JULY 2019 # 56

the Pathologist™

Official society partner of The Pathologist

Upfront
Encouraging the use of point-of-care tests
11

In My View
SP142: its sensitivity and reproducibility
12 – 14

In Practice
Tumor budding as a novel biomarker
28 – 31

Profession
Pathology is truly a clinical discipline
40 – 42

The Picture of Health

A selection of the most beautiful, intriguing, and even humorous images in pathology

16 – 24

Envetec
change more into less

200 series

The new, safe, EPA-approved technology for the on-site treatment of lab-generated regulated medical waste

change more into less

- **40-50% COST REDUCTION FOR RED BAG WASTE**
- **END PRODUCT GRADED AS DOMESTIC WASTE**
- **WASTE TREATED QUICKLY & SAFELY ON SITE**

Meet with us at **AACC booth #3643**

AACC

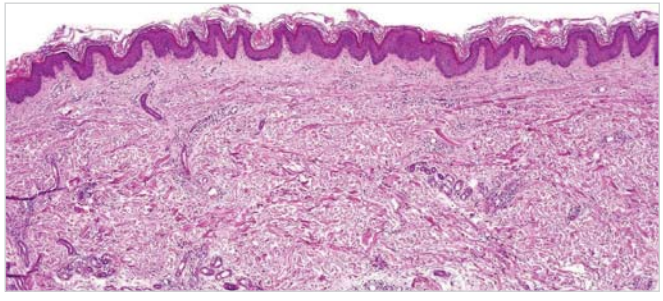
71ST AACC ANNUAL
SCIENTIFIC MEETING
& CLINICAL LAB EXPO

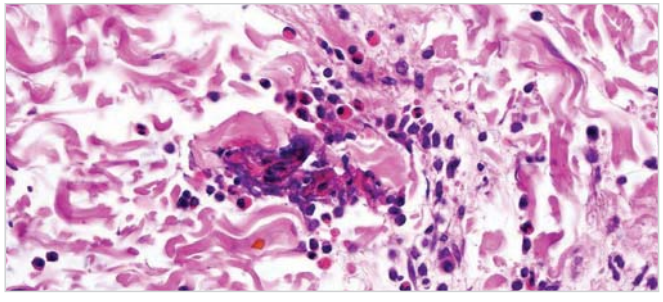
AUGUST 4–8, 2019
ANAHEIM, CA USA

Case of the Month

A 77-year-old man presented with an arcuate, erythematous patch on his lower back. The lesion was painful and pruritic. A biopsy was performed and histology from the case is presented here.

What is the most likely diagnosis?





a. Wells' syndrome

b. Ofuji disease

c. Angiolymphoid hyperplasia with eosinophilia

d. Tungiasis

e. Allergic contact dermatitis

Answer to last issue's Case of the Month…

D. Primary signet ring stromal tumor of the testis

Primary signet ring stromal tumor of the testis (PSRSTT) is a rare benign testicular tumor originally described in 2005 (1). The tumor is typically composed of low-grade epithelioid cells, most of which contain a large cytoplasmic vacuole. These vacuoles compress and displace peripherally the nucleus of each tumor cell, imparting a signet ring-like appearance. PSRSTT must be distinguished from metastatic signet ring carcinoma of the gastrointestinal tract; in this case, negative IHC data were useful for the final diagnosis. Data from the literature indicate that the tumors are positive for β-catenin, cyclin D1, CD10, galectin-3, claudin 7, and neuron-specific enolase (2).

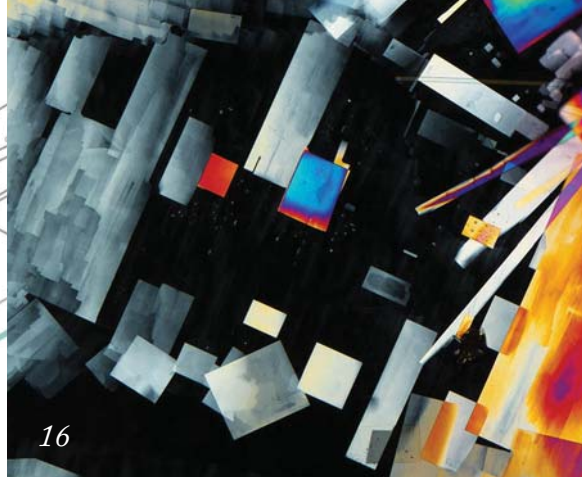
References

1. *M Michal et al., "Primary signet-ring stromal tumor of the testis", Virchows Arch, 447, 107 (2005). PMID: 15909171.*
2. *K Michalova et al., "Primary signet ring stromal tumor of the testis: a study of 13 cases indicating their phenotypic and genotypic analogy to pancreatic solid pseudopapillary neoplasm", Hum Pathol, 67, 85 (2017). PMID: 28739499.*

Submitted by Živka Eri, Professor of Pathology, Medical Faculty, University of Novi Sad, Serbia.

To register your guess, please go to http://tp.txp.to/0719/case-of-the-month
We will reveal the answer in next month's issue!

Contents



Case of the Month

03

Editorial

07 From the Ridiculous to the Sublime, by Michael Schubert

Upfront

08 A Meaningful Microbiome

09 A Microbial Map to Cancer

10 By Our Powers Combined…

11 To POCT or Not to POCT?

In My View

12 **David Rimm** questions the sensitivity and reproducibility of Roche's SP142 assay – and calls on the manufacturers to work with pathologists on a solution.

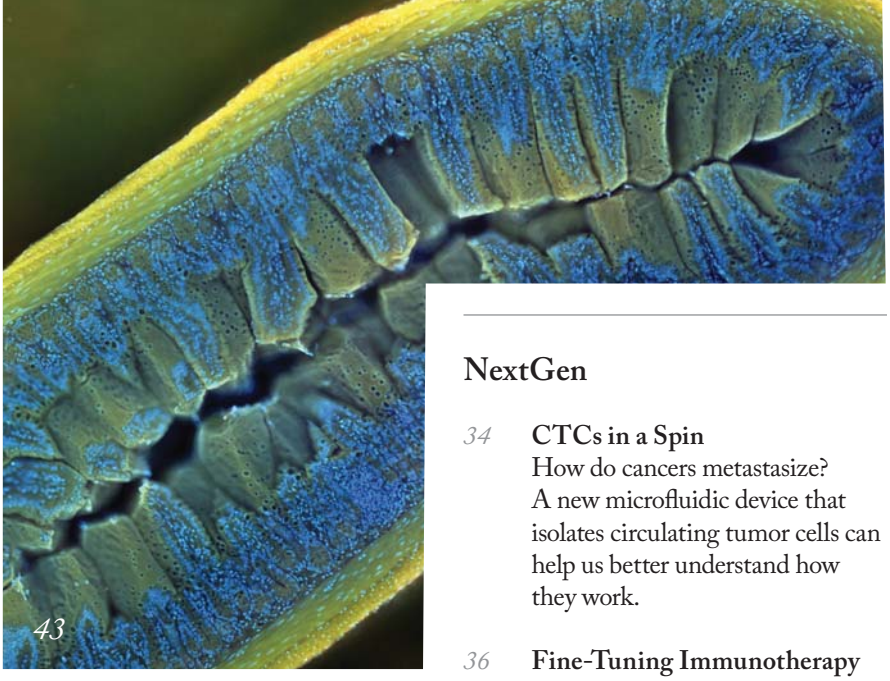
13 Four representatives from Roche – **Eslie Dennis, Eric Walk, Ken Bloom**, and **Mark Kockx** – respond in defense of the SP142 assay and its purpose: to help patients.

From the ASCP

15 **Fostering Quality**
To invest in the success and quality of laboratory medicine, we must invest in its people.

On The Cover

"None of Us Are Free," by Luis Humberto Cruz Contreras. See p. 16 for more.



43

Features

16 **The Picture of Health**
Our annual gallery special feature returns, with beautiful, intriguing, and even humorous examples of the art that comes out of the laboratory – all created by you.

Report

25 **The Value of Extended Availability**
Quality control consumes many of a laboratory's most limited and valuable resources. Reducing the need for lot-to-lot transitions can decrease both monetary and opportunity costs.

In Practice

28 **The Heavy Cavalry of Colorectal Cancer**
To best treat colorectal cancer patients, we must effectively stratify them – and tumor budding, a novel histopathological biomarker, can help.

NextGen

34 **CTCs in a Spin**
How do cancers metastasize? A new microfluidic device that isolates circulating tumor cells can help us better understand how they work.

36 **Fine-Tuning Immunotherapy**
Ensuring the right treatment for the right patient at the right time can be tricky, but advanced liquid biopsy techniques bring us ever closer to that goal.

Profession

40 **Pathology: A Clinical Specialty**
No field of medicine is entirely untouched by pathology, so why do pathologists hold themselves at a distance from the clinic, and how can we remedy that attitude?

43 **Lessons Learned, with Richard Levenson**
Laboratory medicine is a discipline for those who are always learning – so Richard Levenson's strides forward in microscopy, imaging, and diagnostics have much to offer.

Sitting Down With

50 **David Wilbur,** Former Director of Cytopathology and Clinical Imaging at Massachusetts General Hospital and current Chief Medical Scientist at Corista LLC, USA.

the **Pathologist**

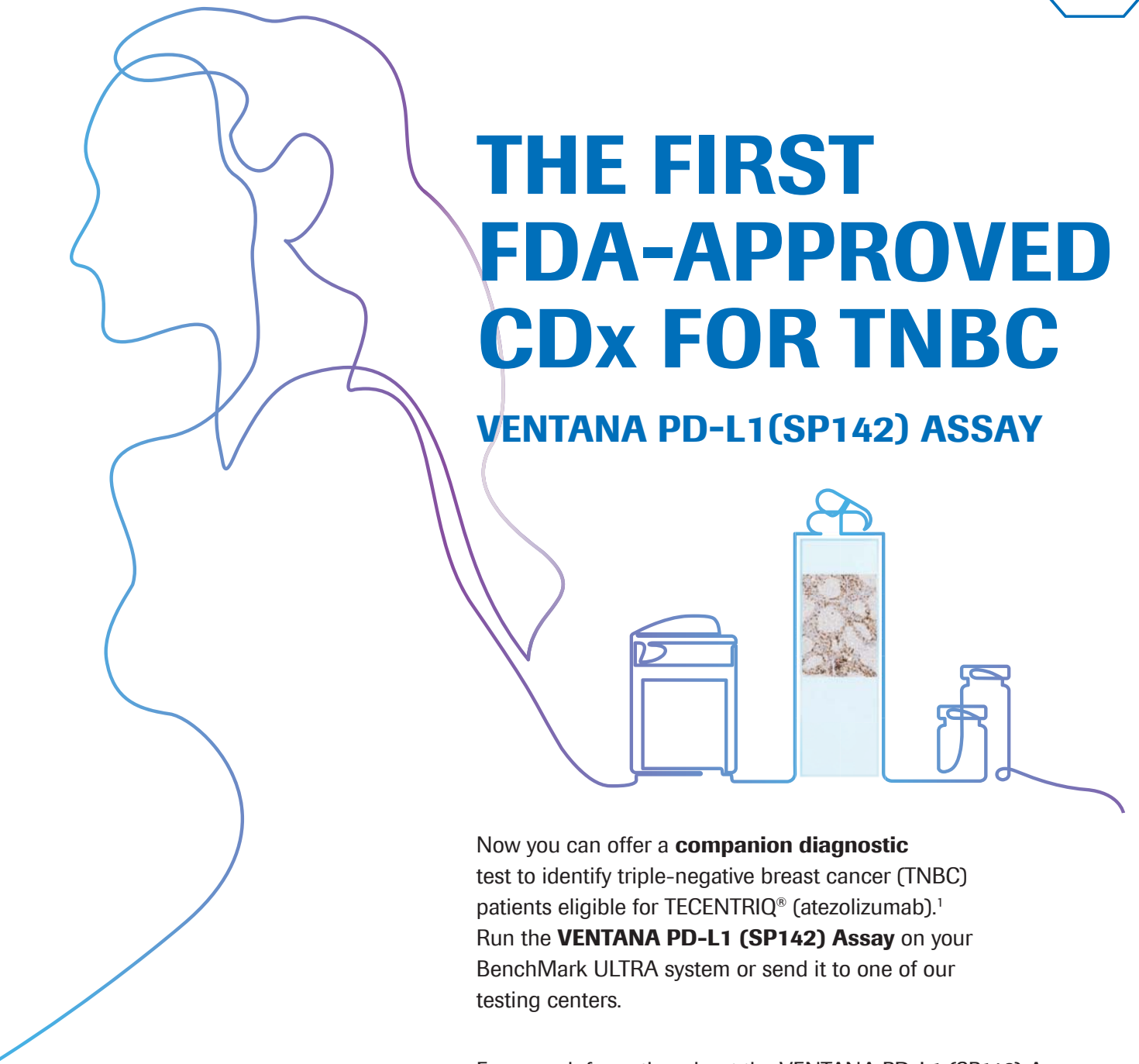
ISSUE 56 – JULY 2019

Change of address/ General enquiries
info@thepathologist.com
Hayley Atiz, The Pathologist, Texere Publishing Limited, Booths Park 1, Chelford Road, Knutsford, Cheshire, WA16 8GS, UK
www.texerepublishing.com
+44 (0) 1565 745200
sales@texerepublishing.com

Distribution:
The Pathologist (ISSN 2055-8228), is published monthly by Texere Publishing Limited, Booths Park 1, Chelford Road, Knutsford, Cheshire, WA16 8GS, UK
Single copy sales £15 (plus postage, cost available on request info@thepathologist.com)
Non-qualified annual subscription cost is £110 plus postage

THE FIRST FDA-APPROVED CDx FOR TNBC

VENTANA PD-L1(SP142) ASSAY



Now you can offer a **companion diagnostic** test to identify triple-negative breast cancer (TNBC) patients eligible for TECENTRIQ® (atezolizumab).[1] Run the **VENTANA PD-L1 (SP142) Assay** on your BenchMark ULTRA system or send it to one of our testing centers.

For more information about the VENTANA PD-L1 (SP142) Assay and a list of testing sites, visit **diagnostics.roche.com/pdl1SP142**

Your Assay Choice Matters

[1] VENTANA PD-L1 (SP142) Assay approved as first companion diagnostic to identify triple-negative breast cancer patients eligible for treatment with TECENTRIQ® in combination with ABRAXANE®.

From the Ridiculous to the Sublime

Art and the laboratory are inextricably intertwined

Summer 2019 marks the fourth year running that The Pathologist has hosted a gallery feature to showcase the artistry that emerges from the lab. Each year, the offerings are stellar, and each year, they become more creative. I've seen abstract paintings, paper quilling, animated GIFs, and even a nine-year-old's mixed media collage of a microscope!

I love seeing the submissions that come in each year – not only because they are beautiful, but because they remind me of one of a diagnostic professional's most vital traits: creativity. It's true that, often, the slide under the microscope (or the peaks on the spectrometer, or the lab values on the computer screen) seems straightforward and easy to label. But that's not always the case – and when it isn't, the ability to look beyond the typical becomes a valuable skill.

Pathology is a deeply visual discipline – so it comes as no surprise that its practitioners are equally so. I've met laboratory medicine professionals who draw, paint, or even sculpt as a hobby. I've met still more who do none of those things, but still find ways to marry the creative with the analytical. Take the popular social media hashtag #PathArt, for example. Those who contribute to it seek out the unusual, the humorous, and the bizarre in the images they see every day. Flowers in colon crypts; the Cookie Monster in a dentinal tubule; a chameleon in a thyroid smear. These ideas may sound silly at first, but consider that these people are training their pattern recognition skills. By looking for the outline of a dog in a frozen section, they may be honing their ability to look for an abnormal finding in an otherwise normal specimen.

So be proud of your paintings, your photographs, your Cookie Monsters! Share your work on the #PathArt hashtag and on the covers of journals! Never underestimate the value of an artist's eye in a scientist's laboratory and – perhaps just as important – never underestimate the value of a sense of humor in a serious field of medicine and research.

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

A Meaningful Microbiome

Cervical microbiota could help diagnosticians spot patients at high risk of cancer

The gut microbiome is increasingly notorious for its diagnostic potential and its effect on overall health. But just because it's the most famous doesn't mean it's the only one; a new study has revealed the potential of the cervical microbiome as a biomarker of cervical cancer risk.

A transatlantic group of researchers from the University of Nebraska–Lincoln and Tanzania's Ocean Road Cancer Institute conducted a study to investigate the relationship between human papillomavirus (HPV), human immunodeficiency virus (HIV), and cervical dysplasia (1). To that end, they collected cytobrush samples from the cervical lesions of 144 Tanzanian women and performed 16S rRNA gene deep sequencing to examine the microbiota present. The goal? To understand how the bacterial community differs between patients with different HIV status and cytology grade.

The researchers discovered that HIV significantly increases the overall richness of the cervical microbiome. HIV-positive individuals showed higher rates of Bacillus and Mycoplasma species, but lower rates of Lactobacillus species in particular.

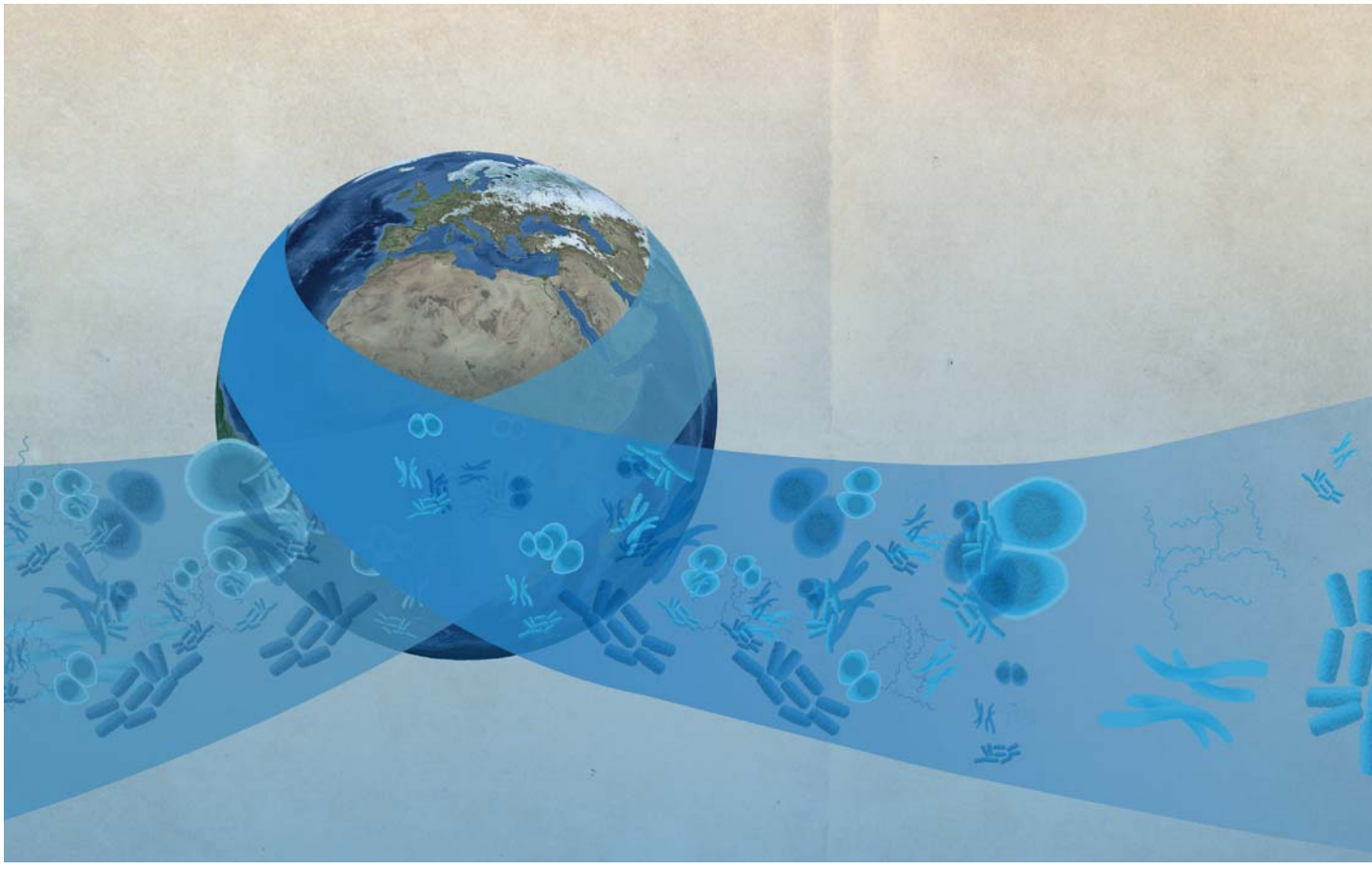
Additionally, different grades of precancerous lesion were associated with different microbiota after separating HIV-positive and HIV-negative groups. Bacteria of the Mycoplasmatales order increased in abundance as lesion severity increased, from 0.2 percent of the total microbiome in the absence of lesions to 3.9 percent in high-grade squamous intraepithelial lesions. The higher-grade lesions also showed increased overall microbial diversity. "There are certain families of bacteria that appear to be associated with the higher grades of precancerous lesions," said lead author Peter Angeletti in a recent press release (2).

Lead author Peter Angeletti and first author Cameron Klein. Credit: Craig Chandler | University Communication

It's possible that, one day, analysis of the cervical microbiome could help diagnosticians better spot patients at risk of cervical cancer – the second-most common cancer in women living in underdeveloped areas, and the fourth-most common in women worldwide. The study's authors are optimistic that, one day, such analysis might even allow for the development of preventative treatments that modulate the cervical microbiome for better health.

References

1. *C Klein et al., "Relationship between the cervical microbiome, HIV status, and precancerous lesions", MBio, 10, e02785018 (2019). PMID: 30782659.*
2. *L Reed, "Link between cervical microbiome and cancer discovered" (2019). Available at: https://bit.ly/2SO5yBW. Accessed June 7, 2019.*



A Microbial Map to Cancer

Machine learning meta-analysis reveals cancer signatures in the gut microbiome

We enjoy a symbiotic relationship with the trillions of microbes that inhabit our bodies. We provide them with a home and food; they assist in digestion, metabolism, and infection protection. But what if they have even more to offer? Recent research reveals that detectable changes in our gut microbiota may give us the opportunity to detect early-stage cancer.

