicdb.org) is a newer, NCI-funded database that focuses exclusively on the clinical interpretation of cancer variants. Whereas ClinGen and ClinVar have historically focused more on germline variants, CIViC spotlights somatic variants. Importantly, CIViC is strongly committed to an open data sharing model, with all content in the public domain. Evidence for variant interpretations and assertions is submitted and moderated through a combination of crowd-sourced curation and expert moderation. CIViC is working closely with the ClinGen

Somatic Working Group and Global Alliance for Genomic Health (GA4GH) to develop standards for somatic variant assessment.

The pros of using CIViC are that you hope to distribute the work somewhat, and that you achieve more of a community consensus – with transparency. Everything is attributed; you always know who said what and when. Existing, centralized resources are often not accessible to everyone and there is generally no provenance. Moreover, there's no real mechanism for feedback if you recognize an issue. You could email an author, but it's not really a direct mechanism for making an improvement to the content.

But like all things, the system isn't perfect. As it's distributed, there's a higher possibility of variable quality, so we editors and moderators need to do a good job of reviewing the content. Additionally, we're not experts in every subject! So, there's a possibility for errors. We're hoping that the transparent model and community allow us to identify such problems quickly.



What are the real-world applications of ClinVar/ClinGen/CIViC?

OG: When you sequence a tumor, you're sometimes faced with thousands of sequences. Generally speaking, 99 percent of these are unimportant – they may be a symptom of a mutation process, but only a fraction of individual mutations are important. Your real aim is to find the few mutations in these thousands that cause the cancer to grow aggressively – essentially, a needle in a haystack. To make finding the needle easier, the CIViC database gathers knowledge from hundreds of previous patients and published studies that have up-to-date knowledge – and gives us a clue as to which mutations we should be thinking about.

HR: In the cancer setting, tumor versus normal tissue can be an incredibly useful filter to help identify what mutations might be associated with tumor growth and cancer progression, allowing us to exclude most of the variation in the germline and focus in. Unfortunately, as Obi mentioned, there's almost never just one variant, so we're still left with the same dilemma: which sequences are passenger mutations that have no role in tumor growth and progression? It's an ongoing challenge. Sometimes we understand the role of variants, but most of the time, we don't. That's where CIViC comes in; it brings together many different somatic cancer databases, doing the same thing we're doing in the germline space.

OG: CIViC is very much the capstone of the knowledge databases. There are hundreds of thousands of studies on cancer genetic data and sample mutations – and the field is expanding every day. If you're dealing with the identification of mutations in a particular tumor, you're going and searching – sometimes in a very manual way – the available literature to help guide your decisions. We understand how laborious that process is, so CIViC was created as a high-level summary of the literature, complete with the potential for clinical colleagues to comment and contribute information based on their experiences – almost like expert-level crowdsourcing.

How is the curation process going to work?

OG: We're really just summarizing existing knowledge in the literature, so what we're aiming for is a faithful or accurate representation of evidence presented in the studies. We're not creating new knowledge; we're simply synthesizing. When someone submits a new variant interpretation or evidence for certain interpretations, we assess whether it's a faithful representation of what is being referenced. Then, as not all papers are created equally, we categorize and weight the level of quality of evidence. For example, a report of one anecdotal case is weighted less than a large clinical trial. Much debate and effort go into those judgments of quality, and then we decide how we should synthesize various competing results into one cogent consensus of the current state of belief for that variant.

HR: Within ClinGen, our Expert Panels encourages labs and locus-specific databases to submit all variant interpretations with evidence to ClinVar. The Expert Panel reviews the evidence on each variant and then either approves interpretations or changes interpretation based on expert review. Sometimes that involves aggregating evidence from multiple labs to move interpretations from uncertain significance to classified (pathogenic or benign); other times, it involves resolving differences in variant classifications between labs.

What could the future hold for this kind of database?

HR: A currently unfunded proposal I have is to actually structure the genetic test reports so that variants are clearly mappable to the genome and are able to interface with electronic health records, using the reported ClinVar data as a reference for the variants. As mentioned previously, medical knowledge changes over time. What if a variant that we previously thought was benign becomes pathogenic? If this proposed system were in place, it could update attending physicians and tell them, "This variant has been interpreted by an expert panel with recent information that differs from the original report. You might want to consider re-contacting your patient." Such high-tier information might be directly used in the healthcare setting in the future.

