SEPTEMBER 2021 # 78

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- Improved cosmesis[6,8]

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 References: (1) Dooley, W.C. and Parker, J. "Understanding the Mechanisms Creating False Positive Lumpectomy Margins." *American Journal of Surgery* 190 (2005): 606-608. (2) Britton, P.D.; Sonoda, L.I.; Yamamoto, A.K.; Koo, B.; Soh, E.; and Goud, A. "Breast Surgical Specimen Radiographs: How Reliable Are They?" *European Journal of Radiology* 79 (2011): 245-249. (3) Molina, M.A.; Snell, S.; Franceschi, D.; Jorda, M.; Gomez, C.; Moffat, F.L.; Powell, J.; and Avisar, E. "Breast Specimen Orientation." *Annals of Surgical Oncology* 16 (2009): 285-288. (4) McCahill, L.E.; Single, R.M.; Aiello Bowles, E.J.; Feigelson, H.S.; James, T.A.; Barney, T.; Engel, J.M.; and Onitilo, A.A. "Variability in Reexcision Following Breast Conservation Surgery." *Journal of the American Medical Association* 307.5 (2012): 467-475. (5) Singh, M.; Singh, G.; Hogan, K.T.; Atkins, K.A.; and Schroen, A.T. "The Effect of Intraoperative Specimen Inking on Lumpectomy Re-excision Rates." *World Journal of Surgical Oncology* 8.4 (2010). (6) Landercasper, J.; Attai, D.; Atisha, D.; Beitsch, P., Bosserman, L.; Boughey, J.; Carter, J.; Edge, S.; Feldman, S.; Froman, J.; Greenberg, C.; Kaufman, C.; Morrow, M.; Pockaj, B.; Silverstein, M.; Solin, L.; Staley, A.; Vicini, F.; Wilke, L.; Yang, W.; & Cody III, H. "Toolbox to Reduce Lumpectomy Reoperations and Improve Cosmetic Outcome in Breast Cancer Patients: The American Society of Breast Surgeons Consensus Conference." *Annals of Surgical Oncology* 22.10 (2015): 3174-3183. (7) Lovrics, P.J.; Cornacchi, S. D.; Farrokhyar, F.; Garnett, A.; Chen, V.; Franic, S.; and Simunovic, M. "The Relationship Between Surgical Factors and Margin Status After Breast-Conservation Surgery for Early Stage Breast Cancer." *The American Journal of Surgery* 197.6 (2009): 740-746. (8) Gibson, G. R.; Lesnikoski, B.A.; Yoo, J.; Mott, L.A.; Cady, B.; Barth, R.J. Jr. "A Comparison of Ink-Directed and Traditional Whole-Cavity Re-Excision for Breast Lumpectomy Specimens with Positive Margins." *Annals of Surgical Oncology* 8.9 (2001): 693-704. (9) Gage, I.; Schnitt, S.J.; Nixon, A.J.; Silver, B.; Recht, A.; Troyan, S.L.; Eberlein, T.; Love, S.M.; Gelman, R.; Harris, J.R.; and Connolly, J.L. "Pathologic Margin Involvement and the Risk of Recurrence in Patients Treated with Breast-Conserving Therapy." *Cancer* 78.9 (1996): 1921-1928. (10) Menes, T.S.; Tartter, P.I.; Bleiweiss, I.; Godbold, J.H.; Estabrook, A.; and Smith, S.R. "The Consequence of Multiple Re-excisions to Obtain Clear Lumpectomy Margins in Breast Cancer Patients." *Annals of Surgical Oncology* 12.11 (2005): 881-885.

Dare to Change

Even good changes can be difficult – so how can we overcome resistance to them?

I recently moved house. Sitting in a room surrounded by cardboard boxes, I was struck by a familiar feeling. Previous house moves? A legacy of my international youth? Not at all; in fact, what I was remembering was packing up my laboratory in graduate school for a move to a larger facility.