Researchers from the European Molecular Biology Laboratory (EMBL) and the University of Trento used machine learning to perform a meta-analysis of eight metagenomic studies of colorectal cancer (1). Their goal? To identify microbial signatures distinct to cancer. "We validated these signatures in early cancer stages and in multiple studies, so they can serve as the basis for future noninvasive cancer screening," explained EMBL's Georg Zeller in a press release (2). Co-author Nicola Segata added, "We not only established a panel of gut microbes associated with colorectal cancer across populations, but also found signatures in microbial metabolism that have similar predictive power."

These signatures not only offer the potential to spot cancer using microbial signatures, they may also allow scientists to understand how gut microbes can contribute causally to the development of disease. Ultimately, Zeller says, the work may even lead to a better understanding of how we can modulate the microbiome to prevent cancer. "But that's very difficult!"

References

1. *J Wirbel et al., "Meta-analysis of fecal metagenomes reveals global microbial signatures that are specific for colorectal cancer", Nat Med, 25, 679 (2019). PMID: 30936547.*
2. *EMBL, "Global microbial signatures for colorectal cancer established" (2019). Available at: https://bit.ly/2HIjg1X. Accessed May 30, 2019.*

By Our Powers Combined…

Using IgM and IgA detection together can lead to better diagnosis of Zika and similar viruses

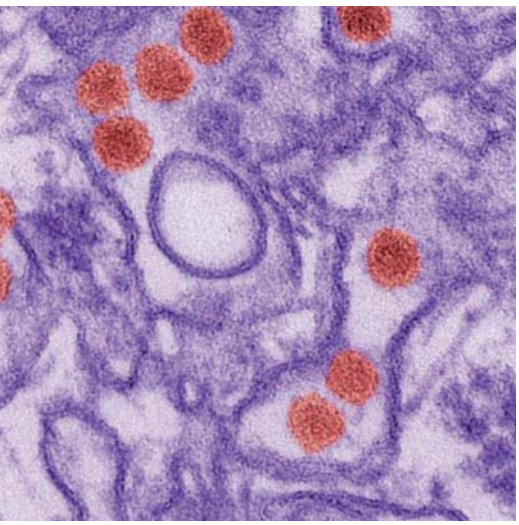
"Will Zika return? What pregnant women and others need to know about this frightening disease"

"1 in 7 babies exposed to Zika in the womb have health problems"

"A 'perfect storm' for the future spread of the Zika virus"

Viruses like Zika have been the subject of intense media attention, particularly since the widely publicized outbreaks in South America began. It's well-known that fetuses and newborns who carry the virus are at risk of permanent health issues – but how can doctors conclusively identify those infected?

Felix Drexler, head of the Virus Epidemiology Group at the Charité – Universitätsmedizin Berlin, explains that current diagnostic methods for Zika and similar viruses face one common denominator: low sensitivity. "This applies to both molecular methods, because viremia is super low and super short-lived, and to antibody tests – the latter in particular in tropical areas," he says. "People with multiple flavivirus infections mount weaker IgM responses, and these people are so full of antibodies against common flavivirus epitopes that serological test specificity diminishes greatly."



These complications can lead to false-positive results – a dangerous situation in general, but especially so for patients in outbreak regions or those without quality health care. Women may choose to terminate a pregnancy that might otherwise be healthily carried to term – and, in cases where safe reproductive care is difficult to access, choices like this can become fatal.

Why are false positives so prevalent? This is partly due to laboratory contamination and partly due to low test specificity. Drexler and his colleagues experimented with using IgA as a marker for acute infection, rather than the standard IgM approach and saw markedly increased sensitivity (1). "At least 50 percent in our study," says Drexler. "IgM and IgA may jointly allow high sensitivity and specificity. IgG increases in paired sera add significantly to this."

Combined antibody testing offers new diagnostic options for not only Zika, but also other viruses, such as Middle East Respiratory Syndrome (MERS) coronavirus. This will help laboratory medicine professionals distinguish between infections caused by different viruses and help patients and primary care providers understand how best to proceed after diagnosis – hopefully, saving lives in the process.

Reference

1. *FA Bozza et al., "Differential shedding and antibody kinetics of Zika and chikungunya viruses, Brazil", Emerg Infect Dis, 25, 311 (2019). PMID: 30666934.*

To POCT or Not to POCT

When commonly ordered tests have reliable POCT alternatives, why are doctors still ordering them from the laboratory?

Point-of-care diagnostics are becoming increasingly valuable; they save both patients and healthcare providers time and money, and they can provide rapid answers to better triage patients' need for care. Nonetheless, some of the most commonly ordered tests are not often performed at the point of care, despite the availability of point-of-care testing (POCT) technology.

For instance, consider C-reactive protein (CRP) and neutrophil count (NC), important markers of inflammation. A recent study from the University of Oxford suggests that both tests are in high demand, with general practitioners ordering an average of 36 CRP and 72 NC tests each week (1). But by sending these tests out to a laboratory, doctors often have to wait up to 24 hours for results that could influence their treatment decisions. POCT equivalents for both tests exist – so why aren't they being used?

The answer might lie in practitioners' familiarity with the laboratory versions of the tests, or a lack of information about POCT alternatives. Regardless, CRP and NC testing is ripe for the move from laboratory to POCT. "Inflammatory marker laboratory tests are requested frequently in the community, particularly in combination, with many patients needing repeat tests," said the study's lead author, José Ordóñez-Mena, in a recent press release (2). "We also find that CRP test requests are becoming increasingly common in older patients. Given that these tests can now be provided by point-of-care technologies, there is scope for this testing to start moving into the community, carried out by general practitioners for results within minutes, rather than being performed by central laboratories."

References

1. *JM Ordóñez-Mena et al., "C-reactive protein and neutrophil count laboratory test requests from primary care: what is the demand and would substitution by point-of-care technology be viable?", J Clin Pathol, [Epub ahead of print] (2019). PMID: 30992343.*
2. *NIHR, "Scope for inflammatory marker laboratory tests to move into community settings" (2019). Available at: https://bit.ly/2EUqtKQ. Accessed June 7, 2019.*

In My View

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

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

Contact the editors at edit@thepathologist.com

Welcome to Our Kitchen

Turning pathologists into chefs

By David L. Rimm, Professor of Pathology and Director of Yale Pathology Tissue Services, Yale University School of Medicine, New Haven, USA

In this food-crazy world, every chef has a special recipe. Unfortunately, that approach has now been forced onto pathologists, who would understandably rather consider themselves scientists than chefs. The difference? A chef's secret recipes are highly successful, but hard to reproduce. Pathologists aspire to high-level assay reproducibility. We often create our own tests (known as lab-derived tests, or LDTs), rather than use a kit, so that we know exactly what is in each component of the assay. It's the protocol – not the recipe – that leads to a high level of reproducibility.

With the FDA's approval of atezolizumab with the SP142 companion diagnostic test, Roche Diagnostics has relegated pathologists to the role of short-order cook. Although it is true that the IMpassion 130 trial shows that atezolizumab improves survival in patients with high PD-L1 (1), their recipe for detection of high PD-L1 is no less secret than a master chef's prize dish. The SP142 assay has been shown by two independent prospective multi-institutional studies not only to have lower sensitivity for detection of PD-L1 protein by immunohistochemistry, but also – when using the prescribed measurement criteria – to be non-reproducible (2,3).

Why would the FDA approve such an assay? Clearly, the drug is the dog and the assay is the tail. No doubt there is great political pressure to make available a drug for triple-negative breast cancer that increases median survival by 10 months in the PD-L1-positive group. So pathologists just need to buy the kit and do the assay – right? Unfortunately, data in the literature suggests that it will be hard to accurately reproduce the assay approved by the FDA. First, pathologists are asked to score "immune cells" – a task shown to be reproducible between the two or three company pathologists in the SP142 summary of safety and effectiveness data from the FDA, but not between the 13 or 25 pathologists participating in statistically powered, prospective studies done in the real world. What will happen when thousands of pathologists around the world

"Whether the pathologist uses the FDA-approved test or an LDT, there is no clear way to standardize and quality control the assay."

are expected to read this assay? Second, the assay is less sensitive than others that detect PD-L1. Because the recipe is secret, we can't be sure why, but both pathologist-read and objective cell line measurement studies have shown the assay to be negative in cases or cell lines that are positive by other assays (4). Whether the pathologist uses the FDA-approved test or an LDT, there is no clear way to standardize and quality control the assay. With the other assays, validation can be done by comparing them to one another. This is especially important for companion diagnostic tests. We know that the SP142 assay is less sensitive, so there is no standard for comparison or validation.

In Roche's defense, they did not know when they began the IMpassion 130 trial that their assay would be less sensitive or that their reading system would show poor reproducibility. Furthermore, with over 900 patients, a 10-month improvement in overall survival is hard to ignore. Nevertheless, the whole trial hinges on the reads of one pathologist in a central lab placing 369 patients into a negative or positive PD-L1 group – hardly a foolproof process.

What is the way forward? One possibility would be for Roche to test an RT-PCR assay that they have, in their Poplar trial, shown to be predictive in lung cancer (5). RT-PCR of three specific mRNAs could be much more objective and probably more reproducible. Another approach would be to use cell lines with known PD-L1 protein concentrations to standardize their IHC assay against other assays, or to test the IMpassion 130 tissues with multiple IHC assays, including their own SP263 assay, to allow harmonization with existing assays.

Because other PD-L1 assays are more sensitive than the SP142 test, we will never know how many patients will receive treatment based on those assays and show no benefit. Nor will we know how many patients who won't receive treatment (due to the challenge of accurately reading the assay) who might have benefited. In all cases, the patients are the potential victims – but this appears to be completely under the radar of the hype surrounding this new drug. It is my hope that Roche/Genentech/Ventana will work with pathologists to find a solution by bringing some science to the table.

References

1. *P Schmid et al., "Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer", N Engl J Med, 379, 2108–2121 (2018). PMID: 30345906.*
2. *DL Rimm et al., "A Prospective, Multi-institutional, Pathologist-Based Assessment of 4 Immunohistochemistry Assays for PD-L1 Expression in Non-Small Cell Lung Cancer", JAMA Oncol, 3, 1051–1058 (2017). PMID: 28278348.*
3. *MS Tsao et al., "PD-L1 Immunohistochemistry Comparability Study in Real-Life Clinical Samples: Results of Blueprint Phase 2 Project", J Thorac Oncol, 13, 1302–1311 (2018). PMID: 29800747.*
4. *S Martinez-Morilla et al., "Quantitative Assessment of PD-L1 as an Analyte in Immunohistochemistry Diagnostic Assays using a Standardized Cell Line Tissue Microarray" – under review.*
5. *L Fehrenbacher et al., "Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial", Lancet, 387, 1837–1846 (2016). PMID: 26970723.*

Improving Patient Outcomes

How PD-L1 testing identifies the right patients for treatment

By Eslie Dennis, Vice President and Head, Global Medical Affairs, Roche Tissue Diagnostics; Eric Walk, Chief Medical & Scientific Officer, Senior Vice President, Medical & Scientific Affairs, Roche Tissue Diagnostics, Tucson, USA; Ken Bloom, Chief Medical Officer/Advanced Pathology & Genomic Services, Invicro and Ambry Genetics, Konica Minolta Companies, Aliso Viejo, USA; and Mark Kockx, Founder, Chief Executive Officer & Chief Medical Officer, Histogenex Laboratories, Antwerpen, Belgium

We thank David Rimm for his comments and the opportunity to respond. As a cancer community unified by a common mission, we have a great responsibility to tackle serious unmet patient needs and deliver effective treatments through innovative and collaborative approaches to drug and diagnostic discovery, development, and integration into clinical practice.

Patients with advanced or metastatic triple-negative breast cancer (TNBC) experience poor outcomes relative to patients with other breast cancer subtypes (1,2). TECENTRIQ (atezolizumab) in combination with nab-paclitaxel was granted FDA Accelerated Approval for the treatment of adult patients with unresectable, locally advanced, or metastatic TNBC whose tumors express PD-L1 – defined as PD-L1 stained tumor-infiltrating immune cells (IC) of any intensity covering ≥ 1 percent of the tumor area – as determined by an FDA-approved

test (3). The VENTANA PD-L1 (SP142) Assay (SP142 assay) is the FDA-approved companion diagnostic to TECENTRIQ (4)*. These approvals were based on the IMpassion130 clinical trial (5) and represent the first immunotherapy regimen for breast cancer and an important new treatment for its most aggressive subtype.

In the IMpassion130 study, patients whose tumors were positive for PD-L1 showed a stratified hazard ratio (HR) of 0.62 ($p<0.0001$) for median progression free survival (PFS) in favor of the TECENTRIQ + nab-paclitaxel combination. Exploratory analysis showed no benefit in PD-L1-negative patients as tested by the SP142 assay, further supporting the assay's role in identifying patients who may benefit from the TECENTRIQ combination. The second interim overall survival (OS) analysis demonstrated a HR of 0.71 in PD-L1-positive patients; OS was not formally tested in the PD-L1-positive population due to the hierarchical design for OS. As with PFS, exploratory analyses showed no benefit in the PD-L1-negative patients (6). Additional exploratory biomarker analysis evaluating PD-L1 expression on tumor cells, stromal tumor-infiltrating immune cells, and cytotoxic T cells concluded that PD-L1 IC expression based on the SP142 assay was the best predictor of clinical benefit (7).

Regarding the differential performance characteristics of PD-L1 assays, it is important to distinguish analytic sensitivity and specificity from the ability of these assays to predict treatment response. The SP142 assay incorporates an amplified detection system with a different staining pattern to other approved PD-L1 assays. This has created challenges for pathologists seeking to harmonize these assays so that they can use one PD-L1 assay to make therapeutic decisions for several different PD-1 and PD-L1 inhibitors across several different indications based on different scoring algorithms. Although the desire for streamlining of testing is understood, the role of a companion diagnostic assay is to discriminate between responders and non-responders for that specific therapeutic product in a specific indication, with a cutoff based on clinical outcomes.

In vitro diagnostic devices (IVDs) are subject to design controls and must be validated and comply with Quality Systems regulations. Companion diagnostic assays must demonstrate both analytic and clinical validity. Results of validation studies approved by the FDA pertaining to the analytical specificity and sensitivity, repeatability, precision, and readability of the SP142 assay are publicly available (8).

The era of precision medicine in oncology reinforces the role of pathologists as partners to oncologists, and Roche wholeheartedly supports the education and training of the global pathology community. Data from the Roche international pathologist training program for the SP142 assay (8) have demonstrated reproducibility in both NSCLC (TC/IC algorithm) and urothelial carcinoma (UC) (IC only algorithm) indications. Average agreement rates were 88.3 and 95.3 percent respectively (9), likely reflecting the simpler IC-only algorithm across two categories in UC. The TNBC algorithm similarly includes IC-only assessment across two categories. Roche has currently trained more than 1,000 pathologists globally through in-person programs for non-small cell lung cancer (NSCLC), UC and TNBC indications; this data will be published shortly.

Although NSCLC is not a surrogate for TNBC, the Blueprint 2 study (10) conducted in NSCLC noted that the SP142 assay was the only assay to show moderate-to-strong agreement between pathologists versus the trainer for distinguishing IC0 versus IC1, 2, and 3, which corresponds with the IC1 percent cutoff.

Roche is dedicated to working with pathologists and the healthcare community to find solutions to challenges in an evolving era of precision medicine and remains committed to advancing scientific innovation to improve clinical outcomes.

**The VENTANA PD-L1 (SP142) Assay may not be available for the TNBC indication in all geographies.*

References

1. *Y Gong et al., "Impact of molecular subtypes on metastatic breast cancer patients: a SEER population-based study", Sci Rep, 7, 45411 (2017). PMID: 28345619.*
2. *NCI SEEER, "Cancer Stat Facts: Female Breast Cancer Subtypes" (2016). Available at: https://bit.ly/2IrIBh7. Accessed June 20, 2019.*
3. *Drugs@FDA: FDA Approved Drug Products, "TECENTRIQ" (2019). Available at: https://bit.ly/2ZK6CpJ. Accessed June 20, 2019.*
4. *Premarket Approval, "VENTANA PD-L1 (SP142) Assay" (2019). Available at: https://bit.ly/2ZH0Fd3. Accessed June 20, 2019.*
5. *P Schmid et al., "Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer", N Engl J Med, 379, 2108 (2018). PMID: 30345906.*
6. *P Schmid et al., "IMpassion130: updated OS from a global, randomized, double-blind, placebo-controlled, Phase III study of atezolizumab + nab-paclitaxel in previously untreated locally advanced or metastatic TNBC". Presented at the 2019 ASCO Annual Meeting; May 31–June 4, 2019; Chicago, USA. Abstract #1003.*
7. *LA Emens et al., "IMpassion130: efficacy in immune biomarker subgroups from the global, randomized, double-blind, placebo-controlled, phase III study of atezolizumab+ nab-paclitaxel in patients with treatment-naïve, locally advanced or metastatic triple-negative breast cancer". Presented at the San Antonio Breast Cancer Symposium; December 4–7, 2018; San Antonio, USA.*
8. *Premarket Approval, "Summary of Safety and Effectiveness Data" (2019). Available at: https://bit.ly/2L4zxAu. Accessed June 20, 2019.*
9. *E Dennis et al., "Robust and reproducible pathologist training for VENTANA PD-L1 (SP142) assay assessing tumor cells (TC) and immune cells (IC) utilizing a novel digital training platform", Virchows Arch, 471 (Suppl 1), 1 (2017).*
10. *MS Tsao et al., "PD-L1 immunohistochemistry comparability study in real-life clinical samples: results of Blueprint phase 2 project", J Thorac Oncol, 13, 1302 (2018). PMID: 29800747.*

Fostering Quality

Laboratory excellence requires investing in our people

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

Pathologists and laboratory scientists are obsessed with quality – and rightly so. Accurate results, data registries such as ASCP's National Pathology Quality Registry, quality assurance reports, and quality control measures… it's safe to say that we all eat, sleep, and breathe quality. All of these elements require the right people to operate and manage them – which means that, if we invest in those people, we invest in quality. How does that concept apply to pathology?

It means investing in cutting-edge technology. We can digitize pathology services, implement telepathology systems to better serve rural and international areas, or expand molecular analytical departments so we are one step closer to providing truly personalized laboratory diagnostics. Investing in technology also means we invest in our communities.

"Laboratory practices that invest in people invest in quality and allow quality to flourish."

It means supporting continuing education efforts. That education can come in a variety of forms, such as teaching courses at a university, accepting residents into our workplace, attending professional society meetings, and engaging in leadership training. Investing your time back into the community enriches us all. By teaching new residents, networking with new colleagues through social media, and providing in-service education to laboratory staff, we improve morale and strengthen relationships. When we support pathologists' lifelong learning, we support quality patient care.

It means being appropriately remunerated. Whether working within merit-based incentive systems, securing fair payment practices from insurance companies, or ensuring competitive pay scales, we need to make it clear that our contributions have monetary value.

It means work-life balance. Burnout in the medical community is an ongoing concern, so it's important to remember that we have a life outside our practice. Taking time away from the microscope or clinical care team meetings allows us to stay emotionally balanced. Time off can often act as a reset button and allow us to remember what brought us to the profession in the first place – helping others through scientific advancement.

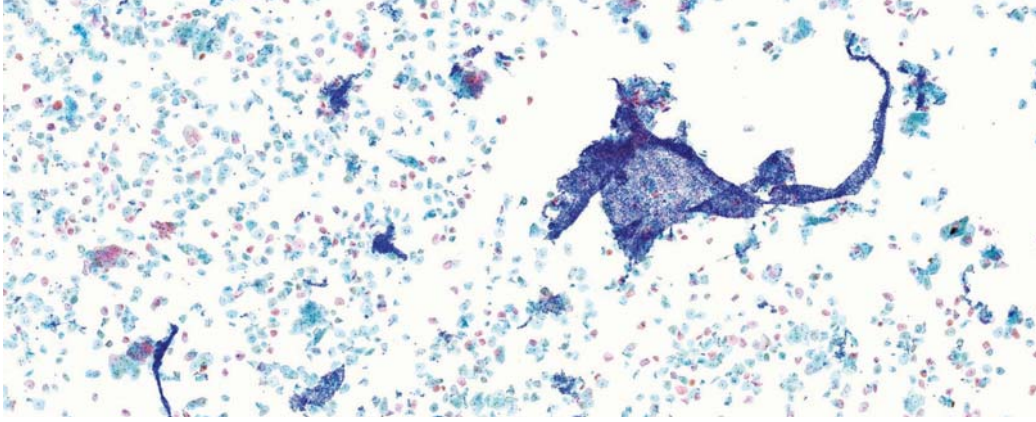
It also means advocating for those working in other roles. Our laboratory professional colleagues – from phlebotomists to pathologists – deserve the best we can provide. Upgrades to equipment, regular continuing education, and competitive pay practices are important for every rung of the career ladder. A rising tide lifts all yachts, so it's imperative that we invest the necessary resources into the entire laboratory ecosystem. Laboratory practices that invest in people invest in quality and allow quality to flourish.

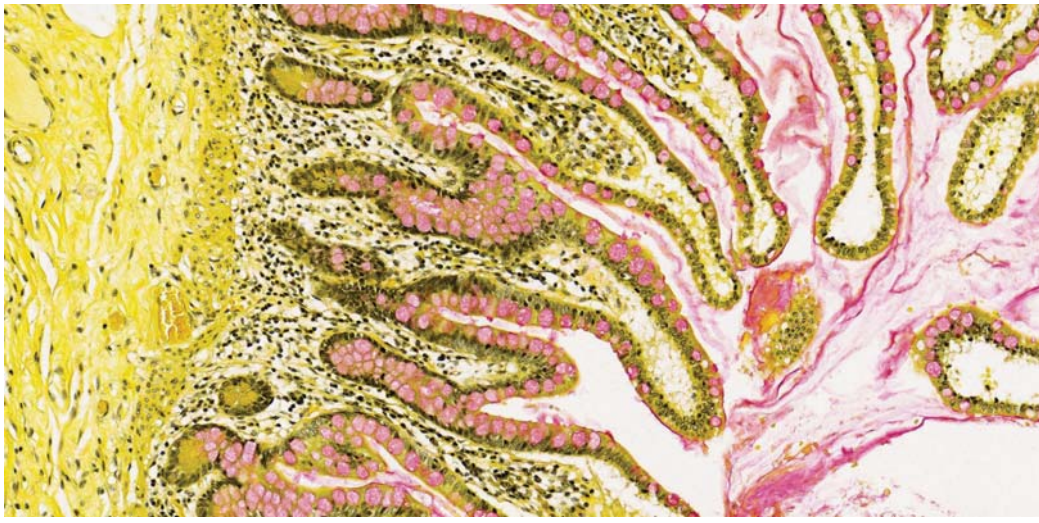

The
PICTURE
of
HEALTH

None of Us Are Free

Heart and lung dissection.