OG: In building resources like CIViC, ClinVar and ClinGen, we are really trying to tackle how genetics impacts human disease in general. We're trying to create resources that will eventually allow these approaches to move out of the research setting and into the patient care setting.

Sequencing is becoming quite common. I think the biggest problem coming into the field is the diversity of options. Do you do exome sequencing, whole genome sequencing, or a panel? Do you go with commercial vendors or not? The regular clinician (who's not a physician-scientist) is unlikely to download a VCF file and interface with databases after they've received a sequencing report. Rather, they're going to depend on the report that's generated from the clinical diagnostic lab they work with. Right now, there are many disparate variant interpretation resources, but there really isn't a single go-to source, so you'd probably need a genome atlas, bioinformatician or another kind of expert to help navigate the diverse field of resources at your disposal.

"What we're aiming for is a faithful or accurate representation of evidence presented in the studies."

The ClinGen group and others have really championed guidelines where variants get categorized as benign or pathogenic, according to very well-described rules. We're trying to be inspired by that, but also do things differently. We want to work within the global alliance and other consortia to get the scientific community involved around the world to work together on this problem, because it's way too big for any one group to solve. Again, that's why CIViC was built, to help facilitate that collaboration – and we've got over 100 contributors to date.

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Heidi Rehm is the Chief Genomics Officer in the Department of Medicine at Massachusetts General Hospital and Medical Director of the Broad Institute's Clinical Research Sequencing Platform, Cambridge, USA.

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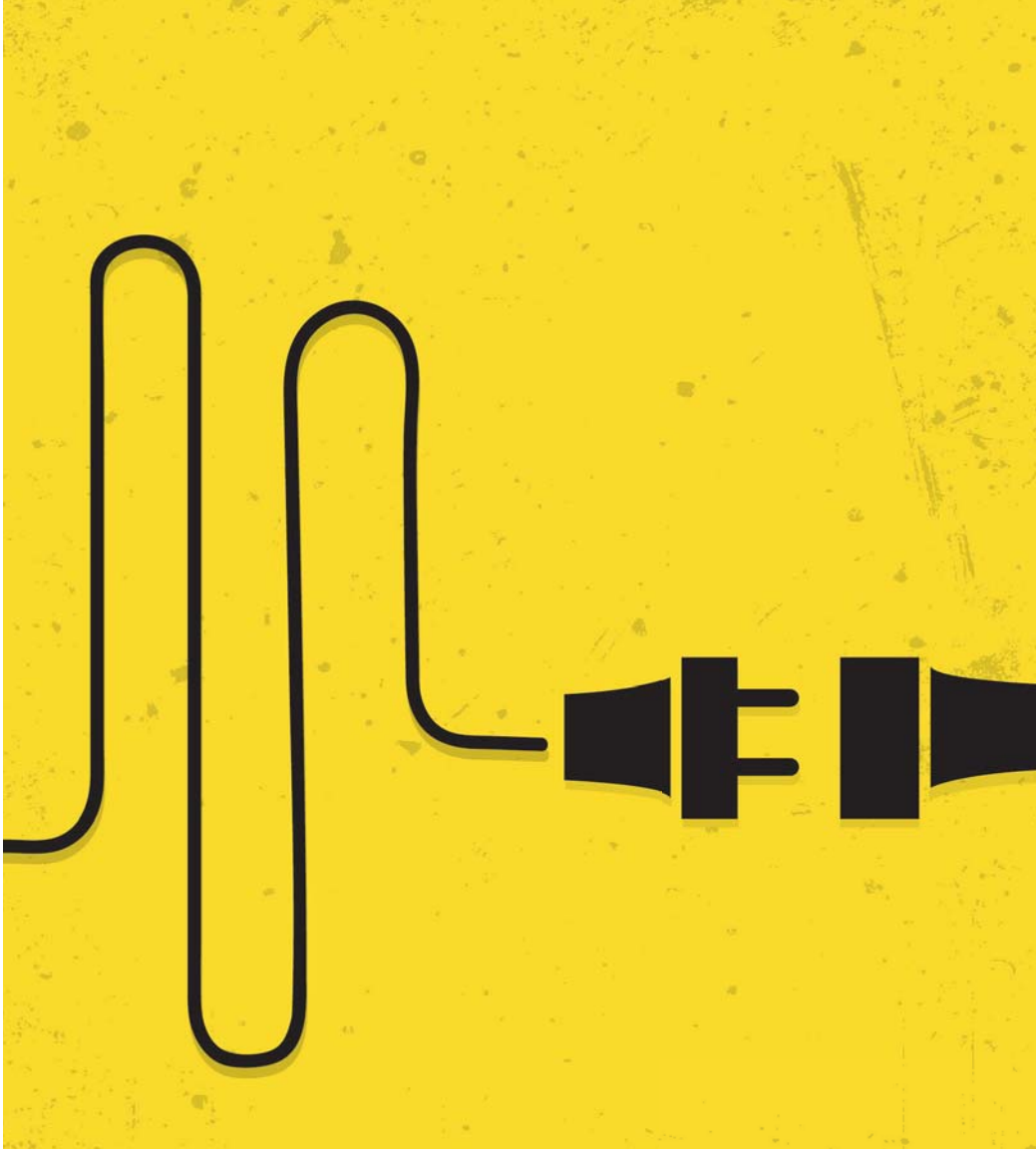
A Call for Connected Diagnostics