Like many changes, lab expansion is often a good thing. More space for research! More space for staff! More space for piles of test results, journal preprints, grants to review, and folded posters from long-forgotten presentations! But all change, even good change, requires an adjustment – and that isn't always easy.

When my laboratory expanded, all of us – from the most senior postdoctoral fellow to the summer student who had been with us for a few weeks – assumed our benchtop space would stay just as it was. "Don't fix what ain't broke" seemed to make sense – so it came as a surprise when the plans to gut and redesign the space included not only the new areas, but also our existing laboratory.

Several months later, we were installed in a bright, airy room with three times as much space as our old lab. The benches were new, the windows were big, the sinks worked properly… It was an adjustment for us all, but a good one. The stress of packing and moving – and changing – was worth it.

Since then, I've noticed that the most forward-thinking among us – the scientists, the doctors, the laboratory professionals – are just as subject to change resistance as anyone else. Old habits die hard, and this can make progress difficult. How can you convince someone to make the move to new technologies, new diagnostics, or new treatments when they have been using the old ones (successfully or otherwise) for years? Often, results are not enough to overcome resistance, and the perceived benefit may be outweighed by challenges: staffing, training, cost, space requirements… the list goes on. And, sometimes, this presents a disadvantage for patients. After all, a rare disease patient may go undiagnosed without newer, more complex technologies – or may go without treatment if the supporting tests are unavailable.

Have you encountered a resistance to change in the laboratory? Tell us about it (edit@thepathologist.com) – and share your tips for overcoming this unique obstacle!

Michael Schubert
Editor

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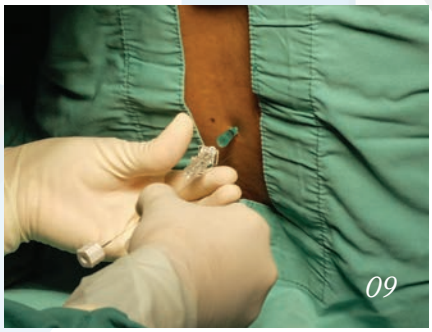
On The Cover

Artist's representation of pathology at war with itself.

Upfront



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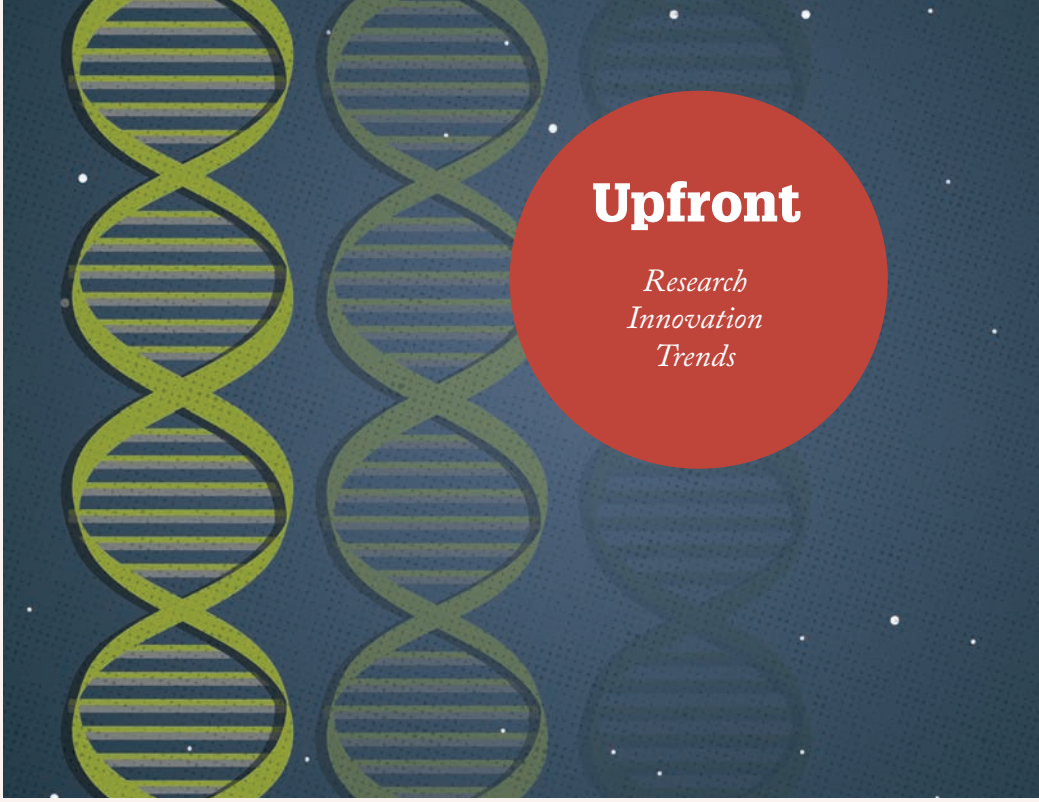