Luis Humberto Cruz Contreras, Hospital Materno Infantil, Irapuato, Mexico



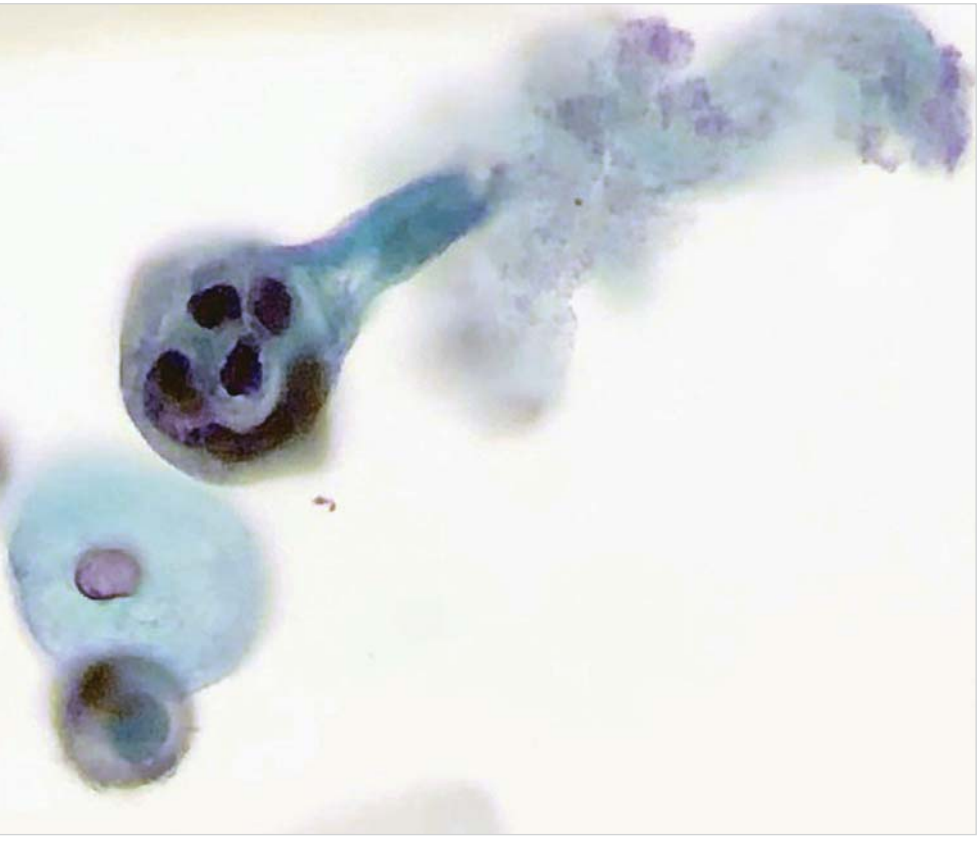


Cytology Thin Prep Slide

Mucin-Stained Stomach Cells

Striking slide scans captured on the MoticEasyScan One digital scanner with a 40X objective.

Casey Wahl, Motic Digital Pathology, San Francisco, USA

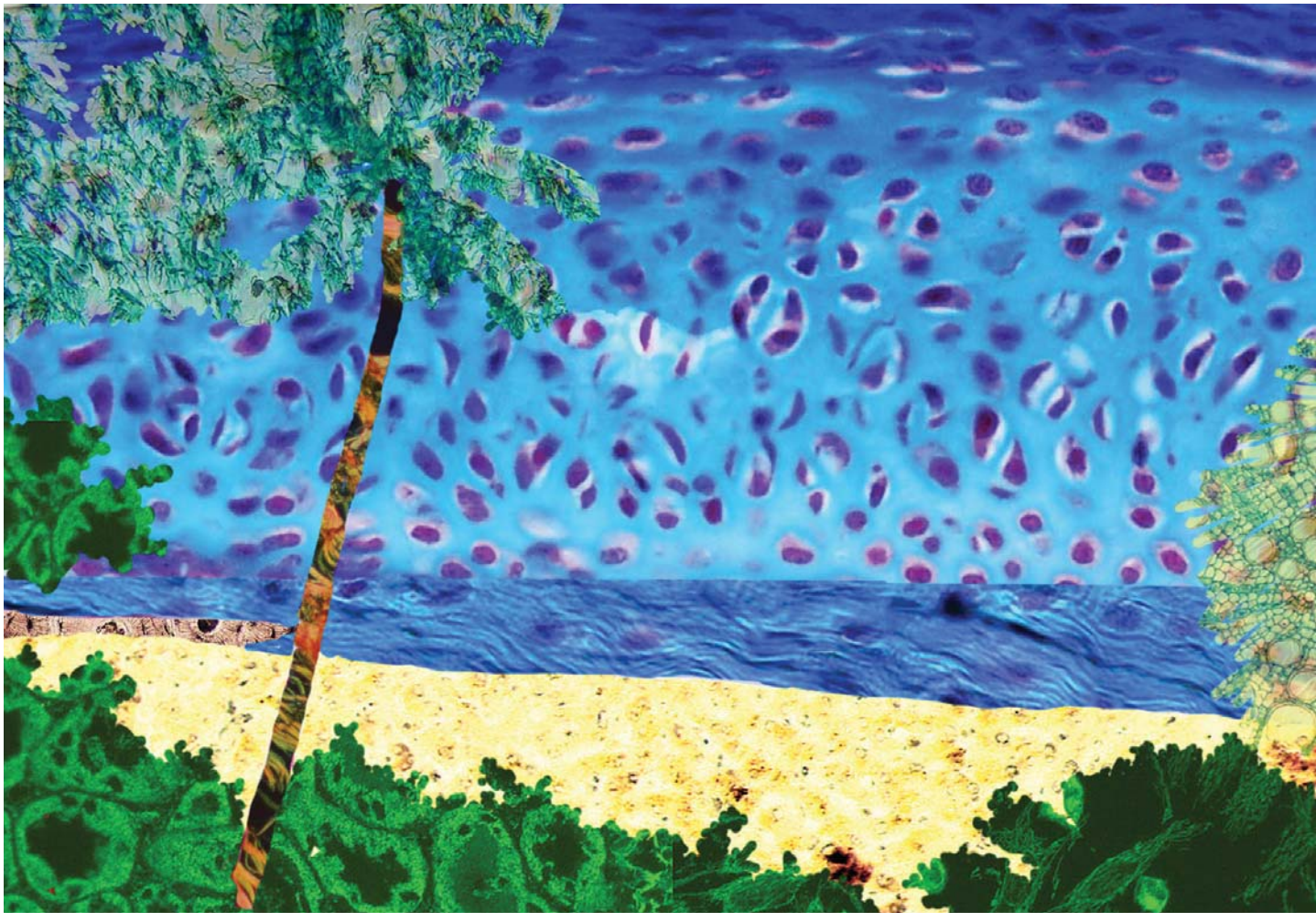


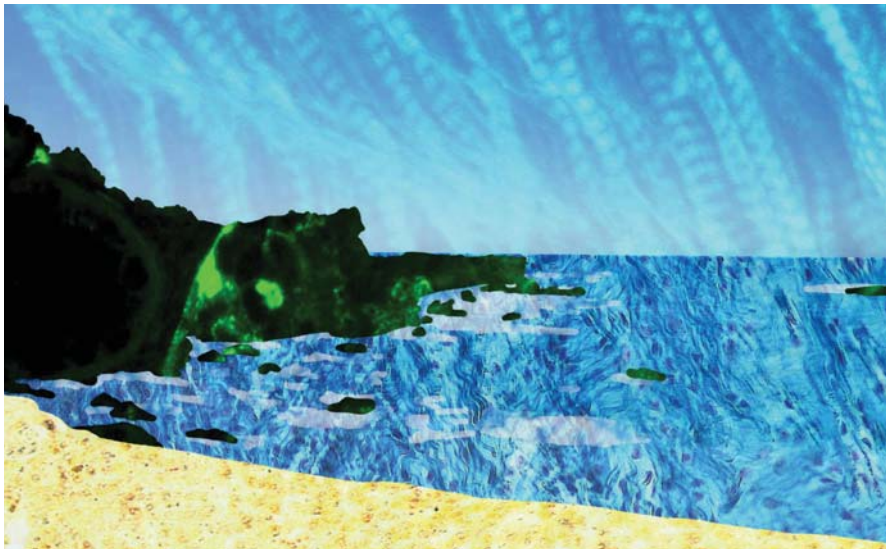
Funny Face From Urine

Epithelium's Fungal Fate

Candida and cells.

Sarah Kelting, University of Kansas Medical Center, Kansas City, USA

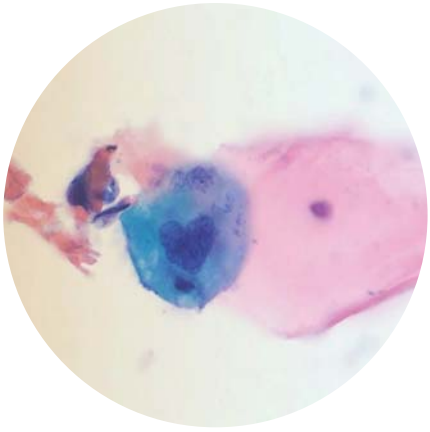




The North Shore

Digital collages inspired by memories of the North Shore of O'ahu. Made with Adobe Photoshop.

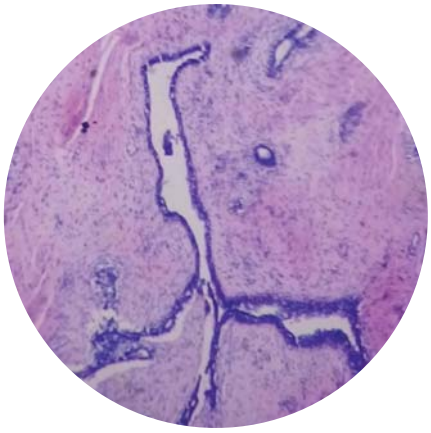
Cooper Schwartz, Alpert Medical School at Brown University, Providence, USA



Heart Shape of a Nucleus

It's easy to be impressed by hidden images in pathology, such as this heart hidden within a nucleus.

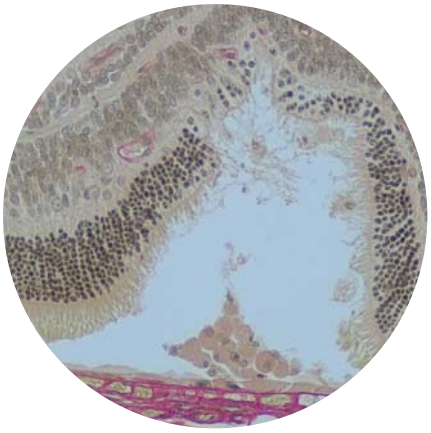
Lara Pijuan, Hospital del Mar, Barcelona, Spain



Kissper

This image is from a fibroadenoma, H&E-stained, 100X magnification.

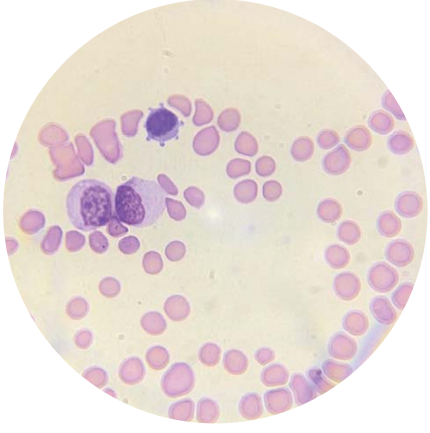
Rico P. Lasaca, Our Lady of Porziuncola Hospital Inc., Calbayog City, Western Samar, Philippines



Pathology of the Eye

A pretty stain of the retina showing vessels.

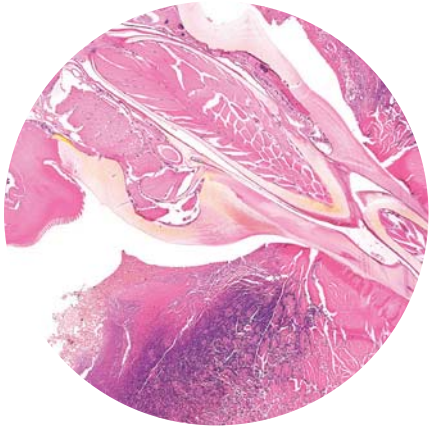
Paula Keene Pierce, President, Excalibur Pathology, Inc.,Norman, USA



Abnormal Lymphocytes

A sample from an acute lymphoblastic leukemia patient stained with Wright's stain, showing anisocytosis and macrocytosis.

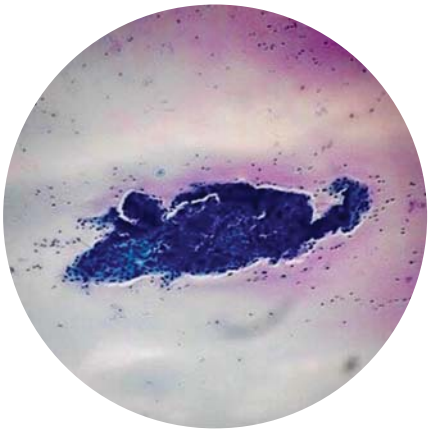
Nina Simonini, Medical Laboratory Technician, AdventHealth Oncology and Hematology Lab, Orlando, USA



Tick Bite

This microscopic image is a skin biopsy showing a tick bite, with the tick's mouth part attached to the skin in its entirety. You can even see the microanatomy of the insect.

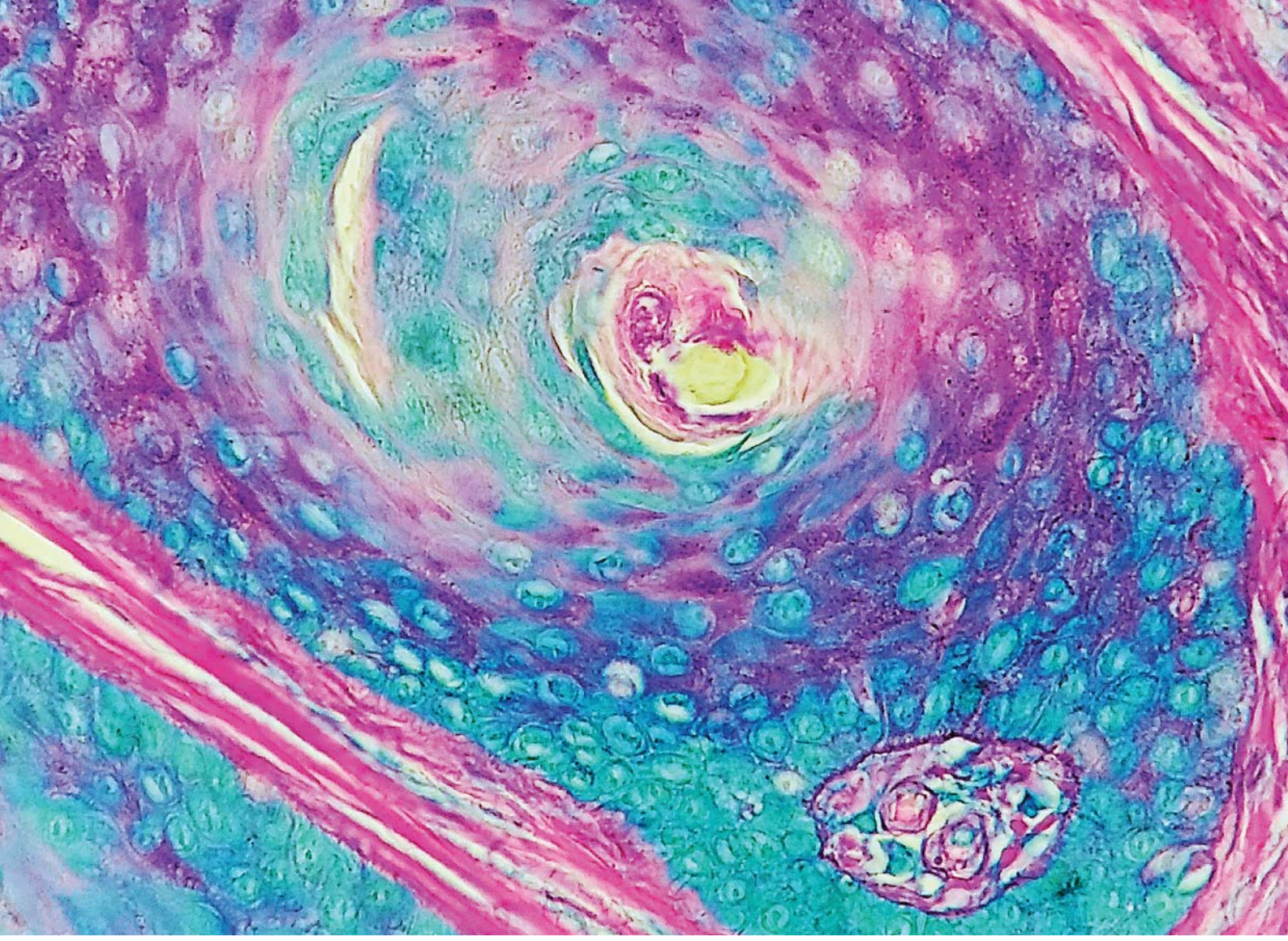
Rola H. Ali (@DrRolaAli), Associate Professor of Pathology, Faculty of Medicine, Kuwait University, and Pathologist, Cytogenetics/Molecular Lab, Kuwait Cancer Control Center, Kuwait City, Kuwait

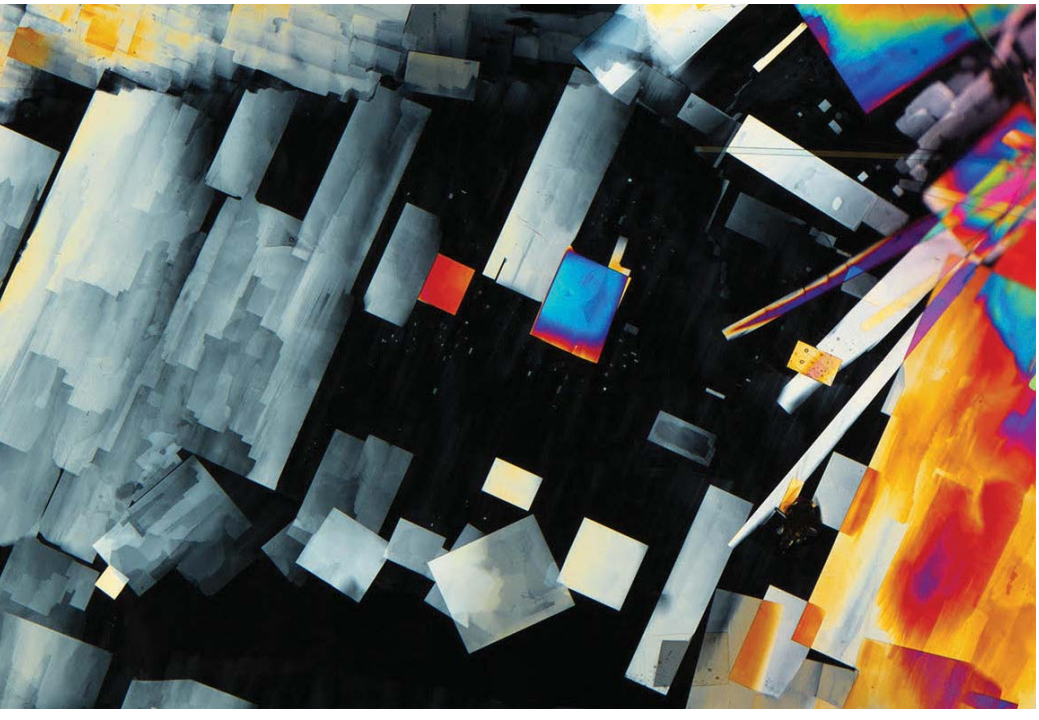


When the Cat Is Away, the Blue Mouse Will Play

This image is from a cervicovaginal Pap smear and shows aggregates of parabasal cells.

Rico P. Lasaca, Our Lady of Porziuncola Hospital Inc., Calbayog City, Western Samar, Philippines





Et Lux Perpetua

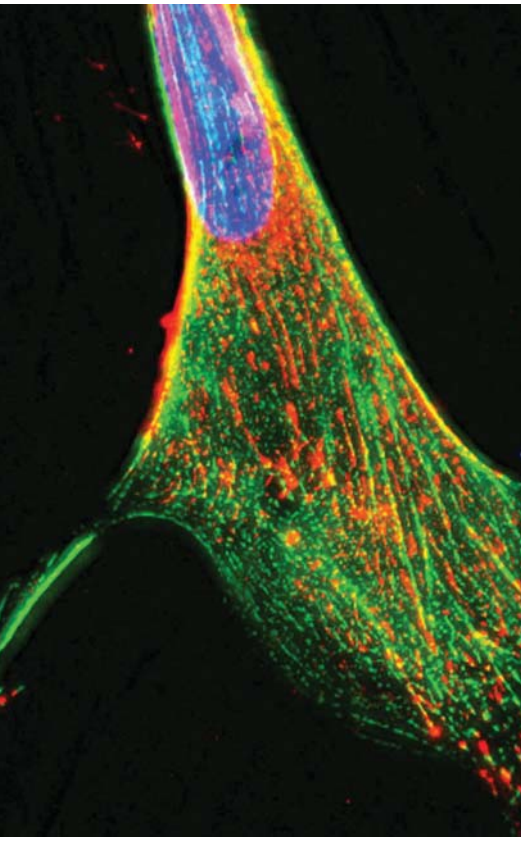
Epidermis stained with Alcian blue.