Laboratories and vendors should take a more practical approach to digital integration to truly help pathologists

By Jane Rendall

Digitization – it's a hot topic across pathology, especially as conversations about artificial intelligence and computer-assisted diagnosis add to the fire. But what does it actually mean to pathologists "on the ground?" What does the process of transitioning to digital involve, and what can it actually do for pathologists right now? Most importantly, how can digital technologies fit into the existing workflow so that they help, rather than hinder, the vital day-to-day work of the laboratory?



At a Glance

- *Connected diagnostics are becoming increasingly important as collaboration becomes essential*
- *Integration has something to offer laboratories at every level of digital maturity, but change must take place logically and sensibly*
- *Interoperability is key; new technologies should be as compatible as possible with a laboratory's existing software and equipment*
- *Computer-based technologies should help pathologists cope with the growing volume of information they must handle every day*

A call to connect

We've been working on our digital pathology product as part of our enterprise solution for a number of years. Now, we're rolling it out in several countries – notably Sweden, the Netherlands and the United Kingdom. Integrated diagnostics is beginning to really capture the imagination of healthcare providers. Particularly since the emergence of the Carter Review on hospital productivity in 2016 (1); the UK's National Health Service (NHS), for instance, is pursuing a variety of strategic initiatives – many of which involve the adoption of digital pathology. There's a trend toward breaking down the silos between departments and bringing together medical professionals from all specialties, and connected tools allow that to happen.

Obviously, there are a number of stakeholders involved in this kind of integration. Often, patients (key stakeholders!) – already expect all of their data to be integrated across departments, and sometimes even across institutions. To them, it seems like a "no-brainer" that everyone who looks after them should have all of their medical information – and in some cases, that's true; in many others, it is unfortunately not.

Information technology (IT) and healthcare professionals are focused on the same questions: how can we collaborate more efficiently? How can we work better? How can technology help us to help our patients? What benefits can a new system offer us that our current systems cannot? At the moment, different departments and specialties may all have different IT

systems, which can make collaboration and information sharing difficult. From an IT perspective, we need to establish which systems are the best – most user-friendly, most affordable, easiest to combine, and so on; however, those may not be the foremost points in the minds of other stakeholders.

System users – doctors, nurses, laboratory technicians, and other healthcare professionals – must also consider how their computational tools benefit them. Radiologists, for instance, may benefit from seeing pathology images and results, and vice versa. In my opinion, it's hugely important for integration and consolidation to go hand-in-hand so that the patient record is as rich as it can be. Such integration and consolidation will bring all the images, reports, and advanced tools to clinicians and support concordance workflows and multi-disciplinary meetings. And for laboratory medicine professionals, the consensus seems to be that digital pathology is the way forward. It's a massive change, but it's also a great opportunity!