CRISPR/Cas9 That's Out of This World

In space, someone can splice your genes

CRISPR/Cas 9 in space: the final frontier? With more astronauts embarking on space explorations (and an obsession for commercial space travel in billionaire circles), there is a growing need to tackle the risk of DNA damage caused by ionizing radiation outside the Earth's protective atmosphere. Researchers have now developed a system that can study DNA repair in yeast cells in space (1) – so we spoke to senior researcher, Sebastian Kraves, to find out more.

What inspired you to develop a CRISPR/Cas9 gene editing system for use in space?

The idea was developed by a team of high school students who participated in the 2018 Genes in Space competition, in which young students propose their ideas for a DNA experiment to help advance space exploration. The group became interested in studying DNA damage and repair in space – and thought that CRISPR/Cas9 would be the perfect tool. Previous studies on the effects of microgravity on DNA repair were limited to using simulated microgravity or inducing breaks on Earth before studying repair in space, so they wanted to design an experiment that would allow scientists to carry out the process entirely in space.

How does the system work – and how did you specifically modify it for space?

The elegance of this study is that astronauts can trigger DNA lesions in a controlled manner using the CRISPR gene editing system. CRISPR allows us to target the DNA damage to a particular gene that, when disrupted and repaired, results in a visible color change in the cells. This color shift allowed the astronauts in our study to report back that the technique was working, which they later confirmed with molecular tools.

What's next for the research?

The experiment can now be repeated and expanded to a large enough sample size to draw valuable conclusions about how DNA repair is altered in space. This has huge implications for the DNA repair that occurs in astronauts exposed to ionizing radiation and can motivate protective measures and future medical advancements for astronauts returning from spaceflight.

Interested? Read the full interview and references at: tp.txp.to/crispr/cas9

INFOGRAPHIC

The 2021 Power List in Numbers

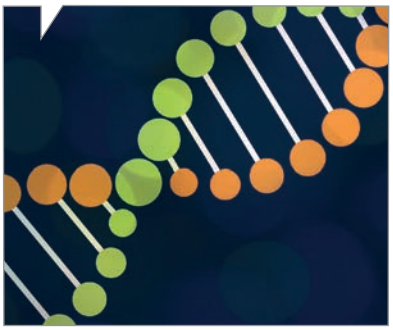
Breaking down this year's Power List by country, gender, and nominations

Finalists by country

US 50
UK 14
Spain 2
Grenada 2
South America 1
Greece 1
Africa 1
Czech Republic 1
Australia 1
Saudi Arabia 1
Canada 1

GENETICS IN FOCUS

The latest research in laboratory medicine, with a focus on genes



Family Ties

Though genomic studies have developed our understanding of heart disease risk, an American Heart Association statement highlights the lack of representation of marginalized racial and ethnic groups and Indigenous people (1). Despite only making up 16 percent of the global population, about 79 percent of genomic study participants are of European descent.