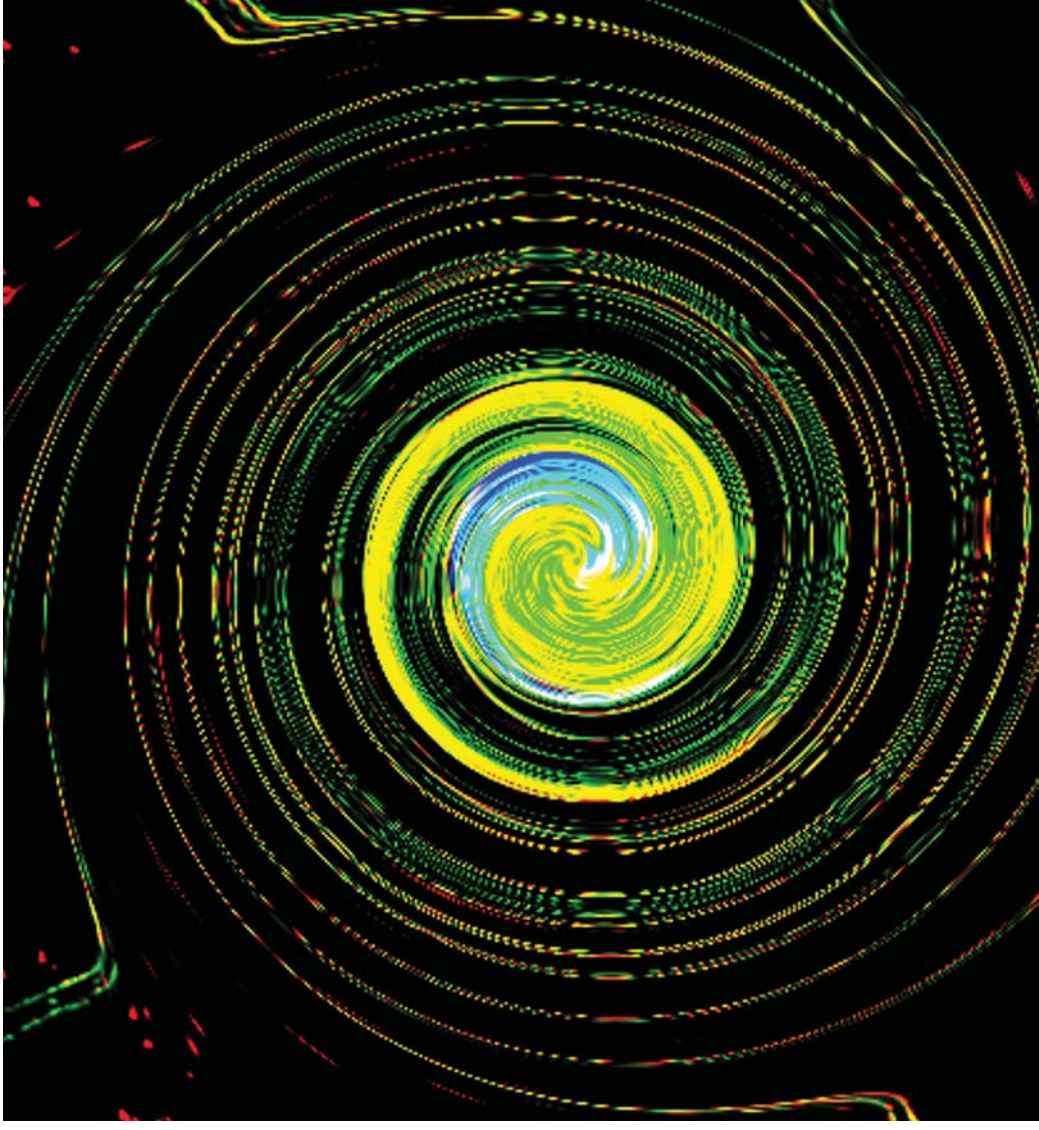
Luis Humberto Cruz Contreras, Hospital Materno Infantil, Irapuato, Mexico

Orbit

Arizona wine crystals photographed through a microscope.

Scott Taft, Tucson, USA

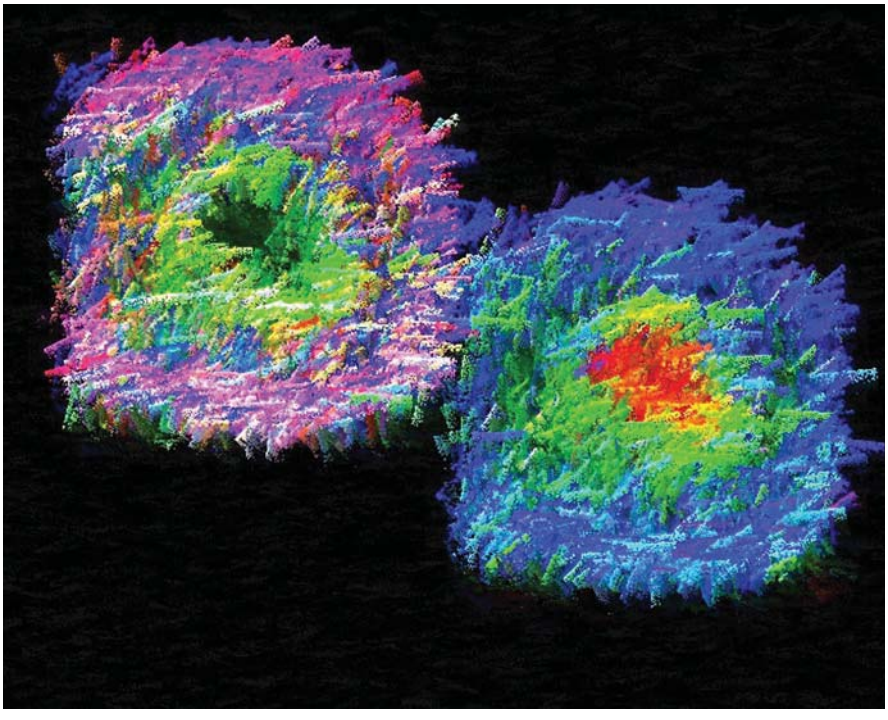


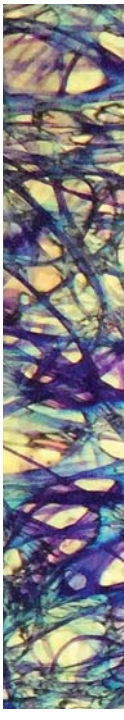


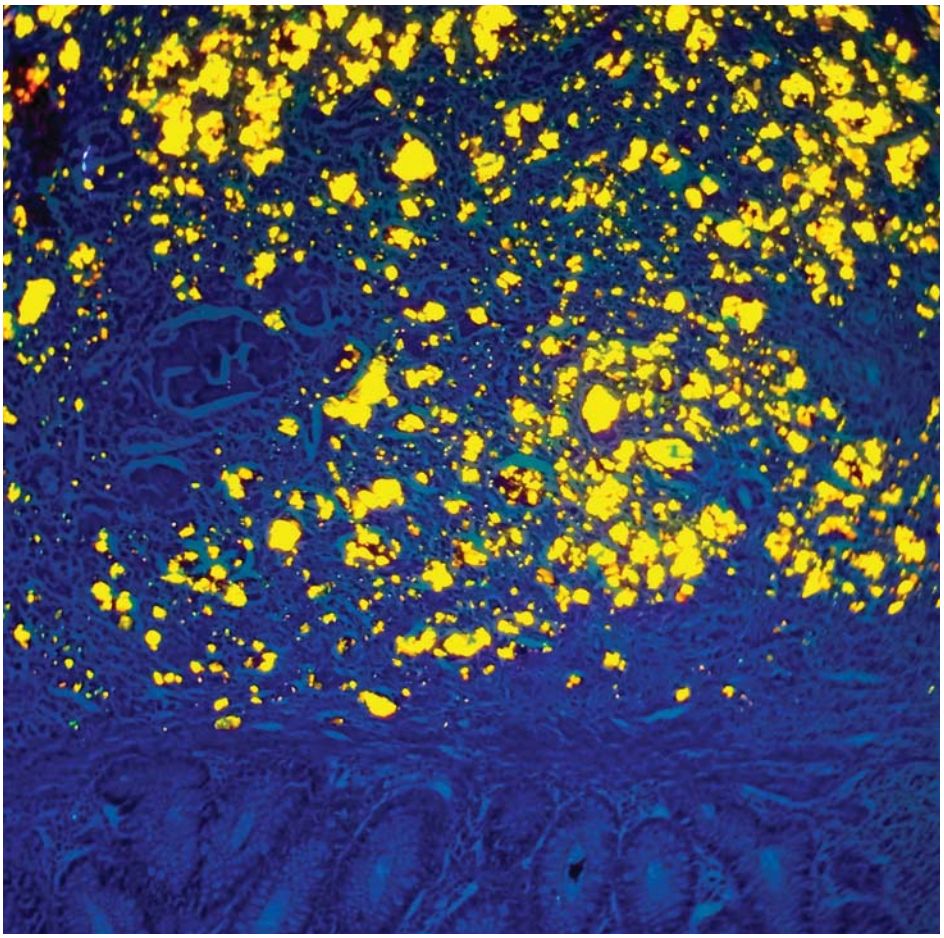
The Art of Fluorescence Deconvolution Imaging, Part III

A series of artistic images created using fluorescence deconvolution microscopy.

Brian J. Poindexter and Roger J. Bick, Multi-User Fluorescence Imaging and Microscopy Core Lab, UT McGovern Medical School, USA







Van Gogh

Bright yellow flecks of hematoidin crystals are strewn across this colonic wall in a patient with aortoenteric fistula, redolent of a madman/genius post-impressionist painter's most iconic work, "The Starry Night."

Randell Arias, Zamboanga City Medical Center, Philippines

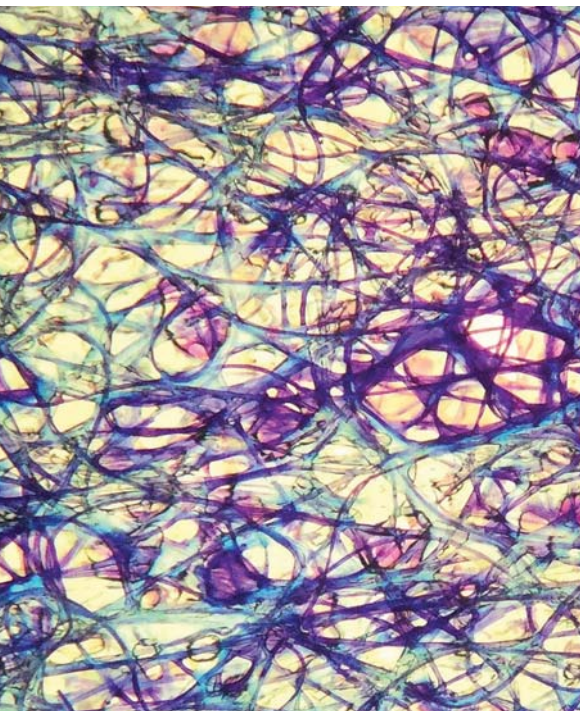
Tangled Web

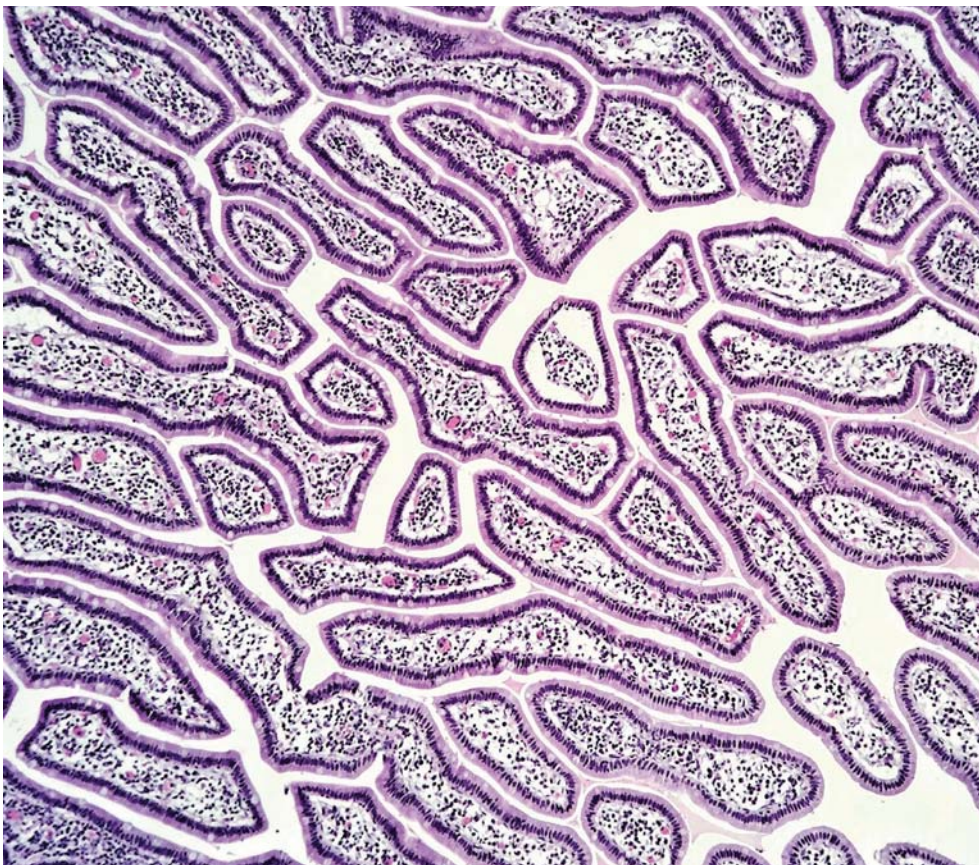
Bottom left: Several laboratories in the Philippines are behind the latest advances in slide labeling. This micrograph features tissue stain caught up in the tangles of micropore tape fibers.

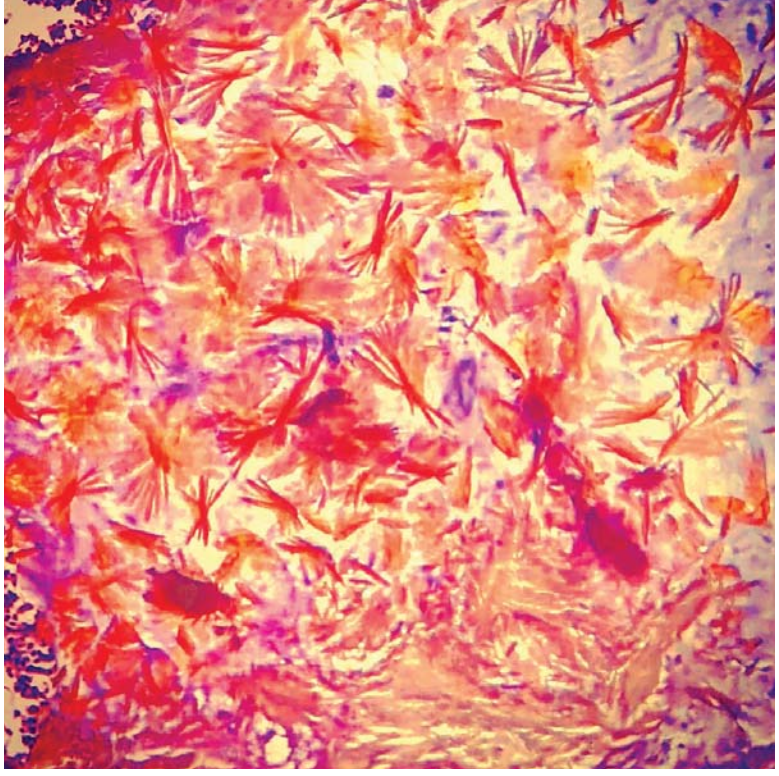
Maze

Bottom right: Scanning hematoxylin and eosin stain of the small bowel.

Othaniel Philip R. Balisan, The Philippine Heart Center, Manila, Philippines



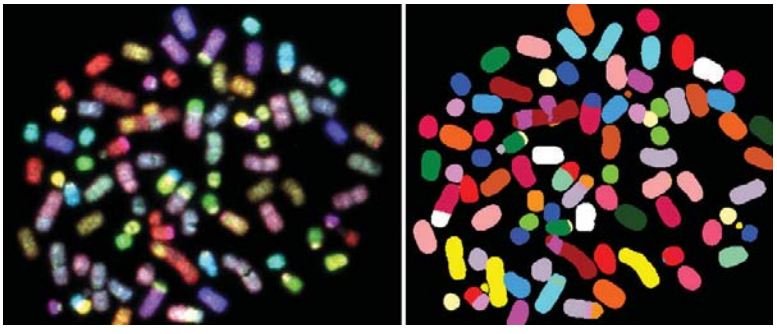




Stream of Fire During Fall in Wilderness

This picture depicts a fragment of bone tissue with tyrosine-like crystals that refract on a polarizing microscope using H&E stain. Colors were enhanced by filters.

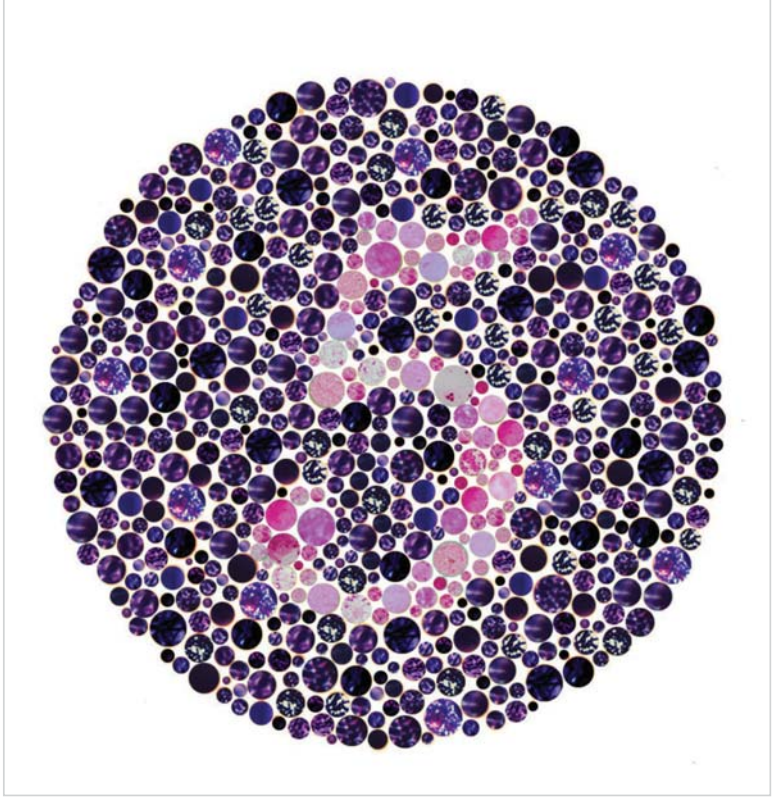
Franz Jobert L. Sebastian, The Philippine Heart Center, Manila, Philippines



SKY Illustration

A SKY picture of mouse cancer models showing extensive chromosomal rearrangements.

Murty Vundavalli, Associate Professor, Institute for Cancer Genetics, Columbia University, New York, USA



Basic Fuchsin Five

This image is an IshiharaGram, an aesthetic concept series I created by merging the Gram stain and the Ishihara test, two different techniques in medicine that use color as a primary mechanism for determining clinical criteria. The pink five is composed of colored dots representing microscopic fields of GNRs or PMNs, whereas the surrounding purple dots represent microscopic fields of GPCs, GPRs, or yeast.

Ansel Oommen, Clinical Laboratory Technologist, New York-Presbyterian Morgan Stanley Children's Hospital, and Research Assistant, New York State Psychiatric Institute, Columbia University Medical Center, New York, USA

The Value of Extended Availability

How QC materials with long-term stability can stabilize the laboratory

By Samuel Reichberg, MD, PhD, FCAP

If you could improve one thing about your laboratory technical operations, what would it be? As someone with decades of clinical laboratory experience, I've found analytical quality control (QC) to place some of the highest demands on laboratory professionals engaged in the unrelenting effort to keep analytical error rates low.

QC materials are one of the most important tools used by the 24 clinical laboratories in our healthcare network to harmonize test results. Because different lots of QC materials have different analyte concentrations, we use the same lots throughout the network – so you can imagine the complexity of coordinating simultaneous transitions.

A QC lot changeover constitutes a significant operational and quality disruption. You need to test the new lot of materials for about two weeks, analyze the results extensively, evaluate stability, and account for the differences among instruments performing the same tests to ultimately roll out new performance targets. Even after you have placed the new lot in operation, you must remain especially vigilant for weeks or months to ensure that the statistical performance holds up. Because laboratory expertise is in critically low supply and these lot-to-lot transitions are managed by our best-qualified staff, each changeover involves not only direct material and labor costs, but also huge opportunity costs by distracting senior laboratory professionals from other pressing tasks.

In a large laboratory, you might be doing this a dozen times a year, each time requiring several weeks of work and a small army of people. In a network of laboratories like ours, these efforts are amplified exponentially. QC lot transitions are even more disruptive when not predicted.

That's why we sought out ways of increasing predictability and reducing lot-to-lot transitions. A first step was to reduce the number of different QC materials: we replaced six chemistry QC materials with two Technopath controls. That change alone cut the number of expected lot-to-lot transitions in three years for the dozens of tests covered by these materials from 10 to zero – but that was not all. The new controls also last longer, not just because of their longer shelf life and the decrease (by about 50 percent) in amount needed, but also because of more reliable availability. Previously, we were often unable to use our controls up to the labeled expiration date because suppliers ran out of inventory prematurely or analytes began to degrade. So far, we have exceeded the longest stability period of the previous materials (two years) with no evidence of degradation.

Many discussions focus on the theory of analytical QC, but it is often the practices – the day-to-day reality I experience in our own labs and see in visits to others – that determine how effective our analytical QC systems really are. How many procedures and record-keeping steps are needed? What do technologists do when the QC is out? How does the lab manage corrective actions? Is QC used to prevent, in addition to detect, test inaccuracy? How disruptive and labor-intensive are lot changeovers? Those things make a big difference, and lot-to-lot changes can throw this system out of balance because they represent periods of heightened risk for errors and uncertainty. For instance, if you switch lots and find yourself out of control, you need to consider an expanded range of possible interpretations: Is it the instrument? A reagent? A test calibration issue? Or unexpected behavior of the new lot?