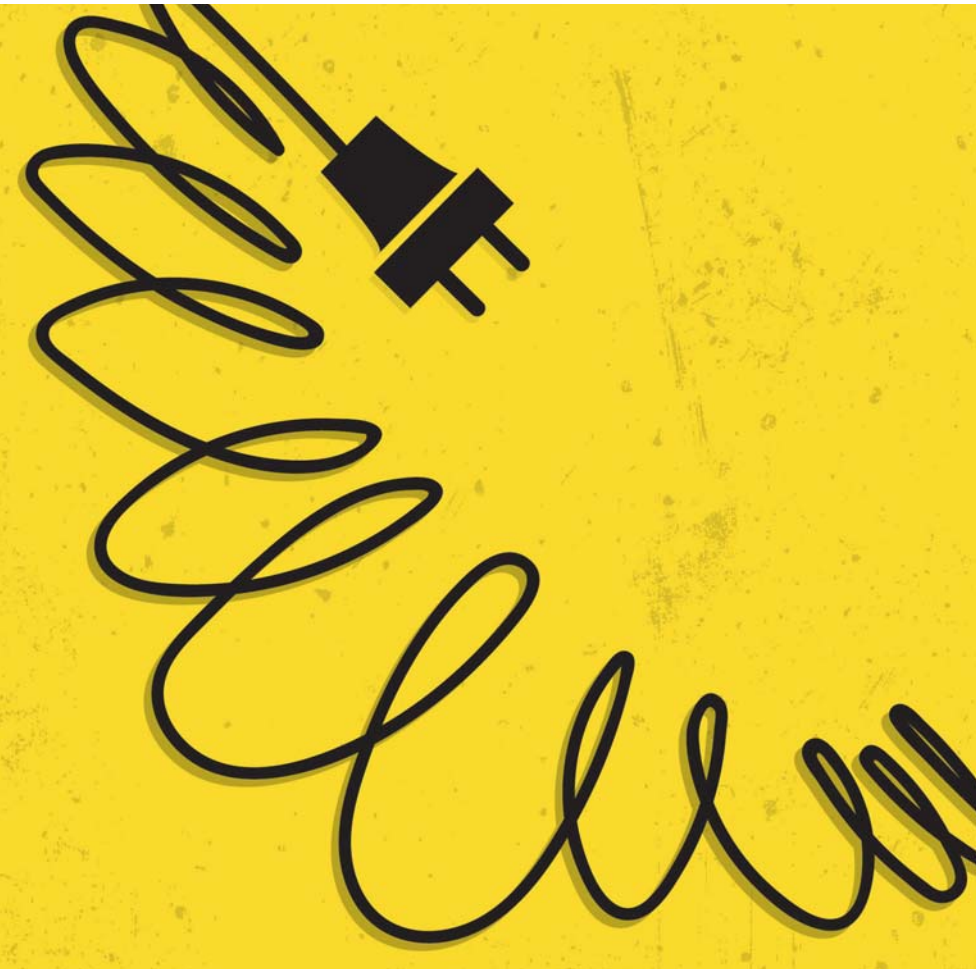
The integration equation

How a laboratory tackles integration will depend on the degree to which its workflow is already digitized; every institution has a different level of digital maturity. Assuming a very analog workflow (thus, essentially, starting from scratch), requests will arrive at the lab on a piece of paper. Samples will be handled manually – fixation to staining – and results will be returned and reported on another piece of paper that then must be delivered back to the requesting physician. Today there are degrees of automation and digitization in a lab (such as automatic processing, embedding, or staining) – but, equally, there are plenty of opportunities to streamline those processes. The good news is that I think most pathology departments truly understand the need for efficiency, and the benefits that agile process changes can bring – so the real hurdle is in the nature of the change, not the need itself.

A good starting point for this theoretical laboratory would be sample barcoding, ensuring that each sample is always denoted by a single identifier. Purchasing a tissue-tracking system might be a good second step, so that the sample is tracked throughout the entire testing process by having its barcode scanned at each station to maintain the chain of custody. The impetus is then to automate as many steps in the process as possible. Then you can say, "We've got brilliant processes; we've got high-tech equipment; we've got a good laboratory infrastructure; now it's time to work toward new digital opportunities – collaborating with other hospitals, for instance, or sharing images with a multidisciplinary team." That's the real benefit of digitization and integration – it builds exponentially. The more you take on, the more ability you have to expand further!