On/Off Switch

Researchers have developed new software to identify meaningful transcription factor activities in cells and reveal underlying gene regulatory mechanisms (2). The software works by combining ChIP-seq transcription factor information and scRNA-seq data analysis.

Eat, Sleep, (Tandem) Repeat

A new software that identifies variable-number tandem repeats (VNTR) with a repeat-pangenome graph solves the problem of VNTR mapping for short-read sequencing (3). It will enable researchers to better identify DNA variants involved in genetic changes that influence cell function and disease.

Forensic Files

Duct tape is a common piece of evidence recovered from water in forensic investigations. New research has found that, as long as there was sufficient cellular material initially, enough DNA can be recovered from folded duct tape submerged in the ocean for up to 336 hours to form a complete short tandem repeat profile (4).

Behind the Disorder

A newly identified genetic pathway may underlie a rare neurodevelopmental disorder (5). "Our findings have given us valuable insight into the role of MeCP2, miR-199a, and BMP signaling in the pathology of Rett syndrome," says team lead Kinichi Nakashima (6), who is working to uncover the mechanism behind disease-causing *MECP2* mutations.

See references online at: tp.txp.to/bite-gen-ed

(AI) Skin in the Game

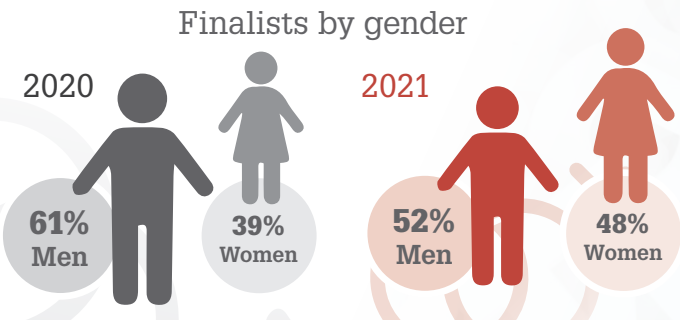
An artificial intelligence-based system can assist with tricky dermatopathology diagnoses

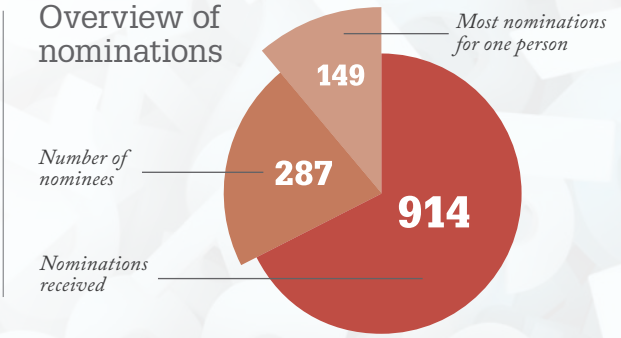
When devastating diseases manifest themselves, patients want answers – fast. Unfortunately, those are not always forthcoming, especially with conditions such as epidermolysis bullosa acquisita (EBA), an autoimmune blistering disease in which antibodies attack type VII collagen in the skin's epidermal basement membrane.

Currently, the disease can take months or years to diagnose, partly because diagnostic antibody patterns can be difficult to identify on a skin biopsy slide. To address the problem, dermatologist Joost Meijer and colleagues designed an AI-based system to spot the serrated, U-shaped immunodeposition patterns characteristic of the disease (1).

The system yielded sensitivity and specificity of 89.3 percent – outperforming many doctors – but the work is still ongoing. "It will take a year to collect the data of new skin biopsies, which will be able to validate the system," said Meijer (2). "Hopefully, we will then have a quicker and easier way to diagnose EBA and prevent the sometimes debilitating scarring."

See references online at: tp.txp.to/ai-skin





Screened at Birth

A new metabolomic profiling approach improves diagnosis of inborn errors of metabolism

Credit: Fé Ngô/Unsplash.com.