When you devote your laboratory staff time to changing lots and then compound potential error sources, you're removing them from other patient testing needs. Just last week, I had an important clinical question for a laboratory supervisor – but I couldn't reach her because she was preparing for a QC lot changeover. I had to make a patient with a possible bleeding tendency wait while the laboratory completed the QC task. Nothing focuses us on the need for efficient QC operation like being unable to immediately address urgent patient needs!

QC is a tool of quality maintenance. Its goal is to reduce or eliminate analytical errors in lab test results. There's often a tendency to forget that quality is free and that the costs of its tools are dwarfed by the cost of potential errors due to poor quality. As physicians, we swore to do no harm – an oath we break if a wrong healthcare decision follows an inaccurate test result. The potential liability risks of these errors might also be significant; the monetary cost of a single such mistake could match or exceed the cost of a lab technician's salary for a decade. But the most impactful costs are the pervasive effects on downstream healthcare expenses and compromised patient outcomes.

That's why our laboratories switched to new QC materials. Fewer controls require fewer changeovers, and the longer they remain stable, the more we can reduce disruptions. We have been using the same lots of Technopath chemistry QC materials for over two years with no issues; our current target is three years before changeover. We can measure that gain in dollars or in hours of time saved, but there's an even more important metric: positive patient health outcomes.

And it's because we recognize this value that, following a thorough evaluation of our QC materials, Northwell Health has partnered with Technopath to help make their products available throughout the US.

Samuel Reichberg, MD, PhD, FCAP is Associate Medical Director of the Northwell Health Laboratories and Professor of Pathology and Laboratory Medicine at the Zucker School of Medicine at Hofstra University in New York, USA.

the Cannabis Scientist™

Meet…

… the people shaping the world of cannabis science.

Explore…

… the technologies and innovations driving advancement across all areas of cannabis science.

Connect…

… with scientists and researchers promoting good science in this growing field.

Visit…

… www.thecannabisscientist.com

Register today to access regular content, to receive our weekly alert, and to benefit from new website features – all for free. www.thecannabisscientist.com/register

In Practice

Technologies and techniques
Quality and compliance
Workflow

28–31
The Heavy Cavalry of Colorectal Cancer
By measuring tumor budding, we can gain new insight into colorectal cancer and how to stratify patients for optimal treatment.

The Heavy Cavalry of Colorectal Cancer

What is tumor budding – and how can this novel histopathological biomarker better stratify colorectal cancer patients?

By Alessandro Lugli

Personalized healthcare requires solid, reproducible biomarkers to stratify cancer patients into prognostic subgroups. One such factor emerging in colorectal cancer is tumor budding, a novel and promising histopathological biomarker now classified as an additional prognostic factor in the eighth edition of the TNM Classification of Malignant Tumors 2017 (1).

Tumor budding describes the presence of single tumor cells or small tumor clusters of up to four cells. These "buds" are detached from the main mass and are usually located in the stroma at the invasive front of the tumor. Studies carried out on patients with colorectal cancer show that a higher number of tumor buds is associated with an increase in vessel invasion, lymph node metastasis, and distant metastatic disease. These associations indicate that tumor buds – or at least a subset of the budding cells – can invade the extracellular matrix and disseminate through blood vessels.

Located within the tumor microenvironment, tumor buds have been shown, at least in part, to display features of epithelial-to-mesenchymal transition (EMT). They also overexpress markers of cell invasion, migration, and survival, as well as deregulating proteins involved in the Wnt signaling pathway. Tumor buds frequently disrupt E-cadherin expression and prevent β-catenin expression at the cell membrane, often along with nuclear translocation of β-catenin (2). These morphologic and molecular characteristics make tumor buds the attacking "heavy cavalry" at the invasive tumor front, counteracted by immune cells as the defenders (2) (3).

A budding practice

Given that colorectal cancer patients within the same disease stage have heterogeneous outcomes, tumor budding could be an important biomarker to better resolve these differences. Put simply, the more tumor buds identified in the histological evaluation of colorectal cancer, the worse the patient's prognosis. Tumor buds are classified as either peritumoral or intratumoral and are visible on standard hematoxylin and eosin (H&E) staining.

In daily practice, tumor budding may impact at least three potential clinical scenarios. For early colorectal cancer that begins in polyps (pT1), peritumoral budding is associated with the presence of lymph node metastasis, which provides an indication that an oncologic resection should be considered to establish the lymph node status (4). In stage II colorectal cancer that doesn't include lymph node metastasis,

At a Glance

- *Tumor budding is proposed as an additional prognostic factor in the eighth edition of the TNM Classification of Malignant Tumors 2017*
- *As a biomarker of tumor progression, budding is part of the tumor microenvironment and involved in the epithelial-to-mesenchymal transition (EMT)*
- *Tumor budding can aid clinical decision-making in early-stage colorectal cancer*
- *A 2016 International Tumor Budding Consensus Conference established a reproducible and cost-effective scoring system*

"Tumor budding seems to be a simple, reproducible, and robust histopathological biomarker."

tumor budding is an independent prognostic factor associated with worse disease-free and overall survival. In stage II colorectal cancer patients with high-grade budding, adjuvant therapy should potentially be considered (5). Finally, another promising clinical scenario has recently emerged: preoperatively treated rectal cancers. Why is tumor budding a useful biomarker in this setting? Because intratumoral budding can be seen in colorectal cancer biopsies and, therefore, identifying this feature in a colon or rectal cancer patient may prove useful in their preoperative management (6).

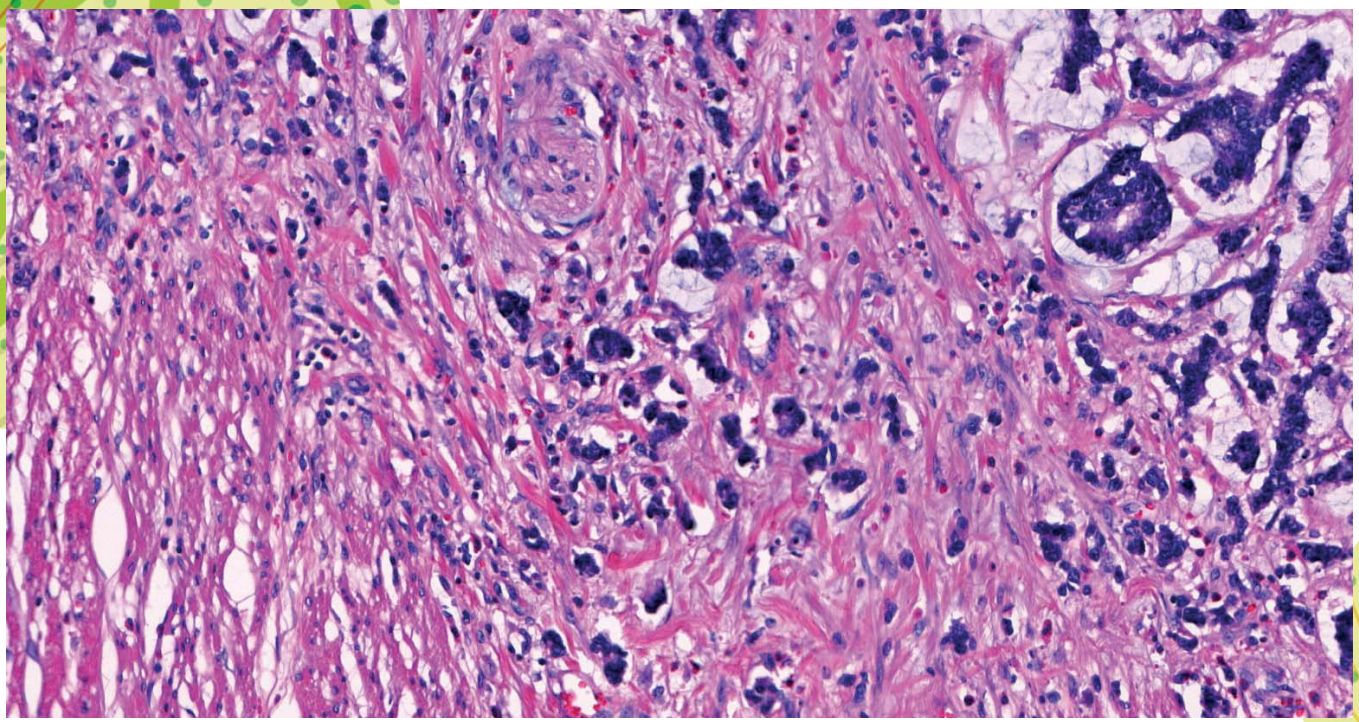
Although tumor budding is still a marker of tumor progression in more advanced and metastatic colorectal cancer (stage III and IV), we are still investigating its role in clinical practice. Patients with stage III colorectal cancer normally benefit from treatment with adjuvant therapy because clinically occult micrometastases may still be present after surgery, leading to disease recurrence. Tumor budding may not play an imminent role in decisions about therapeutic management, but high-grade budding is still an indicator of the potential progression of a local into a distant metastatic disease. It could therefore be used to optimize the clinical management of stage III colorectal cancer patients.

In stage IV colorectal cancer, the liver is the main site for distant metastases. For patients with isolated colorectal cancer, the regional treatment of liver metastases – including surgery alone or in combination with systemic chemotherapy – may be considered. In this clinical scenario, tumor budding could potentially be assessed in biopsies of colorectal cancer liver metastases (intrametastastic budding), or in resected colorectal cancer liver metastases (perimetastatic budding). Nevertheless, there is not enough data in the literature to make any conclusions on the prognostic or predictive role of tumor budding in colorectal cancer liver metastases.

Introducing new guidelines

Given the amount of evidence that supports the clinical value of tumor budding in colorectal cancer, it is somewhat surprising that this feature has not yet been universally accepted into diagnostic practice. The main reason for the absence of tumor budding in previous colorectal cancer guidelines and protocols has been the lack of an international standardized scoring system. For this reason, the International Tumor Budding Consensus Conference (ITBCC) was held in Bern, Switzerland, in April 2016. Its goal? For 23 experts in GI pathology from all over the world to meet and propose a reproducible, cost-effective scoring system based on the available data. The group reached a consensus based on the following statements (7):

- Tumor budding is defined as a single tumor cell or a cell cluster of up to four tumor cells.
- Tumor budding is an independent predictor of lymph node metastasis in pT1 colorectal cancer.
- Tumor budding is an independent predictor of survival in stage II colorectal cancer.



H&E stain showing tumor buds at the invasive front of colorectal cancer.

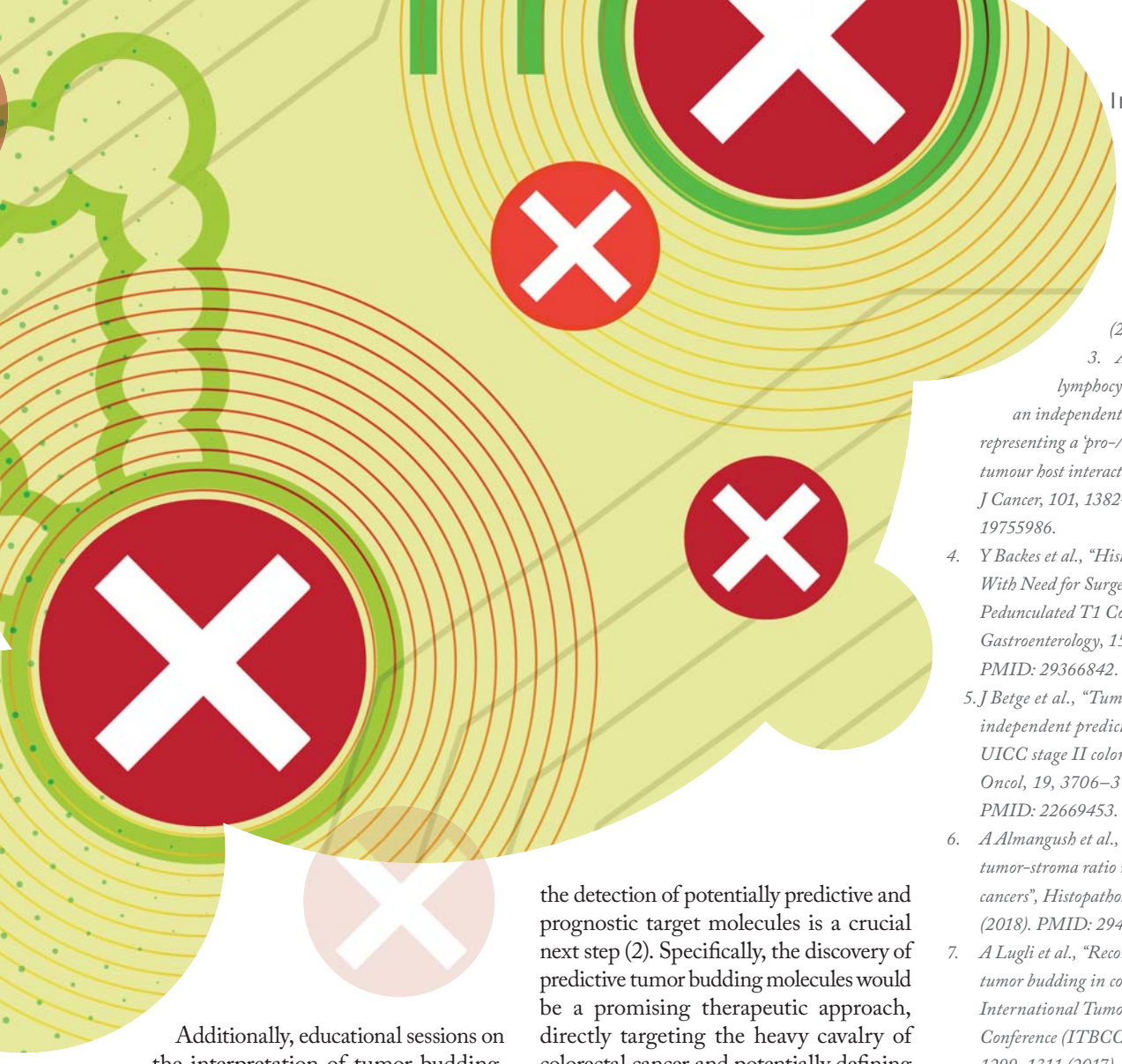
- Tumor budding should be taken into account along with other clinicopathological factors in a multidisciplinary setting.
- Tumor budding is counted on H&E.
- Intratumoral budding in colorectal cancer has been shown to be related to lymph node metastasis.
- Tumor budding is assessed in one hotspot (in a field measuring 0.785 mm^2) at the invasive front.
- For tumor budding assessment in colorectal cancer, the hotspot method is recommended.
- A three-tier system should be used along with the budding count to facilitate risk stratification in colorectal cancer.
- Tumor budding should be included in guidelines and protocols for colorectal cancer reporting.
- Tumor budding and tumor grade are not the same.

The results of the ITBCC were published in 2017 in Modern Pathology (7). Five practical steps are proposed for scoring tumor budding in daily practice:

1. Determine the field (specimen) area for the 20X objective lens of the microscope based on the eyepiece field number diameter.
2. Select the H&E slide with greatest degree of budding at the invasive front.
3. Scan 10 individual fields at medium power (10X objective) to identify the "hotspot" at the invasive front.
4. Count tumor buds in the selected "hotspot" (20X objective).
5. Divide the bud count by the normalization factor to determine the tumor bud count per 0.785 mm^2.

These five steps allow the pathologist to select the budding category (BD1 – low, BD2 – intermediate, or BD3 – high) based on bud count and indicate the absolute count per 0.785 mm^2.

The ITBCC guidelines should not be regarded as the endpoint of tumor budding, but as a basis for large retrospective and prospective clinical trials. Indeed, the ITBCC guidelines have already been applied in several studies and are also included in guidelines and protocols (8) (9) (10). Many pathologists working with tumor budding in daily practice are asking themselves why it was not defined based on immunohistochemistry, as this better visualizes tumor buds – especially in a highly inflamed peritumoral environment. This was discussed in depth at the ITBCC in 2016; the main argument for using H&E was based on the data available in the literature, which clearly favors the use of H&E stains to assess tumor budding in colorectal cancer.



Additionally, educational sessions on the interpretation of tumor budding, similar to those organized for PD-L1 scoring, may not only further develop digital image analysis, but also minimize the inter-observer variability for tumor budding in colorectal cancer.

An increase in the number of biopsy studies that focus on tumor budding will support the implementation of intratumoral budding in preoperative biopsies of colon and rectal cancer. From the tumor microenvironment perspective, the inclusion of tumor budding and immune cells into a prognostic score is an interesting approach (3) (11). Indeed, the inclusion of tumor- and host-related biomarkers better reflects the "attacker–defender" approach and the role of cancer and immune cells in the tumor microenvironment of colorectal cancer. From a molecular perspective, the detection of potentially predictive and prognostic target molecules is a crucial next step (2). Specifically, the discovery of predictive tumor budding molecules would be a promising therapeutic approach, directly targeting the heavy cavalry of colorectal cancer and potentially defining an anti-budding therapy in the future.

Tumor budding seems to be a simple, reproducible, and robust histopathological biomarker. Its use is applicable not only to colorectal cancers, but also to other solid tumors, such as cancer of the oral cavity, lung, pancreas, esophagus, breast, and urinary bladder. In my opinion, there is a promising outlook for this new approach!

Alessandro Lugli is Professor of Tumor Pathology and Vice-Chair of the Institute of Pathology at the University of Bern, Switzerland.

References

1. *J Brierley et al., TNM classification of malignant tumors, 8th edition. Wiley: 2017.*
2. *I Zlobec, A Lugli, "Tumor budding in colorectal cancer: molecular rationale for clinical translation", Nat Rev Cancer, 18, 203–204 (2018). PMID: 29376521.*
3. *A Lugli et al., "CD8+ lymphocytes/ tumour-budding index: an independent prognostic factor representing a 'pro-/anti-tumour' approach to tumour host interaction in colorectal cancer", Br J Cancer, 101, 1382–1392 (2009). PMID: 19755986.*
4. *Y Backes et al., "Histologic Factors Associated With Need for Surgery in Patients With Pedunculated T1 Colorectal Carcinomas", Gastroenterology, 154, 1647–1659 (2018). PMID: 29366842.*
5. *J Betge et al., "Tumor budding is an independent predictor of outcome in AJCC/ UICC stage II colorectal cancer", Ann Surg Oncol, 19, 3706–3712 (2012). PMID: 22669453.*
6. *A Almangush et al., "Prognostic impact of tumor-stroma ratio in early-stage oral tongue cancers", Histopathology, 72, 1128–1135 (2018). PMID: 29427291.*
7. *A Lugli et al., "Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016", Mod Pathol, 30, 1299–1311 (2017). PMID: 28548122.*
8. *SJ Cho, S Kakar, "Tumor Budding in Colorectal Carcinoma: Translating a Morphologic Score Into Clinically Meaningful Results", Arch Pathol Lab Med, 142, 952–957 (2018). PMID: 30040461.*
9. *J Selves et al., "[Guidelines for the management of malignant colo-rectal polyps (pTis and pT1) treated by endoscopic resection]", Ann Pathol, 38, 331–337 (2018). PMID: 30056944.*
10. *A Lugli et al., "Qualitätsrichtlinien SGPath Kolon und Rektum". Presented at Schweizerische Gesellschaft für Pathologie (SGPath); November 8–10, 2018; Lugano, Switzerland.*
11. *C Lang-Schwarz et al., "Budding and tumor-infiltrating lymphocytes – combination of both parameters predicts survival in colorectal cancer and leads to new prognostic subgroups", Hum Pathol, 79, 160–167 (2018). PMID: 29787819.*

ASCP 2019
ANNUAL MEETING

SEPTEMBER 11-13 | PHOENIX, AZ

EDUCATION
ADVANCEMENT
DISCOVERY

Attend the ASCP 2019 Annual Meeting

Take advantage of the world-class education at the ASCP 2019 Annual Meeting to gain practical, immediately-applicable knowledge and bring advancement to your lab today with strategies to improve outcomes, become more cost effective, and enhance patient care.

Specialized Content Created for You!

Pathologist Education

- 90+ Sessions for Pathologists
- Laboratory Stewardship
- 12 New Video Microscopy Tutorial Sessions
- ASCP/SH Day with a Focus on Hematopathology
- Wellness and How to Prevent Burnout Sessions
- Women in Pathology Empowerment Sessions
- Laboratory Management

Laboratory Professional Education

- 70+ Sessions for Laboratory Professionals
- Laboratory Stewardship
- Laboratory Professional Track Developed by Laboratory Professionals
  - » Microbiology
  - » Clinical Chemistry
  - » Transfusion/Blood Banking
  - » Hematology/Coagulation
- Laboratory Management

The ASCP 2019 Annual Meeting | September 11-13, 2019
—we invite you to an oasis of knowledge!