When looking into digitization and integration, it's important to remember that what we see at the clinical level is just the tip of the iceberg. Patient care considerations are obviously first and foremost, but we also need to begin taking into account aspects like accessibility, scalability and security. How safe is the patient's data? What level of support does a given product have? What effort is required in implementing and maintaining a particular solution? Ultimately, we need solutions that can deliver benefits while fitting into existing patient pathways and workflows. The more a technology can mesh with what's already in the lab, the more likely it is to present a useful solution to an existing problem. We're now seeing the rise of technological solutions based on artificial intelligence and machine learning – so these kinds of things will need to integrate smoothly into the existing laboratory infrastructure. The goal is for pathologists to understand their tools and for IT departments to be able to set them up for immediate use.

"It's hugely important for integration and consolidation to go hand-in-hand so that the patient record is as rich as it can be."



Standardization (of file formats, for example) is another problem we need to tackle – ideally as soon as possible. In my opinion, digital pathology platforms should all have the ability to work with one another's file formats – or, better yet, we should define a standard format for all platforms to use going forward. These "bleeding-edge" technologies suffer from enough barriers to entry; we should aim to remove as many as possible.

Flexible – but robust – solutions

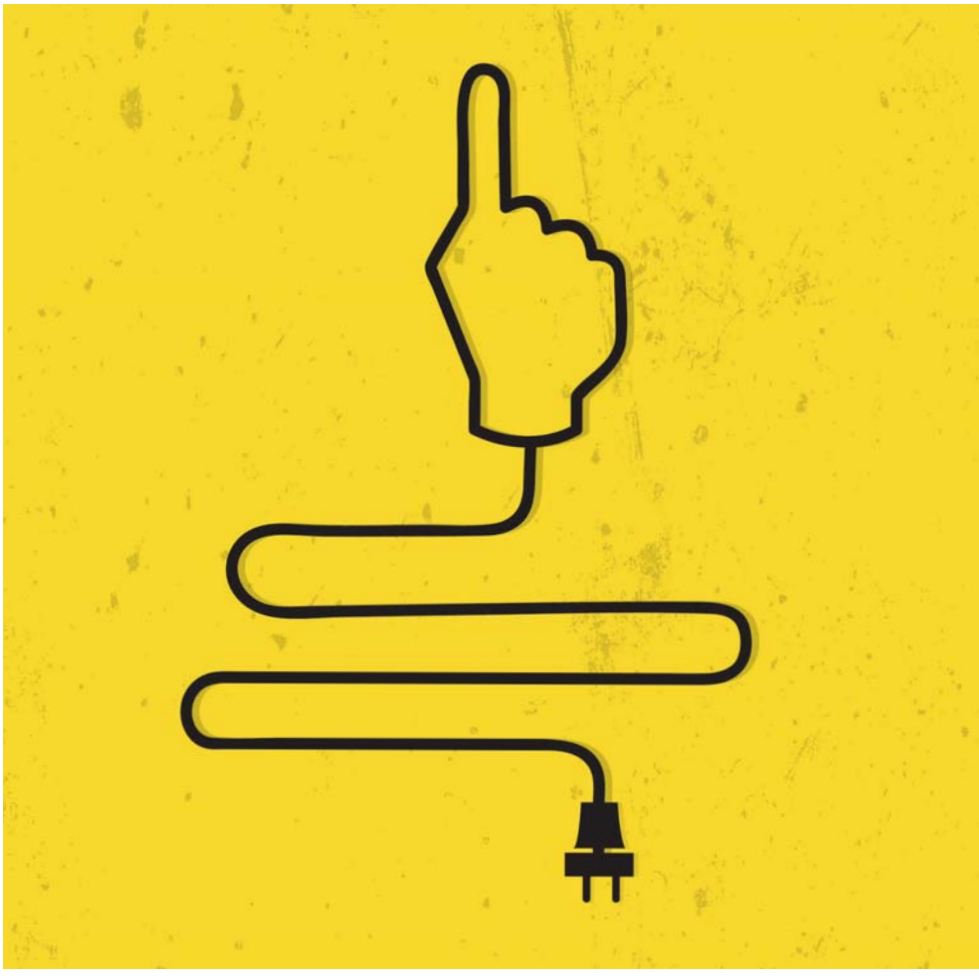
Many pathologists may think, "I'm in a very strong standalone department. I don't see the need (or there's no pressure on me) to collaborate or take in work from anywhere else." But even those who don't think they have an immediate need for collaboration can still benefit from digital technology – so the best way to approach purchasing is to make sure your chosen IT solutions are flexible. They need to be scalable, of course – but they also need to allow users to "cherry-pick" the technologies they want. You should be able to say, "I don't want to buy a solution that only works with a certain scanner. I want to buy one that lets me have this scanner here, that scanner there, and yet another scanner for other types of work." The more compatible a solution is with the equipment you already use – or may want to use in the future – the better it will serve you.