In the US, every newborn baby is screened at birth for rare (but serious) disorders; however, screening for inborn errors of metabolism (IEM) is not covered in standard panels or first-line biochemical testing. "Clinical genomic sequencing was becoming the norm, and I suspected that inborn errors were more common and not readily identified with current approaches," says Sarah Elsea, Professor at Baylor College of Medicine. "Genomic testing was identifying patients that should be diagnosed with metabolic screening, but the right tests were not ordered (or not available). It was clear that metabolic testing and screening were not keeping up with genomic screening, which delays diagnosis."

Recognizing the need for improved diagnostics, Elsea's team investigated whether untargeted metabolomic profiling was associated with increased diagnostic rates of IEMs compared with traditional metabolic screening approaches (1). "Our data show that some conditions are much more common than originally thought and that the phenotype spectrum can be quite broad," says Elsea. "We knew the overall diagnostic rate would be low, but we were somewhat surprised at the discrepancy between the traditional targeted testing and the global metabolomic approach. However, we also knew that serial testing, typically done for patients who tested negative using traditional approaches, delayed diagnosis."

So what's the next step? Elsea says, "New goals emerged from the analysis of our diagnostic testing data. Several conditions are clear candidates for newborn screening and we will be focusing on these genetic conditions – as well as targeting additional disorders – to identify disease-specific biomarkers and assess the phenotypic spectrum."

But the team won't stop there. "We will also use this testing approach to monitor treatment and management of individuals with IEMs, and we are investigating other types of biological samples that may be required for diagnosis and monitoring of some conditions – as well as correlation of metabolomics data to genomic variants to improve interpretation of variants of uncertain significance," Elsea says. "We only know what we know. If we do not look beyond those limits and assess where we are, then we will never discover anything new, and we will not improve diagnosis, treatment, or quality of life."

Reference

1. *N Liu et al., JAMA Netw Open, 4, e2114155 (2021). PMID: 34251446.*

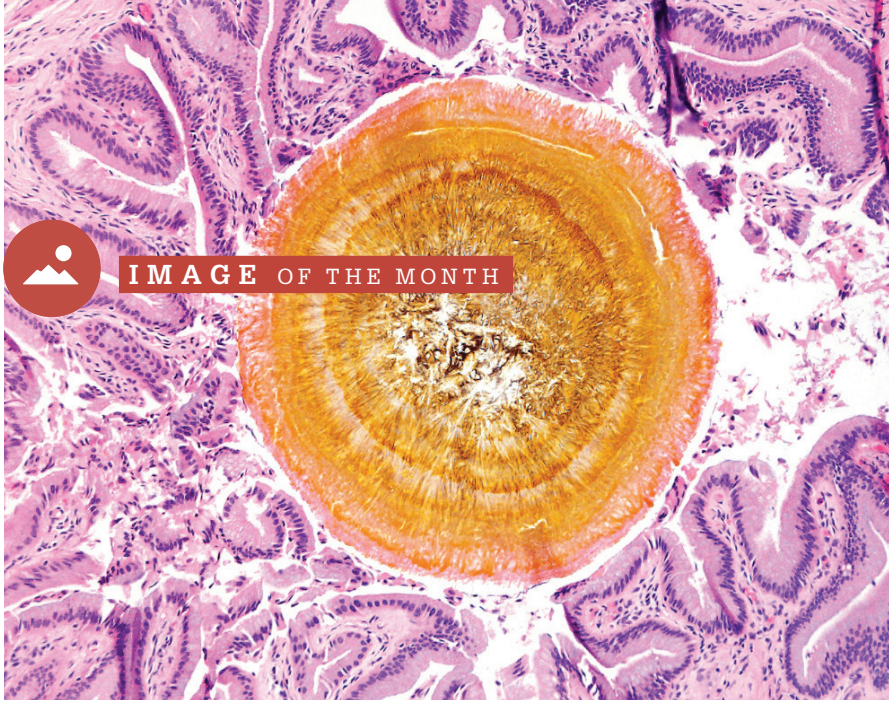
A Gut (Microbiome) Feeling

Potential novel biomarkers found in the gut microbiomes of children with autism spectrum disorder