For more information visit: **www.ascp.org/2019**

NextGen

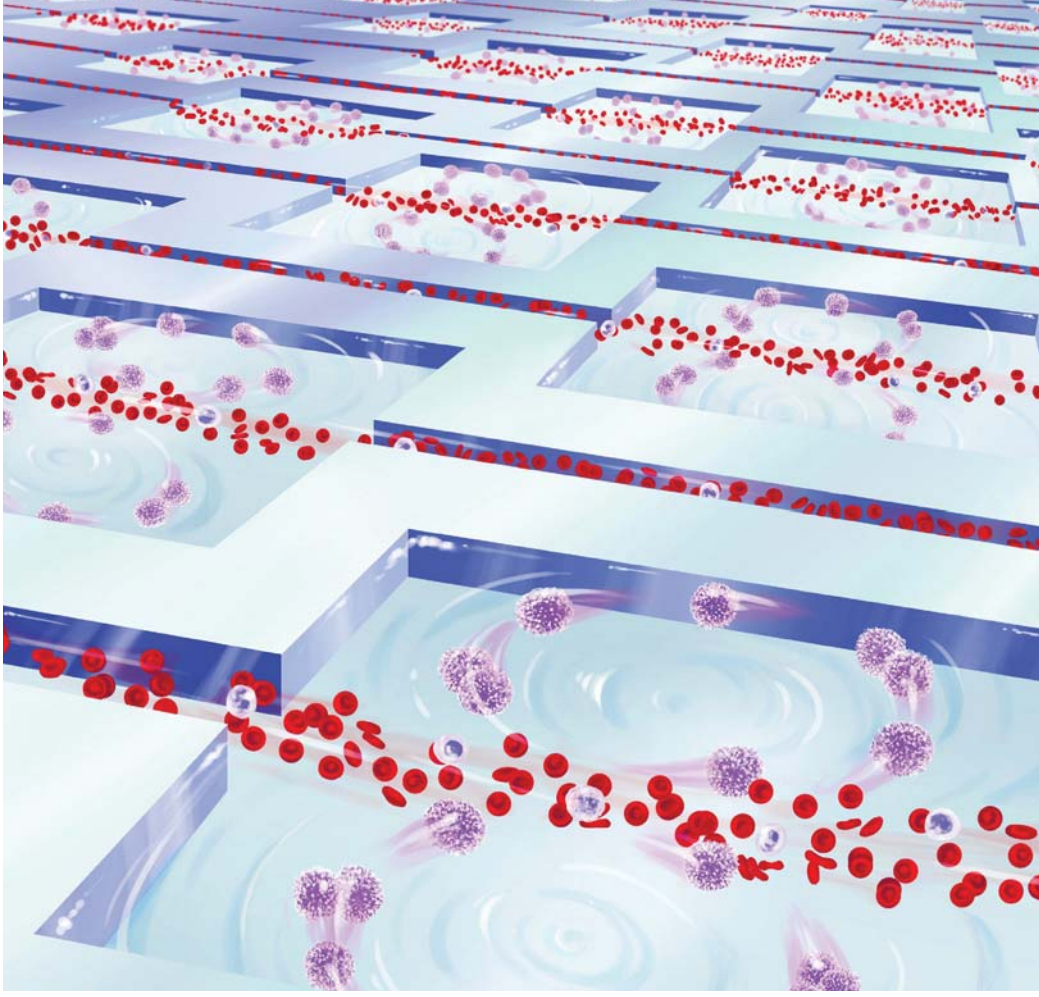
Research advances
New technologies
Future practice

34–35
CTCs in a Spin
A new device that isolates circulating tumor cells can yield insight into their role in cancer metastasis.

36–38
Fine-Tuning Immunotherapy
Liquid biopsy techniques are rapidly advancing – and, by using them, we can ensure that every patient receives the most appropriate therapy.

CTCs in a Spin

A microfluidic device isolates circulating tumor cells from the blood with micro-whirlpools in 15 minutes, facilitating further research into the link between CTC protease expression and metastasis



Australian pathologist Thomas Ashworth first described "cells identical with those of the cancer itself" in the blood in 1869 (1). Today, the presence of such circulating tumor cells (CTCs) is associated with the aggressive spread of a tumor, which is thought to occur when CTCs secrete proteolytic enzymes that facilitate invasion. These matrix metalloproteases (MMPs) are synthesized as inactive preproenzymes, but become activated by pro-matrixins once secreted and then degrade extracellular matrix barriers.

Previous efforts to quantify the number of CTCs in patients, with the goal of predicting treatment effectiveness, have yielded mixed results. This is partly because of CTC phenotype heterogeneity; not all cells have a phenotype optimized for extravasation. Similarly, clinical trials of MMP inhibitors have not been overwhelmingly successful thus far, because CTCs vary in their secretion of MMPs. What if we could establish the level of MMP secretion by patients' CTCs – rather than simply measuring the number of CTCs? And would such a tool allow us to identify patients who would benefit from MMP inhibitors?

At a Glance

- *Liquid biopsies currently focus on the number of circulating tumor cells (CTCs) in the blood to detect the spread of a tumor*
- *Cancer cells secrete proteases that are linked with metastasis; however, the success of protease inhibitors has been inconsistent because of tumor cell heterogeneity*
- *A new microfluidics device that isolates CTCs can analyze the proteases they secrete to gain insight into their function and heterogeneity*
- *Using the technology, researchers found that matrix metalloproteases secreted by CTCs indicate active malignant processes*

One in a million

A new technique developed by Dino Di Carlo and his team at the University of California, Los Angeles, uses liquid biopsy and a microfluidics device to isolate and analyze CTCs in the blood. "Solid tumors generally produce between one and 100 CTCs per milliliter of blood – a volume that contains around five billion red blood cells and 10 million white blood cells. That rarity is a critical challenge when attempting to pick out these cells for analysis," Di Carlo says. "Existing technologies to isolate these cells are numerous, but don't go any further – and, once they are isolated, there is a lot of downstream work using traditional techniques, such as staining. Unfortunately, most of the CTCs are lost in this process, leading to poor performance and the inability to quantify the properties of these cells."

Di Carlo's team combined the isolation and analysis of CTCs into a seamless integrated system. Their microfluidic technique captures CTCs from blood, exchanges the fluid around them to eliminate contaminants, adds an MMP substrate, and encapsulates them into droplets on the nanoliter scale. The process reduces cell loss, resulting in the ability to analyze individual cells to detect the secretion and activity of particular enzymes, such as MMPs. "Instead of looking at genetic information or protein levels in their non-functional form, we are able to study the activity of these CTCs in terms of proteases that they are secreting or expressing on their surfaces. The key breakthrough is that this device tells us

how active the proteases are – because they can be secreted in an active or inactive form – which provides crucial information on the actual function of MMPs."

The integrative aspect of the technology means that the process – from whole blood sample to isolated CTCs – takes just 15 minutes to complete. In addition, the sensitivity of the device enables as few as seven protease molecules to be counted per droplet, allowing for high levels of precision. The isolation technique, based on fluid dynamics, was discovered by Di Carlo serendipitously. "I began developing new types of microfluidic tools based on fluid inertia when I was a postdoctoral researcher. Such an approach was unheard of at the time, because everyone thought that small amounts of fluid were characterized by smooth, constant motions known as low Reynolds number flows. We challenged that way of thinking and found that, in rapid-moving inertial flows, randomly distributed cells will migrate across fluid streams, ordering themselves into preferred locations."

Putting tumor cells in a whirl

The same inertial flows form the basis of the microfluidic device, in which rapid streams produce a jetting flow that stems off the main channel upon exposure to sudden expansions of small reservoirs. Micro-whirlpools form in these reservoirs; small cells (such as red and white blood cells) can enter the whirlpools and exit downstream, whereas larger cells (such as CTCs) become trapped inside by fluid dynamic lift forces. "The nice thing here is that, once they're trapped and circulating, you can lower the flow in the channel and then the whirlpools dissipate, so all of the larger cancer cells are released into very small, highly concentrated volumes."

Using the microfluidic system in their research, Di Carlo's team applied the assay to analyze MMP secretion by cells in seven metastatic prostate cancer patients. Along with CTCs, other circulating cells that are known to secrete MMPs in prostate cancer patients include leukocytes, which do so at the sites of tissue inflammation and tumors. MMP activity from CTCs was found to be 2.6 ± 1.5 times higher than that from leukocytes in the same patients (2). The results of this study indicate that the relative increase in MMP secretion by CTCs compared with a leukocyte baseline could signal the presence of active malignant processes, helping to inform the prognosis of metastatic prostate cancer.

Future applications of the technique could include its use in studying subcategories of cancer-specific proteases, facilitating a better understanding of the proteolytic pathways associated with patient-specific disease. "It would make sense that there is variation in the mix of enzymes secreted by different cancer types, and there could be differences in the activity of these cells," Di Carlo says.

MMPs are also involved in helping cancer cells evade the immune system – another interesting application of the new device. MMPs secreted in this instance cleave stress proteins expressed on the cell surface, preventing natural killer cells from identifying the tumor and eradicating it. "We're seeing the development of more and more drugs that block the cleavage of these stress proteins, so that the immune system can start to re-attack those cancer cells. By characterizing the activity of MMPs, we will gain a better understanding of these processes and get one step closer to identifying patients who will benefit from certain treatments."

Development in full flow

"As an engineer, I'm particularly excited by fact that we are able to identify CTCs that are 'one in a billion' in the blood – and then measure a few molecules from each of those single cells. My hope now is that we can get this device into laboratories to help pathologists and oncologists make treatment decisions," Di Carlo says. The vortex trapping technology has been licensed and is currently being developed into a complete assay that Di Carlo hopes will be on the market within the next three years. "The technology is getting ever closer to that stage, and we're currently developing more downstream assays. Our ultimate aspiration is to help select the most effective drugs and improve the lifetime of patients, such as those who are helping us with our research studies."

The trapping technology is a class I FDA-registered device currently being sold as a research instrument in the US. Alongside the analysis of proteases secreted by CTCs, the format of the technique – confining cells within droplets – is well-suited to nucleic acid level measurements, single cell sequencing, and other single cell-based assays. No wonder Di Carlo is optimistic for the future. "In the field of precision medicine, one of the key goals is obtaining samples that are as informative – but not as invasive – as traditional methods. And that's where our device fits in. Now that we can receive functional information from individual cancer cells, I think it's going to be an exciting new area with plenty to explore."

Dino Di Carlo reports the following relevant disclosures: Board Member of Vortex Biosciences, the company that has taken the trapping technology to market in the US.

Dino Di Carlo is a Professor in the department of Bioengineering and Director of the Cancer Nanotechnology Program of the Jonsson Comprehensive Cancer Centre at the University of California, Los Angeles, USA.

References

1. *TR Ashworth, "A case of cancer in which cells similar to those in the tumors were seen in the blood after death", Aust Med J, 14, 146–149 (1869).*
2. *M Dhar et al., "Functional profiling of circulating tumor cells with an integrated vortex capture and single-cell protease activity assay", PNAS, 40, 9986–9991 (2018). PMID: 30224472.*

Fine-Tuning Immunotherapy

How advanced liquid biopsy techniques can help determine earlier than ever when cancer immunotherapy is effective – and when it isn't

By George Karlin-Neumann

In a field that does not see breakthroughs often enough, immunotherapies have revolutionized cancer care. Instead of the broad, untargeted effects and acquired resistance patients experience with toxic chemotherapy and gene-targeted therapies, immunotherapies can promote a tumor-directed response wherein the patient's own immune system fights off the cancer. When it works well, patients can achieve deep, long-lasting responses (1).

However, immunotherapies do not work in all patients. This is because the chosen therapy often doesn't fully address the reason the patient's cancer has escaped their immune system. The efficacy of checkpoint inhibitors, for example, varies widely across different cancer types, ranging from 15 percent in small-cell lung cancer to 85 percent in Hodgkin's lymphoma (2,3). Furthermore, immunotherapies can produce severe immune-related adverse effects in patients. And that's why it's critical to select the proper therapy for each patient – and to confirm that the patient is seeing a benefit as early as possible after treatment begins.

But most diagnostic tests cannot adequately identify who will benefit from a given immunotherapy. Why? Because current cancer biomarker approaches are not clinically specific and sensitive enough by themselves. Some approaches, such as microsatellite instability testing or estimating tumor mutational burden, involve assessing the likelihood that a tumor is highly antigenic and therefore detectable by the immune system. Others, such as immune gene expression profiling, test whether an inflamed tumor shows evidence of tumor lymphocyte recognition and infiltration. But none of these approaches fully reveals whether or not the patient's immune system is likely to respond to immunotherapy, nor do they explain why it has not responded on its own.

A well-known example of this is PD-L1 immunohistochemistry tests, which can result in a large number of false positives and false negatives that, in turn, lead to errors in the use of checkpoint inhibitors (4). Not all tumors express high levels of PD-L1, and even the time of sampling and the sample's location within the tumor may impact the PD-L1 expression levels seen in tests (5). Accurately predicting responders and non-responders prior to treatment – even imperfectly – will almost certainly require combining multiple tests.

Check your answers

Science still has a long way to go before it can accurately predict the optimal treatment regimen for each patient, so oncologists must begin monitoring the patient's response as early as possible – and, if not favorable, discontinue or adjust treatment accordingly. Traditional diagnostic methods, unfortunately, are unsuitable for this purpose. Regular monitoring requires repeated testing, which makes tissue biopsies risky and impractical – and tissue biopsies may not reveal the genetic characteristics of the entire tumor, nor of secondary metastases (6). This is especially a concern in late-stage cancer, as heterogeneity increases through the course of the disease (7). Blood protein markers are often used to monitor therapy response, but many of these markers, such as serum lactate dehydrogenase for melanoma and prostate specific antigen for prostate cancer, are not very sensitive or specific (8, 9).

Imaging techniques, the current standard of care in assessing response to therapy, provide a phenotypic indicator of cancer progression. They are effective at determining tumor location and size, making cancer staging, tissue biopsies, and surgery possible. But their inability to reveal more than phenotypic information limits their accuracy. For instance, false positives can be caused by pseudoprogression, a phenomenon wherein a tumor appears to be growing when the patient is actually improving. In fact, this "growth" is the result of T cells infiltrating the tumor and causing temporary inflammation. An oncologist could stop or change a patient's therapy prematurely if they misinterpret this as true progression.

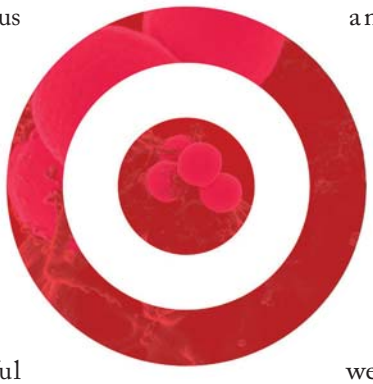
Another common approach to monitoring cancer progression in response to checkpoint inhibitors is to measure protein expression using immunohistochemistry (IHC). PD-1/PD-L1, in particular, is expressed in several tumor types and its presence is often used to guide treatment decisions. But IHC tests for PD-L1 expression are not well-standardized (10); labs may use different antibodies, detection methods, and thresholds, leading to inconsistent results. Furthermore, not all

At a Glance

- *Cancer immunotherapy can be very effective, but many patients do not respond to such treatments*
- *Immunotherapy's poor efficacy in these patients is largely due to the imprecision of methods intended to predict who will benefit*
- *Because of the treatment's limited success rates and potential severe side effects, oncologists should determine its efficacy as early as possible and adjust treatment accordingly*
- *Droplet digital PCR technology, which directly quantifies the concentration of circulating tumor DNA, can help*

tumors express PD-L1 at the same high levels, and there can even be differences in expression within a single tumor. Thus, although traditional diagnostic techniques reveal useful information about a patient's tumor, many of them have limitations that make them insufficient or unreliable for monitoring immunotherapy response.

But there is a promising alternative method: liquid biopsy. This approach can often accurately track cancer progression and distinguish who is and isn't responding to various types of immunotherapy. Using plasma collected from a simple blood draw, liquid biopsies quantify circulating tumor DNA (ctDNA), both a highly specific genetic marker for the tumor and a phenotypic biomarker of successful tumor turnover. Because ctDNA concentration in most cases directly correlates with tumor burden, physicians can distinguish pseudoprogression from true disease progression. Additionally, liquid biopsies are minimally invasive and can be used for serial monitoring with lower risk to the patient. Liquid biopsies can even deliver results within days or weeks following treatment initiation, unlike imaging, which takes place six to 12 weeks after treatment begins.



To monitor cancer progression with liquid biopsies, we must identify tumor-specific ctDNA mutations. If the tumor's mutations are unknown, next-generation sequencing (NGS) is a comprehensive method for initially profiling tumor-associated genetic mutations in the blood. Once the mutations are determined, tests based on droplet digital PCR (ddPCR) and related technologies can quickly and cost-effectively quantify and track these mutations in blood or other body fluids as biomarkers of treatment response and disease progression.

ddPCR liquid biopsy in practice

Several investigations over the past few years have demonstrated the ability of ddPCR-based tests to distinguish responders from non-responders, often within weeks of treatment initiation. This capability has been demonstrated for several different types of immunotherapies across multiple cancer types, including melanoma, non-small cell lung cancer (NSCLC), and cervical cancer. Specifically, several studies have shown how a liquid biopsy based on ddPCR can predict and monitor the effectiveness of checkpoint inhibitors – currently the most widely used class of immunotherapies – as well as CAR T cells and tumor infiltrating lymphocytes (TILs), which are expected to be a major part of the next generation of immunotherapies.

In one case, Jenny Lee and colleagues developed a ddPCR liquid biopsy to track BRAF, NRAS, and KIT mutations. They evaluated the test's ability to distinguish between responders and non-responders to anti-PD-L1 (+/- anti-CTLA-4) therapy in 105 patients with stage IV melanoma. They assayed these mutations in both training (n=76) and validation (n=29) cohorts at the start of therapy and at regular intervals for up to 12 weeks and found that longitudinal monitoring of ctDNA was an effective means to identify patients who responded to the checkpoint inhibitors (11). Ultimately, in this study, most patients who did not have detectable ctDNA by 12 weeks survived to at least a median of 17.5 months following the start of therapy. In contrast, patients who still had detectable ctDNA after 12 weeks had poorer outcomes, with a median overall survival of 9.7 months.

The researchers followed this with another study of ctDNA monitoring, this time to evaluate its ability to identify pseudoprogression in a 125-patient cohort, 29 of whom were identified by CT scans as having progressive disease (12). In contrast to the imaging results, ctDNA profiles measured using ddPCR-based liquid biopsy revealed that nine of these 29 patients had favorable ctDNA profiles and were actually exhibiting pseudoprogression. They also correctly identified unfavorable ctDNA profiles in 18 of the remaining 20 patients. Consequently, ctDNA monitoring could accurately separate those who exhibit pseudoprogression from true progressors who might benefit from changing or discontinuing treatment.

Researchers at the Groningen University Medical Center in the Netherlands recently reported the use of a similar technique to measure ctDNA levels of KRAS exon 2 mutations in 16 (since expanded to 29) NSCLC patients undergoing nivolumab (anti-PD-1) treatment (13). Patients with a positive response to nivolumab, an anti-PD-1 treatment, showed a distinctive ctDNA kinetic profile in which ctDNA levels spiked one week after the start of therapy and dropped to undetectable levels a week later. Additional measurements in the first three to seven weeks validated the initial findings. Conversely, patients whose tumors did not respond to nivolumab (as evidenced by increasing RECIST 1.1 scores) showed steadily increasing levels of ctDNA in their blood.

Alternative approaches

Another biomarker under evaluation for

immunotherapy response is PD-1 mRNA in exosomes released by cancer cells into the blood. Marzia Del Re and colleagues at the University of Pisa demonstrated that a liquid biopsy based on ddPCR could track melanoma and NSCLC progression in patients treated with the PD-1 inhibitors nivolumab and pembrolizumab after only two months of treatment (14). Among the 26 patients in the study, PD-L1 mRNA levels decreased by an average of 71 percent in patients who entered complete or partial remission, whereas levels increased by an average of 104 percent in patients with progressive disease.

Beyond checkpoint inhibitors, ddPCR liquid biopsy has also been used to monitor responses to adoptive cell therapy approaches such as CAR T cell therapy and tumor-infiltrating lymphocyte (TIL) therapy. In CAR T therapy, ddPCR liquid biopsy could potentially monitor the persistence of CAR T cells, which, in preclinical models, is a predictor of overall survival in cases of acute myeloid leukemia (AML). CAR T therapy involves removing a patient's T cells, genetically modifying them to express a chimeric antigen receptor (CAR), and injecting them back into the patient to attack tumor cells. For CAR T cells to work in the long term, however, they must persist in the body for months in an intermediate concentration; too high a dose can be toxic, but too low a dose may not be effective.

Mayumi Sugita of Weill Cornell Medicine found that ddPCR liquid biopsy very effectively monitored the persistence of CAR T cells (15). Typically, CAR T cell kinetics are followed using multi-parameter flow cytometry (MFC), but this technique is hard to validate because it is less sensitive than liquid biopsy. Sugita and her team were able to predict overall survival in a cohort of 20 patient-derived xenograft mice with established human AML by simultaneously monitoring minimal residual disease (via the NPM1 tumor mutation) and CAR T cell persistence using ddPCR liquid biopsy. In fact, the ddPCR method was able to detect CAR T cells that could not be evaluated by MFC in peripheral blood.

TILs are another type of immunotherapy that has long been studied as a potential option for treating several different cancers, including cervical cancer (16). In a study examining the validity of using human papilloma virus (HPV) cell-free DNA (cfDNA) to monitor cervical cancer, Zhigang Kang from the National Cancer Institute found that cfDNA exhibits a distinct pharmacokinetic response to TIL therapy; in a cohort of nine patients, the three that experienced objective regression exhibited a spike in HPV cfDNA after two or three days, followed by a drop to undetectable levels in about a week (17). This kinetic profile reflected the anti-tumor activities of the TILs, suggesting that ddPCR-based testing can be used to monitor their effectiveness within a few days of TIL administration.

The future of ddPCR-based liquid biopsies

Liquid biopsies provide sensitivity, accuracy, and reliability where current pre-treatment predictive tests may not – but they still have their limitations. Because ddPCR relies on circulating genetic material to monitor cancer progression, it may be less reliable for monitoring tumors in locations where they cannot slough ctDNA into the blood (for instance, intracranial tumors). In most cases, though, ctDNA is a more direct indicator of tumor load than imaging or immunoassays – and, because liquid biopsies are minimally invasive, they can generally serve as a dependable method for serial monitoring of immunotherapy effectiveness. Ultimately, this method could enable physicians to check their initial treatment decisions early and often, and to adjust or change each patient's therapy to maximize their chances of living a long and healthy life.