I find that the questions users ask at the start of their digital journeys aren't the same ones they ask after they've gained a level of familiarity. When you first invest in technology, you ask things like, "What core features does it have? What can it do?" When you're in your second or third generation, as radiologists are now, you ask, "How stable is it? What's your customer satisfaction like? What's your downtime? How often do you upgrade? What support services do you offer?" The biggest negative effect on efficiency and productivity is unexpected downtime – something you only find out through experience. And when clinicians and patients are depending on your results, you quickly learn to avoid anything that might create delays.

"The more compatible a solution is with the equipment you already use, the better it will serve you."

Consider what would happen if all of a hospital's radiology services were unavailable for a day – or even just a few hours. Some patients wouldn't be able to undergo surgery; emergency departments would be compromised in their ability to diagnose or treat; injured patients could be left without medical care until services were restored.

Pathology is an equally critical service – without the laboratory, critically ill patients go undiagnosed, cancer treatments are postponed, and infections may be left to spread unchecked because clinicians can't select the appropriate antibiotic. To avoid these kinds of issues, our technological solutions must be robust – and that may mean looking for technologies with good interoperability, so that each lab can create its own custom setup without building in known restrictions ahead of time. The monolithic approach is not always the right approach. A solution shouldn't just offer its own, homegrown solution that may lag behind the curve of technology development; it should be open to plugging in bleeding-edge technologies and exciting new AI tools developed by small, agile companies. Integration and interoperability are the way to future-proof your service.



Developing a digital future

The next step forward is to incorporate artificial intelligence and machine learning into the available platforms for digital pathology. Fortunately, I think the researchers who are developing these technologies now understand that, to take them from a research lab and put it into a clinical setting, they have to understand the practical considerations involved. So now, many of those researchers are partnering with health service providers, such as the NHS to ask: "How can we make our algorithms work in such a crazy, chaotic environment? (Or – in other words – how can we take something that works in a research lab and make it work for all kinds of users in a much less controlled setting?) How does it fit into the existing workflow?" We want to see these technologies help medical professionals by taking away tedious chores (that machines do well and humans often dislike), freeing up time for clinicians to tackle more difficult tasks and deliver better patient care.

When I started my career as a radiographer, we used to print out magnetic resonance images on film and look at every single image individually; nowadays, that would be impossible with the thousands of images involved! Thankfully, in the meantime, we've developed solutions and algorithms that work with viewing technologies to manage the volume of data, highlight aberrances, and direct the human's attention where the need is greatest, which helps modern radiologists cope with the sheer volume of data they see every day. It's my hope that the same will be true for pathology; I want digital and computational methods to support people coping with a tsunami of information. Anything that can merge (almost) seamlessly into the existing workflow and go hand-in-hand with existing processes and technologies is welcome – that's how new technologies become not just shiny new toys, but powerful enhancers of patient care.

Jane Rendall is Managing Director at Sectra, Ltd., Stansted, UK.

Reference

1. *Department of Health and Social Care, "Productivity in NHS hospitals" (2016). Available at: http://bit.ly/1FU1TzC. Accessed January 31, 2018.*

Chemistry Connections

Sitting Down With… Carolyn Bertozzi, Anne T. and Robert M. Bass Professor of Chemistry, and Professor (by courtesy) of Chemical & Systems Biology and Radiology,Stanford University, USA.