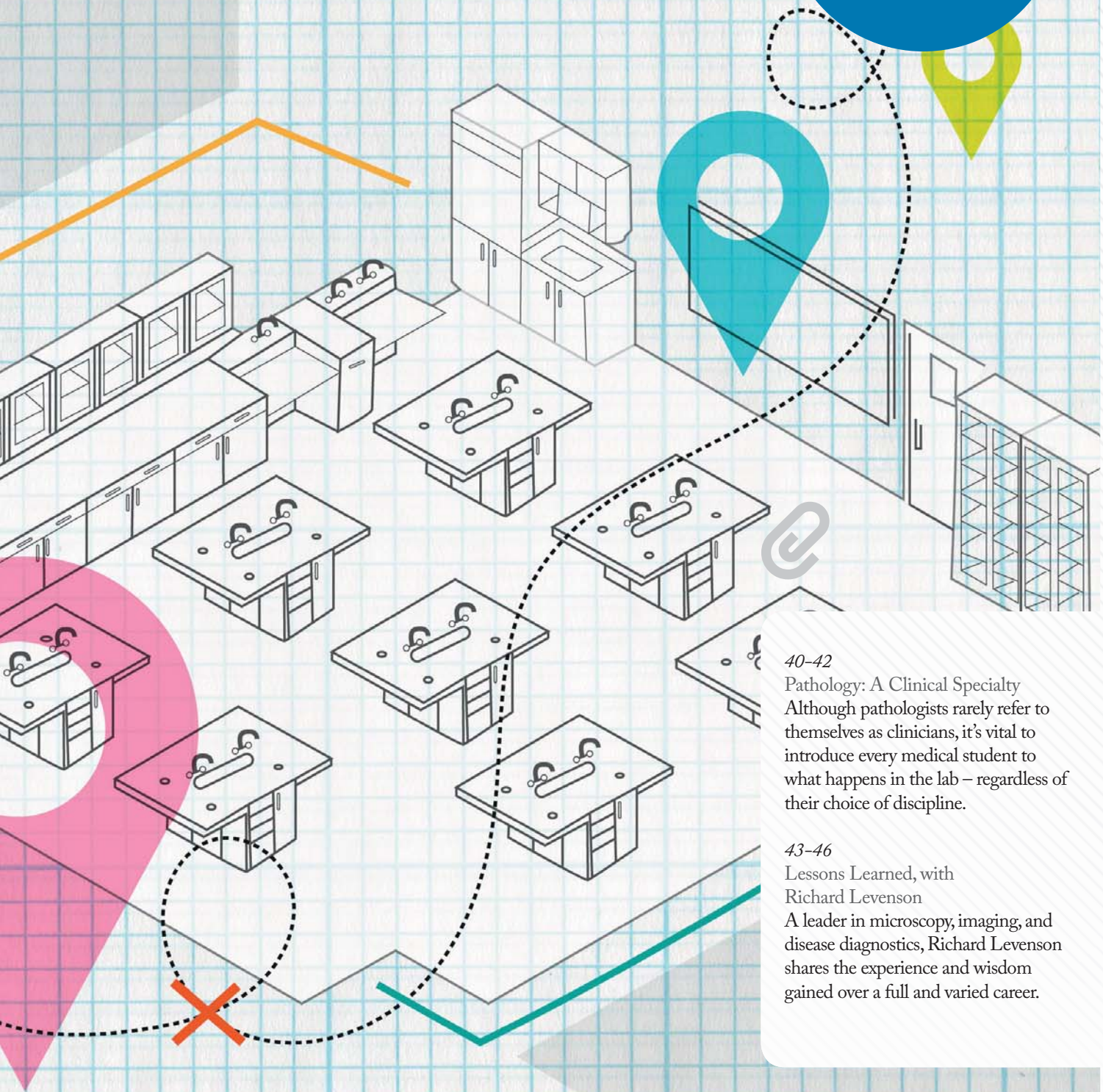
George Karlin-Neumann is the Director of Scientific Affairs for the Digital Biology Group at Bio-Rad Laboratories.

References

1. *NCI Staff, (2015). Available at: https://bit.ly/2lfjvU0. Accessed April 18, 2019.*
2. *SJ Antonia et al., J Immunother Cancer, 3, 376 (2015).*
3. *ER Plimack et al., J Clin Oncol, 33, 4502 (2017).*
4. *M Udall et al., Diagn Pathol, 13, 12 (2018). PMID: 29426340.*
5. *D Escors et al., Signal Transduct Target Ther, 3, 26 (2018). PMID: 30275987.*
6. *C Mao et al., Medicine, 94, e775 (2015). PMID: 26020382.*
7. *I Dagogo-Jack, AT Shaw, Nat Rev Clin Oncol, 15, 81 (2018). PMID: 29115304.*
8. *SC Tsao et al., Sci Rep, 5, 11198 (2015). PMID: 26095797.*
9. *M Adhyam, AK Gupta, Indian J Surg Oncol, 3, 120 (2012). PMID: 23730101.*
10. *L Li et al., Am J Cancer Res, 8, 1947 (2018). PMID: 30416847.*
11. *JH Lee et al., Ann Oncol, 28, 1130 (2017). PMID: 28327969.*
12. *JH Lee et al., JAMA Oncol, 4, 717 (2018). PMID: 29423503.*
13. *TJN Hiltermann et al., Poster presented at the AACR Annual Meeting; March 31, 2019; Atlanta, USA. Poster #399.*
14. *M Del Re et al., Br J Cancer, 118, 820 (2018). PMID: 29509748.*
15. *M Sugita et al., Poster presented at the AACR Annual Meeting 2018; April 14–18, 2018; Chicago, USA. Abstract #5681.*
16. *BC Sheu et al., J Immunol, 167, 2972 (2001). PMID: 11509647.*
17. *Z Kang et al., Clin Cancer Res, 23, 6856 (2017). PMID: 28899967.*

Profession

Your career
Your business
Your life



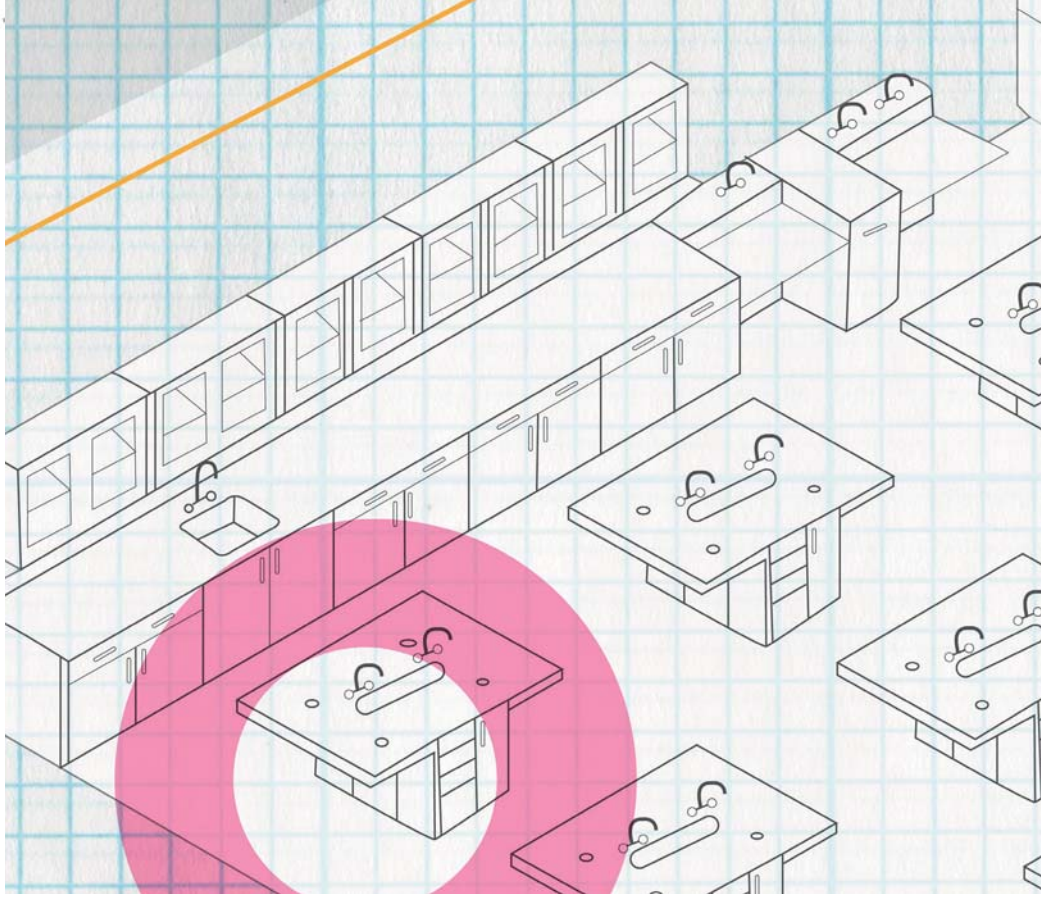
40–42
Pathology: A Clinical Specialty
Although pathologists rarely refer to themselves as clinicians, it's vital to introduce every medical student to what happens in the lab – regardless of their choice of discipline.

43–46
Lessons Learned, with Richard Levenson
A leader in microscopy, imaging, and disease diagnostics, Richard Levenson shares the experience and wisdom gained over a full and varied career.

Pathology: A Clinical Specialty

Although we don't work within a clinic, pathologists and laboratory medicine professionals are indeed members of a clinical discipline

By Aadil Ahmed and Kamran Mirza



Most pathologists and laboratorians refer to patient-facing providers as "clinicians," and to the information garnered from the interactions these healthcare workers enjoy as the proverbial "clinical correlation." What do we mean by this? Is it because we reserve this term for only those healthcare providers who run their practice from an actual clinic? Does that mean that the information coming from our microscopes and our laboratories is not clinical? And, if that is the case, what is it? Research?

This issue came to the frontlines a few years ago, when we found out that the incoming third-year (M3) medical students at our institution have an introduction to the "clinical" aspects of the hospital in a two-week long orientation at the beginning of the academic year. Meticulously designed and laboriously executed, when we saw the details of this orientation, it seemed a practically superhuman feat to put such an event together for hundreds of students. But despite its comprehensive nature regarding most components of clinical medicine, it missed one big thing: the entire world of pathology and laboratory medicine. Every aspect of this orientation conveniently skirted around the labs. The students were introduced to each step in the process of ordering labs in a patient's electronic medical record; in fact, they even learned how to "call and complain" to the lab – but, despite this long orientation process, our discipline itself remained a black box to them. In fact, until a few years ago, we're fairly certain that medical students could spend their entire time on our health sciences campus, see patients, order and retrieve hundreds – if not thousands – of lab tests, get their degrees, and leave… all without even knowing where the lab was actually located!

The problem… and our solution

So why was this the case? A quick review showed us that it was obviously not malicious intent on the part of the orientation's coordinator. It was probably a complex issue with several layers, but we believe that the simplest explanation is as follows: our specialty isn't thought of as clinical medicine. Our patient-facing colleagues don't think so – and, each time we say "clinical correlation is recommended," we help perpetuate that thought. We are truly out of sight and out of mind, a "black box" that spits out patient

"We are truly out of sight and out of mind, a 'black box' that spits out patient results."

At a Glance

- *Although we don't often refer to ourselves as clinicians, we are, at our core, a clinical discipline*
- *Students entering the medical profession must know where the laboratory is located and what it does*
- *An orientation that walks students through pathology and laboratory medicine can help them understand our function*
- *Such an orientation may even increase overall interest in pathology as a career*

results. This happens in part because we let it happen. And so, my colleagues and I decided to change that.

In April 2017, at the beginning of their orientation to the clinical aspects of the hospital, the third-year medical students on our health science campus were introduced for the first time to the pathology laboratories! The proposal we made to the Director of the M3 curriculum was met with enthusiasm and positivity, and we were given precious time out of a tightly packed schedule to split the entire M3 class into two groups and deliver each group an identical orientation to the mysteries of the clinical laboratory.

In hindsight, the start of M3 was the ideal point in the four-year US medical school system for such an introduction. Students have already completed their combined medicine–pathology course in M2 and, as a result, many of them feel that their brief foray into pathology is over. To them, our specialty is just "Robbins" or "Pathoma" or something that needs to be put into short-term memory for their USMLE Step 1 exams. Lack of a required clerkship in pathology further supports this misconception. What we set out to do in the "M3 orientation to the pathology labs" was clarify that pathology and laboratory medicine are at the heart of all medicine. No matter which rotation students may be on in their third and fourth years of medical education, they are concurrently (effectively) on a longitudinal pathology clerkship as well.

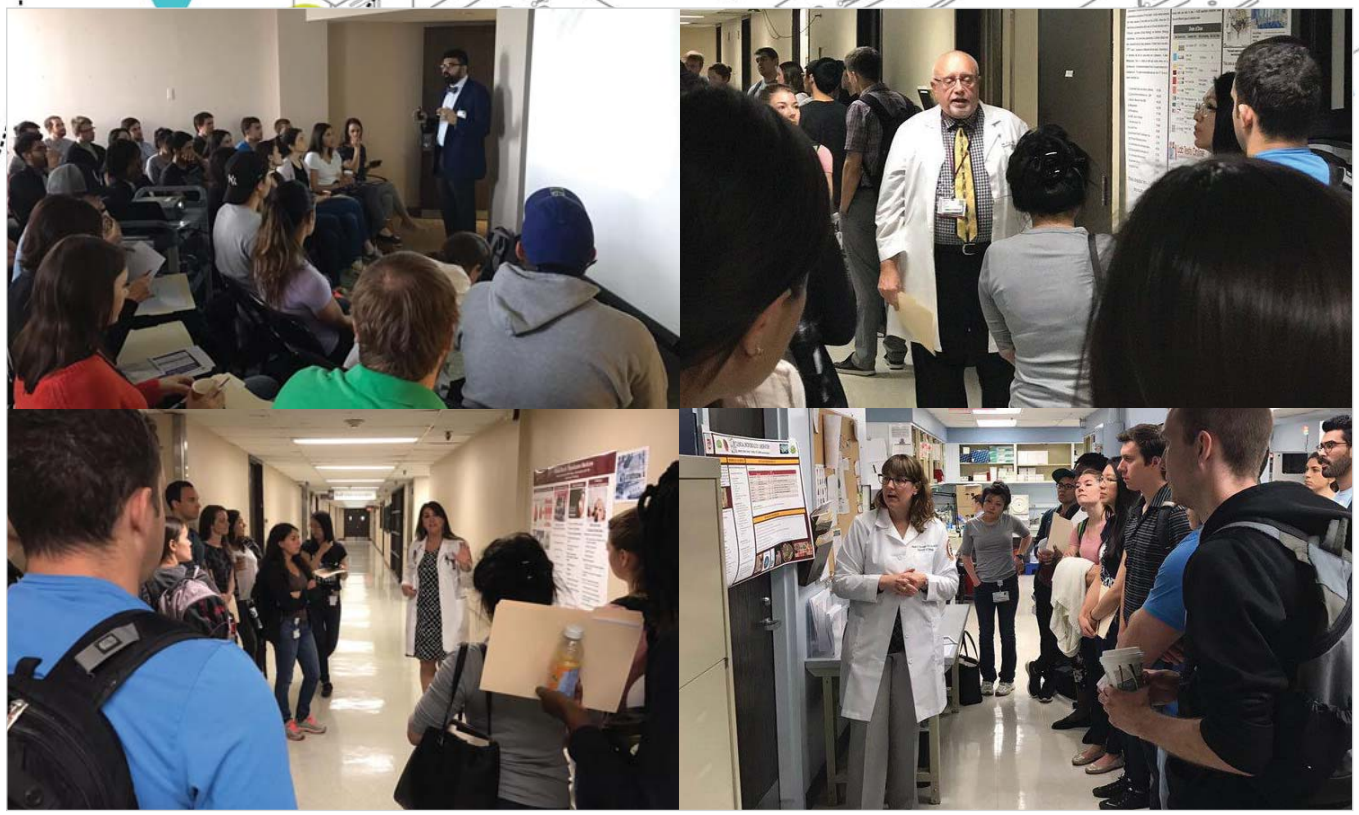
Building an orientation

This project demanded attention to detail, as well as careful coordination between the medical school, the hospital administration, the educational directors, and even the fire marshal! The pressure was on. We could not risk the project's turning out to be a bust, because that would have been devastating to the field of pathology. And because time was so precious, every minute had to be worth it; we couldn't give the students the sense that their time had been wasted. We had to relay a sense of the importance of pathology and, at the same time, convey that we – and our discipline – are fun. Now that is a tall order.

We decided to make the process mirror reality. To represent how "clinical" we truly are, the entire project revolved around a patient case. Our only condition was that the students come to us in the labs. This was on purpose. For every other orientation session, students were expected to go to the relevant department in the hospital – and we knew that, if we didn't encourage them to come down to the basement now, it would never happen. That doesn't mean it was easy. Even split in half, each orientation group was between 70 and 90 students, and attendance was required by the clerkship director. Fitting so many additional people into our department was tricky, but we made it!

The day started off with a working breakfast and a 25-minute presentation of our case. A middle-aged gentleman presents with a cough. Eventually a mass is found in the lung. The session guided the students through intraoperative analysis (frozen section), permanent section and FFPE processing, immunohistochemical staining, FISH and molecular studies, and personalized diagnostics in anatomic pathology. After that portion of the event was complete, the cohort was split into four groups and escorted by residents to one of four stations in the clinical pathology labs.

Those of you who work in smaller laboratory spaces are most likely already cringing – after all, groups of 20 or more students entering and making their way through the lab would certainly cause delays and interruptions. That was true of our department as well, so laboratory directors or their representatives for hematology, the blood bank, core lab/chemistry, and microbiology were waiting outside their labs to greet the students. We placed large posters in the hallways at all four stations to demonstrate how our patient passed through them. In the course of his postoperative care, he required routine monitoring discussed at the core lab/chemistry station; he needed a CBC (which showed lymphocytosis and needed flow cytometry) that was discussed at the hematology station;



Clockwise from top left: Kamran Mirza leads the 25-minute orientation to the case while medical directors stand at their respective stations; Stephen Kahn mans the chemistry/core lab station; Amanda Harrington handles the microbiology station; and Marisa Saint Martin takes charge of the blood bank station. (The fourth station, hematology, is not pictured.)

he developed fevers and sepsis discussed at the microbiology station; and, finally, an unforeseen complication led to the need for a blood transfusion and a possible transfusion reaction that was dealt with in immunohematology/at the blood bank. We created 10 to 12-minute talks at each station and the students rotated through all four sessions before being dismissed from the orientation. The entire process, from start to finish, was around 2 hours per group.

Is it working?

The M3 orientation to the labs was a labor of love, and yes – it has been successfully implemented every year since with great feedback! Now entering its third year, the event is an opportunity to showcase the importance of the laboratory, remind medical students of our role as consultants and diagnosticians, and to make sure that, if nothing else, they know the importance (and the location!) of the pathology laboratories. We use it as an opportunity to advertise the field, emphasize proper laboratory test utilization, and promote programs such as Choosing Wisely. The students leave with the understanding that, even if they choose not to become pathologists, their future careers are intimately related to the information coming from the lab. Their granular understanding of how things function "in the basement" remains essential for our mutual provision of the best and most cost-effective patient care.

As educators, we find the questions and intrigue our students display at these sessions uplifting. We have also seen an increase in the number of elective requests in pathology over the past few years – and, when asked, many of the students credit their attendance at our orientation session as one of their reasons for thinking of us. The aim of the pathology elective, and of the M3 orientation to the labs, is not to coerce medical students into becoming pathologists, but to instill an understanding of the role pathology and laboratory medicine play in patient care. At the same time, these things stand as a reminder to us all that pathology is, at its heart, a clinical specialty.

Aadil Ahmed is a PGY-4 AP/CP pathology resident at Loyola University Chicago Stritch School of Medicine.
Kamran Mirza is Assistant Professor of Pathology and Laboratory Medicine and Medical Education at Loyola University Chicago Stritch School of Medicine, Maywood, USA.

Lessons Learned, with Richard Levenson

More than just pigeons: Levenson has a vast and varied career in pathology, microscopy, and computational tools. Here, he shares his experience and his thoughts on the future.

Pathology has possibilities

I was doing reasonably well as a medical student at the University of Michigan and, at the time, the expectation was that the top-performing students automatically went into internal medicine. That was considered the "prestige track," but I wasn't very inspired by it. A friend of mine asked, "Have you considered pathology?" I hadn't given it a moment's thought. My friend said, "Pathology is great. You don't have to pick a particular specialty; you can do whatever you want. It's a research-oriented profession, so you can have a full-time lab and spend 20 percent of your time performing your service duties on the autopsy service." That seemed like a good idea to me, so I switched my path to pathology, and it has served me well ever since.

I had some very good avenues into research early on. I had the great fortune to work in Judah Folkman's lab – and he, of course, was the founder of angiogenesis research, so that was an amazing experience. After that, I had another fantastic opportunity while at Michigan to work for John Niederhuber, who eventually became the Head of the National Cancer Institute. Eventually, I made my way to Duke University as an assistant professor, where I was able to establish my own cell biology laboratory while spending (less than) 20 percent of my time on the autopsy service – exactly what my friend had originally predicted!

Finding inspiration in imaging

Before arriving at Duke, I was a Wilmot Cancer Research Fellow at the University of Rochester, working in the laboratory of Donald Young. He had developed a technique called "giant two-dimensional gel electrophoresis." Regular 2D gel electrophoresis was performed on postcard-sized gels – but his gels were so large that we had to use chest X-ray film to develop the autoradiographs! Analysis, especially quantitative analysis, was a challenge; there were two to three thousand grey-to-black

At a Glance

- *Pathology is an excellent career choice for those who want to focus on research as well as clinical work*
- *Novel techniques that solve many of pathology's logistical problems may be the way forward for clinical microscopy*
- *The field's transition to digital is promising, but has yet to overcome significant challenges*
- *When digital pathology allows its practitioners to be free of the slide, rather than simply adding steps to its processing, its popularity may spread*

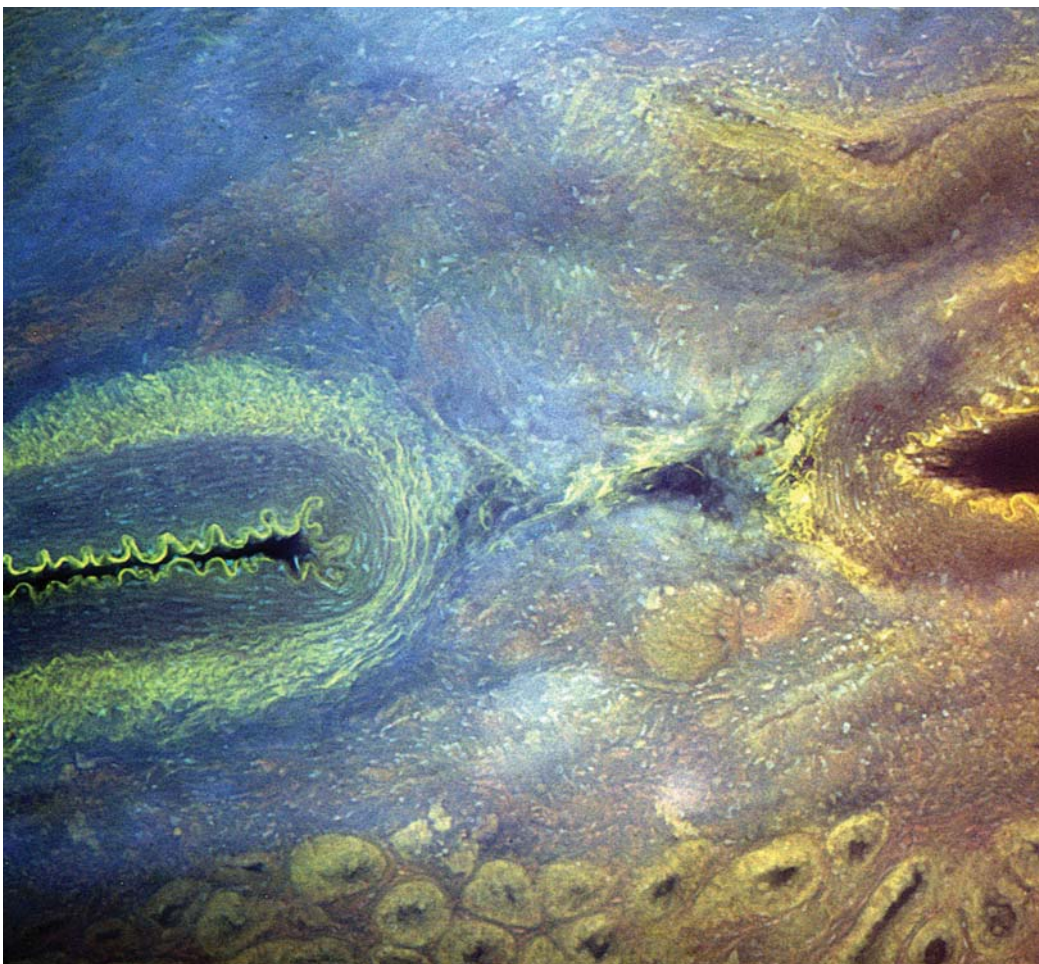
spots (translated proteins) on each film, and in the mid-1980s, we really had no straightforward way to determine how dark each spot was. One day, it occurred to me that we could try to build something we subsequently called a "pen-sitometer."