Were you always interested in chemistry?

I think it started in college. As a pre-med student, I studied biology and took the courses required to go to medical school, but I unexpectedly found myself drawn towards one particular topic – inorganic chemistry – so I changed my major to chemistry. One of the reasons I found the subject inherently interesting was the connection to human health. The chemistry of medicine, the chemistry of life – that's what drew me in.

You've worked across chemistry, glycobiology, oncology, nanoscience, biotech, and more... How?!

I've been very lucky – I've had access to many interesting opportunities, both academic and entrepreneurial. And, in my good fortune, I've been at great universities where there has been a lot of intellectual energy, with motivated, smart co-workers and students. But ultimately it comes down to a matter of time. If you want to explore different areas and pursue many activities, you need the right environment and the right people around you. But if you are fortunate enough to have the time, bandwidth, and support to do so, then I'd absolutely go for it. It's enriching to have that kind of exposure, both for students and fellow scientists.

Do you think collaboration across multiple fields is becoming more important?

It's definitely happening at the training level. For example, I know that PhD programs have been trending towards interdepartmental disciplinary programs; I even launched one at Berkeley that interfaced between chemistry and biology. We also have one here at Stanford that's even more broad – connecting engineering and medicine departments. I think there's a whole cohort of scientists who are going to come out of training in this decade and the next who will be able to easily bypass barriers. It's already happening in biopharma and I believe other biosciences are going to see the benefits soon. But, as with exploring different branches of bioscience, it ultimately comes down to how much time you have and, unfortunately, a lot of doctors don't have much to spare.

What field currently interests you?

Glycoscience has been a highly interesting field to work in, compared with some other areas in biomedicine. It often feels like glycoscience runs in cycles – one moment everyone's excited and enthusiastic, the next moment things cool down for a while and seem harder than people thought. But at the moment, there's a lot of enthusiasm and optimism. It's a big period of excitement because there's research coming out from different areas that are starting to gain a better understanding of glycobiology's contribution to cancer immunology, and that's a really hot area right now. I think it's putting the spotlight on glycoscience again, which is cool.

What do you think of the digital direction of pathology?

Stanford is kind of a hotbed of AI-based image processing at the moment, and there has been a big collaborative program between some Stanford pathologists/radiologists and people at Google. It's a big AI team and I think if it leads to accurate, actionable diagnoses for patients, we should use whatever tools are at our disposal. I'd hope that all pathologists would embrace that, but I think there's a universal human trait that means whenever a new technology breaks on the scene, there's some part of the human psyche that has a little bit of pushback. But I don't think pathologists are ever going to be replaced. I don't think anyone is suggesting we let robots take pictures and make diagnoses while we just become servants to them...

What advice would you give to your younger self?

Don't sweat the little stuff. It's easy to get stressed out about loads of minor details, especially in glycoscience. It's easy to get frustrated with the complexities of the moment, so make sure you never lose your perspective.

You've already achieved a great deal – where to next?

I've never thought, "I've achieved so much." It's more a case of, "Oh god, we still don't understand this?!"

When I first moved to Stanford about two and a half years ago, I had the privilege of taking on a bunch of new projects that arose through new colleagues that have reached out to me. Some are based on the curiosity around glycoscience, some are non-profit collaborations, others are focused on my HIV work.

I moved to Stanford partly because it has a medical center and a focus on the clinical sciences. Basic science-oriented projects that I started at Berkeley have taken a more translational path here. One example is the platform that we developed for ultrasensitive antibody detection, which is the same platform used for the oral fluid HIV test that we recently published (1). HIV is just one of several things that we can test for with such platforms – other infectious diseases, for example, or early detection of type 1 diabetes.

I'm hoping to accelerate down the home stretch towards achieving something I haven't been able to before: producing a therapeutic. Ultimately, I'm hoping that the science we're doing is going to have a beneficial impact on human health – after all, that's always been the end goal.

Reference

1. *W Aryitey, "Super saliva test", The Pathologist, 39, 11, (2018). Available at: http://bit.ly/2FGl2OL.*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