The idea was to put the autoradiograph on a support table with a little hole in it and a light source below the hole, and then take the device – which was originally shaped more or less like a pen with a photodiode at the tip – and rub it over each spot we were interested in and it would tell us how dark the spot was. Don Young figured out that you could actually use a VIC-20, the predecessor to the only slightly less ancient Commodore 64 personal computer and, by hooking up the pen-sitometer directly into the game-port analog-to-digital converter, send a digital stream directly into the PC. We programmed the whole thing in Commodore BASIC – another ancient relic, but still the peak of my coding experience. Our setup allowed us to do some really serious research, though – for instance, we quantitated proteins that were responding to growth factors and steroids in cells in tissue culture.

That was my first introduction to the problems of image analysis. How do you capture information in image form and then extract useful data from it? That's why, when I left Don's lab and went to Duke, I took an adjunct appointment in the computer science department – so I could continue to work on image analysis.

Quite early on, I was interested in the use of confocal microscopy as a tool in pathology. In fact, I was the proud middle author of a paper on the subject back in the early 1990s. That was the genesis of my interest in optics, and my inspiration to move into technology development. Truth be told, my heart was in the tools, although I only realized that late in life. My next destination was

Above: A thick, hand-cut (not microtomed) cross-section of mouse small bowel stained with rhodamine and Hoechst and imaged via MUSE. Below: Vessels, kidney. Thick specimen, stained with rhodamine and Hoechst and imaged via MUSE.



"Don't worry if you don't know something – learn the vocabulary and collaborate."

Carnegie Mellon University, which doesn't have a medical school. There, I worked on a technique called multispectral imaging, which is now part of the armamentarium for people trying to do multiplexed immunohistochemistry or immunofluorescence for cancer immunotherapy. And after that, I made the transition into industry, spending 10 years at Cambridge Research and Instrumentation (recently spun out of PerkinElmer). I was involved in developing the leading multispectral whole-slide scanner for multiplexed imaging, whose descendants are still commercially available.

After my stint at CRI, I consulted for 3 years. One day I got a phone call: "Would I like to be a professor at UC Davis?" My future chair, Dr. Lydia Howell, had a vision of bringing an emphasis on novel, imaging-based technologies into pathology, and fortunately I had come to her attention. I have had the distinct pleasure and opportunity to work with her and colleagues at UC Davis Health for the last seven years.

Currently, I work on microscopy with ultraviolet (UV) surface excitation, or MUSE microscopy, which has turned out to be an interesting and powerful new way of looking at tissues. Just after I arrived at Davis, a friend and colleague of mine from Lawrence Livermore National Laboratory showed me the work that he had been doing on UV-based imaging of tissues and pointed out the basic principle of it: namely, that ultraviolet light at the right wavelengths only penetrates tissue a few microns deep. That allows you to take a big chunk of tissue and image just a thin section of it from the surface down – approximately the same depth as a microscope slide.

Of course, I thought it was great – but he was mostly doing in vivo imaging via autofluorescence, to which I said, "I'm a pathologist. I can cheat and use stains." Together, we started trialing substances that stain tissue in more or less the same way as hematoxylin and eosin, but are fluorescent. The result? It turns out that MUSE allows you to take almost any piece of tissue, cut a flat surface with a scalpel or razor blade, and still generate microscopy results that look as good as – or even better than – an H&E slide… in under three minutes.

I'm looking forward to seeing how MUSE microscopy plays out, because it solves a lot of logistical problems in pathology – speed, cost, and, most importantly, availability of histology facilities. And it's not the only technique my colleagues and I are working on at the moment. Others – although it's too soon to talk about them – are based on new ways of extracting additional information from existing slides. All I can say about that is, "Stay tuned!"

Digital pathology: proceed with caution

For now, I would have to say that the prospects are guarded. Why? For two reasons.

1. *The business case*

   There are great advantages to going digital, most of which are logistical – not having to track down missing slides or repair broken ones, for instance, or having an easy way to share information across long distances. But the sad fact is that, currently, switching to digital involves a very large capital outlay, lots of retraining, and ongoing expenses, such as equipment maintenance, data storage, and so on. It's hard to come up with a realistic return on investment, depending on your financial environment and how costs are calculated and allocated.

   My own university has no immediate plans to go digital, and I think the institutions that have are still relatively few and far between. That's because our transition is not like radiology's. When radiology went digital, it replaced procedures; it replaced film. But when pathology went digital, it needed additional equipment and handling steps. Instead of eliminating slide preparation, the digital transition added another level of complexity to scanning, viewing, and storing information. So it's not a simple story – especially not when trying to convince those who hold the purse strings. There needs to be a solid story on how money is saved by increasing pathologist efficiency and eliminating the problems of finding, storing, and retrieving slides – but it's by no means a slam-dunk.

   Digitization feels modern. It feels technical. It feels like a good solution. But as a way of serving up images to pathologists to make manual diagnoses, it has its costs as well as its benefits, and every pathologist and institution has to weigh that up. Jennifer Hunt, chair of pathology at the University of Alabama, said at one meeting, "Digital pathology is not going to take off until you can get rid of the slide." Perhaps one day, technologies like MUSE microscopy will help us reach that point.

2. *The nascent technology*
   Digital pathology provides transformational value when you add the computer and automated or enhanced analysis. Essentially, that means it's valuable when the computer can do things the pathologist cannot do alone or do efficiently. Some of those applications already exist; for instance, we have computational support for quantitating nuclear staining (ER/PR, Ki-67, and so on). These are not things that humans do particularly well, but computers – if properly programmed and utilized – do. Unfortunately, these applications are seen more as adjunct tools; on their own, they don't make a billion-dollar industry.
   So will artificial intelligence (AI) sweep in and provide value when our slides are digital? Eventually, I anticipate that we will see automated diagnostics and enhanced prognoses, because computational tools can "see" patterns that humans can't.
   We humans also have a limited professional lifespan; we only see so many slides and cases over the course of a career, so we may be perplexed when we see something we haven't previously encountered. Computers don't have that problem; properly trained, they can be exposed to everything we, as a collective, know about, so they can be – at least theoretically – more expert than the best human.

But AI is still fragile. Because there are so many different use cases, each one is a small application that takes a great deal of work to actually bring to clinical utility and achieve regulatory approval. AI with subspecialty expertise would be expensive to develop and validate across multiple institutions. With such a vast range of different computers, laboratory information systems, images, formats, and reports, it's hard at this point in the technology's evolution to imagine it playing out in real life, as opposed to in the academic laboratory. That said, once the FDA gives its blessings to the first anatomic pathology application, the equation will begin to change.

Why do I care about statistics?

This is going to sound very old-school, but statistics should really undergird what we do. In other words, we should have a reasonably refined understanding of what it means to obtain a particular test result in a particular situation, and of how to interpret that in the real world, where things like prior odds and posterior odds really affect the meaning of a test.

Pathologists are responsible for assembling a patient's clinical and histological data and presenting their conclusions to the clinicians, who then move forward with treatment. Unfortunately, it's often clear that humans don't necessarily know how to combine information elements properly. Doctors may chase single aberrant lab tests or have difficulty integrating potentially contradictory data from multiple sources (DNA, RNA, histology, immunohistochemistry, and any other lab tests, plus the clinical situation).

That's why it's so important to understand that the world is a statistical environment, and that our intuition is usually wrong because we don't understand probabilities and risks and benefits. In fact, presentation matters as much as substance. If you present data in a certain way, it suggests a corresponding response – but if you then take the same data and express it in a different way, you get a different response. "70 percent of patients benefit from this intervention" sounds very different to "30 percent of patients received no benefits."

Thanks to human psychology, expressing exactly the same information in different ways can lead to different outcomes. And yet, there is almost no training in probability statistics in medical school, or even in university.

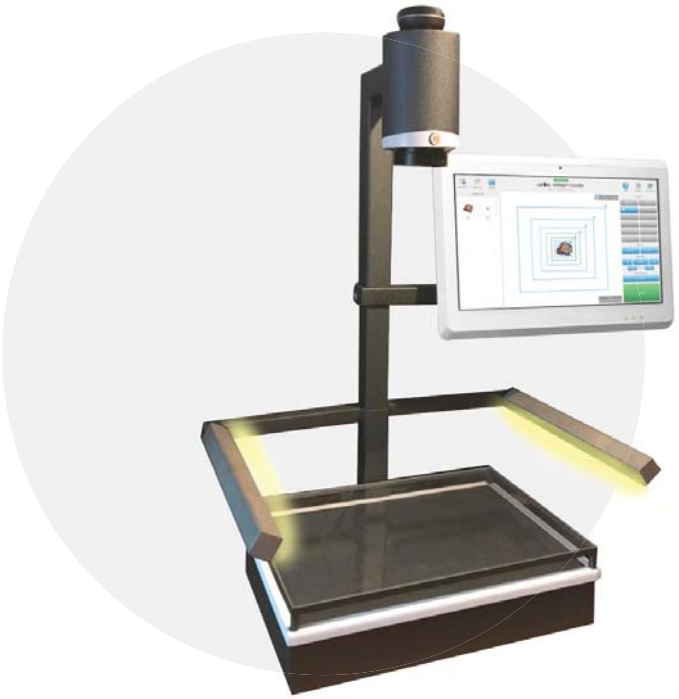
Roll with the punches

People sometimes ask how I maintain a work/life balance. Truthfully, I've found that work is what I like to do best. Email regrettably fills many available hours and, when I'm not doing that, I'm catching up on reading (short) articles that keep me up to date without being overwhelmed. Also, interacting with other researchers and pathologists at conferences (local, national, and international) is tremendously fun and inspiring. I enjoy going from project to project, always looking for new experiences at and beyond the limits of my knowledge. Doing new things, especially when I lack the relevant credentials, allows me to collaborate and learn from others. My wife (and cats) are long-suffering, but we (minus the cats) do manage to get up to the mountains or off to Ireland or Australia when possible.

If I could go back to the start of my career and give myself some advice, I would say three things. One: "Go to class." I slept through most of my classes. One semester, I attended three lectures from an entire physics course. Two: "Don't plan." Things will happen and you will adapt – because where I am now was certainly not planned. And three: "Don't worry if you don't know something – learn the vocabulary and collaborate."

Richard Levenson is Professor and Vice Chair for Strategic Technologies in the Department of Pathology and Laboratory Medicine at the University of California Davis Health, Sacramento, USA.

Professional Quality Gross Imaging Made Easy



Quality: The PATHpix gross imaging systems from Virtus Imaging are designed to make anyone a photography expert. Whether it's the PATHpix XLS stand alone system or the PATHpix XL system integrated into the PMT Scientific grossing station, you can easily capture research quality images with our unique zooming lens design.

Simplicity: Designed for use by experienced and novice photographers, the easy to use interface can be controlled via touchscreen, foot pedal, or voice command. The large depth of focus allows for a two step imaging experience, just zoom and capture.

Efficiency: With a full annotation suite, it's simple to add measurements, labels, text, etc. into your images. PATHpix also integrates with most LIS/PACS systems to fit seamlessly into your existing workflow by allowing direct acquisition into patient reports.

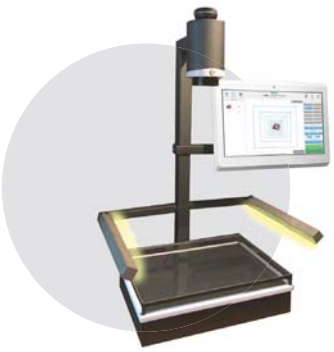
Spotlight on... Technology

Milestone's RoseSTATION: Standardized FNA WorkSTATION

Rapid On-Site Evaluation (ROSE) is a service that cytopathology laboratories provide for patients and their physicians. Historically, there has never been a mobile unit for ROSE.

The RoseSTATION is Milestone's solution to arriving rapidly on-site with a standardized work platform containing all tools required to accomplish this important diagnostic task.

www.milestonemedsrl.com/product/rosestation/



PATHpix XLS Stand Alone Gross Imaging System

Whether you are a novice or professional photographer, you can capture publication quality macro images with the PATHpix XLS. Designed with simplicity in mind, you can control the camera via foot pedal, touchscreen, or even your voice. Our two-step zoom and capture process makes imaging easy.
www.virtusimaging.com/thepathologist

MarginMarker™ Sterile Ink The Global Standard For Tissue Orientation

Surgeons use the MarginMarker sterile ink kit to define margins of excised tissue while in the OR; the pathology lab receives a clearly marked specimen. Unlike suture or metal tags, MarginMarker inks completely define each margin plane, resulting in more accurate re-excisions and potentially lower recurrence. Please request a sample.
Contact us at info@vectorsurgical.com or see VectorSurgical.com

Visualize Pathology With the Hyperion™ Imaging System, powered by CyTOF®

The Hyperion™ Imaging System, powered by CyTOF technology: simultaneous detection of up to 37 protein markers in one tissue section using highly pure metal isotopes conjugated to antibodies. Measure protein markers at subcellular resolution without spectral overlap or autofluorescence for a comprehensive view of the tissue microenvironment.
www.fluidigm.com/applications/imaging-mass-cytometry

Work Comfortably with Olympus Ergonomic Solutions

Microscope ergonomics is a priority for clinical routine microscopy. Sitting in an uncomfortable position for long periods of time can cause pain to the back, neck, shoulders, arms, and hands. The BX46 is specifically designed to meet the demands of repetitive routine microscopy.
https://www.olympus-lifescience.com/en/landing/ergonomic/

BioFire® FilmArray® Torch

The high-throughput BioFire Torch is a fully integrated, random access system designed to meet your laboratory's syndromic infectious disease testing needs. The BioFire Torch is compatible with all existing BioFire FilmArray Panels, providing the quick, comprehensive, and accurate results you have come to expect from BioFire products.
biofiredx.com

Patient Safety Redefined with Signature Cassette Printer

The Signature Cassette Printer of Primera Technology significantly increases the lab's efficiency while helping to reduce the risk of specimen misidentification by directly printing onto cassettes. It is available as a standalone manual printer or as a completely automated system consisting of a printer and a robotic picking system called Autoloader.
www.dtm-medical.eu

Revamping the Digital Interface

Sitting Down With… David Wilbur,
Former Director of Cytopathology and Clinical Imaging at Massachusetts General Hospital and current Chief Medical Scientist at Corista LLC, USAK

What changes have had the biggest impact on your pathology career?

I've borne witness to several revolutions in the field – the biggest of which has been the transition from standard H&E morphology to immunohistochemistry. That huge shift changed not only the way we practice, but also our confidence in making diagnoses. A very similar change is happening today with the molecular revolution; I would say that almost all of our oncology cases now receive some form of molecular testing. But, most excitingly, we are on the doorstep of a third major revolution – at least in Europe – with digital pathology.

We haven't quite seen the dawn of the digital age in the US yet, but it is starting to make an impact in certain instances; for example, with the remote reading of cervical cytology cases from a clinic called CerviCusco in Peru. For the past seven years, we have diagnosed images sent by the clinic every Friday morning via digital pathology, providing huge benefits for the people of Cusco, who don't have a local pathologist to read their cytology. We were also able to remotely train a cytotechnologist in Cusco, which is amazing for the local area and would never have happened before digital pathology.

Why has the US been slower to adopt digital pathology?

Although there is no question about the versatility and accuracy of digital pathology, there is a question surrounding its business case; most departments consider it a cost rather than a revenue-generating concept. European institutions have an advantage over those in the US because their health care systems incorporate digital pathology into their plans and fund these projects over a period of time. Here, we have to demonstrate that it can generate revenue, provide efficiency to save money, or improve patient safety before allocating the money and resources.

I believe that one of the main things holding back the adoption of digital pathology is the poor user interface between the pathologist and the viewing station – something that I have been trying to address over the last 15 years. As the product of over a century of development and fine-tuning, the microscope is exceedingly efficient and ergonomic for the user, and this is difficult to replicate in digital viewing systems. Inspired by the "Powerwall" at Leeds, which is a fantastic way to view digital slide images, I wanted to translate the way pathologists handle slides under the microscope into digital systems.

That's where the laser box virtual slide stage comes in. An artificial slide on top of a small, laser-controlled platform allows you to move the digital image around on the screen as if it were a real glass slide. We have successfully tested this prototype and pathologists have consistently commented on the improved efficiency of the technique compared with using a mouse, which may be excellent for navigating a computer screen, but is quite slow and laborious for diagnostic review of a whole slide image. At the moment the laser box only works with the Corista viewing system but, in the future, I would like to see it as a piece of standalone equipment that can be plugged into any system.

What do you hope to achieve as Chief Scientific Officer at Corista?

In addition to optimizing the interface between pathologists and digital viewing systems, my ambition is to further the potential applications of artificial intelligence (AI). Our first foray into AI has been in screening renal biopsies to identify glomeruli. Although that sounds like an arcane task, for a renal pathologist who has to find and evaluate every glomerulus on a biopsy, the ability to navigate to those glomeruli immediately is highly desirable. In addition, our work includes the registration of all special stains, meaning that the same glomerulus is co-located on each special stain for digital review simultaneously. We're now striving to go one step further and classify the glomeruli based on diagnostic annotations that renal pathologists have given us. If successful, we will then be able to apply those same tools to a variety of biopsies such as prostate, breast, GI, and urinary tract. Through digital "prescreening," we hope to address the sorts of scenarios that will make the pathologist much more efficient and potentially more accurate.

What's your outlook on the future of pathology?

There are a lot of naysayers who believe that pathology is going to disappear because of AI, but I am of the firm belief that it will make us far more productive. Genitourinary (GU) pathologists, for example, will use AI to pre-screen prostate biopsies on arrival to identify "hotspots," or areas of high probability that the pathologist needs to examine most closely. Many of these areas will be false positives, but that isn't a problem – the key part is that there will be a pathologist to look at those slides and discriminate between cancer and benign mimics. This guided screening will mean that, instead of only having time to look at a handful of prostate biopsies in a morning, GU pathologists will be able to look at many more, greatly improving efficiency.

What is the proudest moment of your career?

Interestingly enough, that moment actually happened a few months ago when my son – a practicing radiologist – and I led a workshop together at the American Society of Cytopathology's annual meeting. It was a pathology and radiology correlation conference that we ran as a seminar; my son presented the radiology part on one side of the room and cytologists presented the pathology from the other side. We talked about differential diagnoses from both the pathology and radiology aspects, showing how important it was for patient care to have both perspectives. Seeing my son take his place as a teacher and clinician really was an incredible experience.

ALL THE PIECES ARE IN PLACE.

—Includes—
ClearLLab Controls
The First L&L Application-Specific Control Cells

Presenting the ClearLLab 10C System, including application-specific control cells. If you've been looking for a new, simple and powerful way to expedite and standardize compliant Leukemia and Lymphoma* (L&L) immunophenotyping, you've found it. The **ClearLLab 10C System** is the first FDA cleared IVD and CE marked L&L immunophenotyping solution for both lymphoid and myeloid lineages—reducing the need for extensive validation and cocktail preparation. Beckman Coulter's ClearLLab 10C panels are validated with our **Navios** and **Navios EX** flow cytometer, **Kaluza C** flow analysis software, **ClearLLab Control Cells** and provide the educational **Casebook** to form an integrated, standardized system—enabling more-efficient lab operations and simplifying compliance.

Learn more about ClearLLab 10C System now, at
www.beckman.com/clearllab-10c-system

BECKMAN COULTER
Life Sciences

*Non-Hodgkins Lymphoma only.

FLOW-5128ADV03.19

Diagnostic Pathology is Embracing NGS for Actionable Variants

Interpreting variants top-funded NGS pathology projects

Explore Alissa Clinical Informatics

Adrienne McCampbell, PhD
Pathologist and Research Scientist
Agilent

Diagnostic pathology is engaging downstream NGS workflow for actionable variants

Alissa Interpret is sought for clinical-grade support

Downstream NGS data workflow is the primary focus identified by most participants in Agilent's NGS eSeminar Series hosted by The Pathologist. Tapping into actionable insights, delivering clinical-grade reports is accelerating their NGS adoption and Agilent Alissa Interpret is the leading the way.

But don't take our word for it, let our customers share how they are upleveling their clinical-grade SOPs. Better yet, test drive Alissa Interpret and see for yourself.

Check out our customer Case Studies on our Pathologist Partner Channel.
http://thepathologist.com/agilent/alissa

Alissa Interpret is a USA Class I Exempt Medical Device, Europe CE IVD, Canada and Australia Class I IVD Device.
Alissa Interpret Research is For Research Use Only. Not for use in diagnostic procedures.

DEMO Alissa Interpret at:

https://thepathologist.com/agilent/alissa

the Pathologist

Agilent Technologies