To identify novel fecal biomarkers for predicting autism spectrum disorder (ASD), researchers have investigated the distinct features of the gut microbiota in children with ASD and in typically developing children (1). They found that ASD and chronological age were significantly associated with characteristic microbiome changes independent of diet. These changes included increased bacterial richness and alterations in microbiome composition in children with ASD. Five bacterial species and a depletion of neurotransmitter biosynthesis-related pathways in the gut microbiome were also found in children with ASD, but not in the typically developing group.

Though the study was small (and the gut microbiome also varies geographically), the researchers' findings demonstrate marked differences, including underdevelopment, in the gut microbiota in children with ASD compared to age-matched peers – and may pave the way for better prediction and treatment of the disorder.

See references online at: tp.txp.to/gut-micro



Like the Rings of a Tree

What mysteries might gallstones contain?

This microscopic image depicts a gallstone sitting in the neck of a gallbladder – a rare sight, because gallstones are usually hard to cut and only rarely show their stunning rings microscopically. Maybe the rings of this gallstone, like those of a tree, carry a meaning – but, as far as I know, nobody has ever described the meaning of these rings.

Credit: Jagmohan Sidhu is Medical Director and Chairman of Pathology and Laboratory Medicine and Medical Director of Transfusion Services at UHS-Wilson Medical Center and Binghamton General Hospital, and Clinical Professor of Pathology, SUNY Upstate Medical University, Binghamton, New York, USA.

Do you have a photo suitable for Image of the Month?
Send it to edit@thepathologist.com

TWEET of the month

"A lot of people don't realize that a hospital can't function without a lab and the work that I and my colleagues do. We're the ones that give your doctor the results they need to diagnose/treat you. So next time you pee in a cup, know that there's a person behind the test."

Angela Darby
Read the original tweet here: tp.txp.to/kikileesi-twt

Credit: Casa nayafina/Shutterstock.com

Expanding the Framework

Adding new biomarkers to existing tests for neurodegeneration can expand our diagnostic capabilities

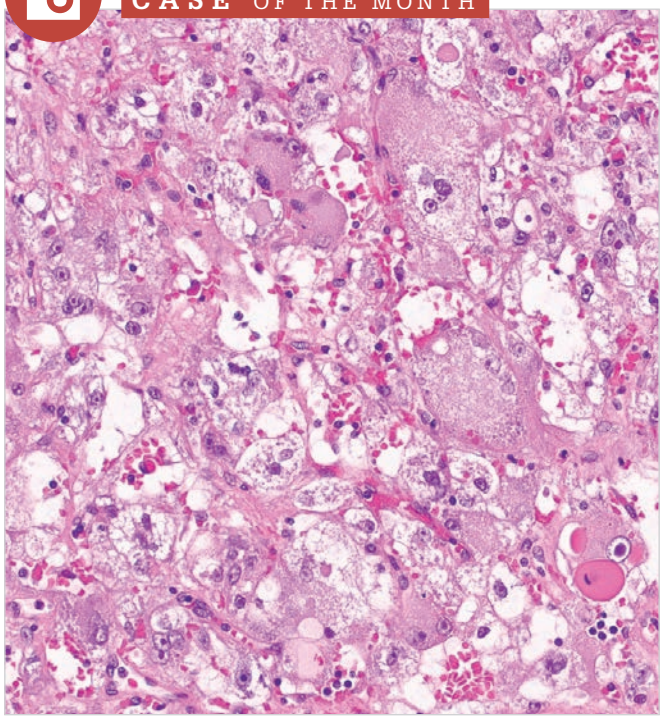
Diagnosing Alzheimer's and related diseases is tricky; these conditions are still diagnosed clinically and can only be confirmed on autopsy. However, the field of biomarkers for neurodegenerative diseases is promising – and a test known as the ATN framework can help.

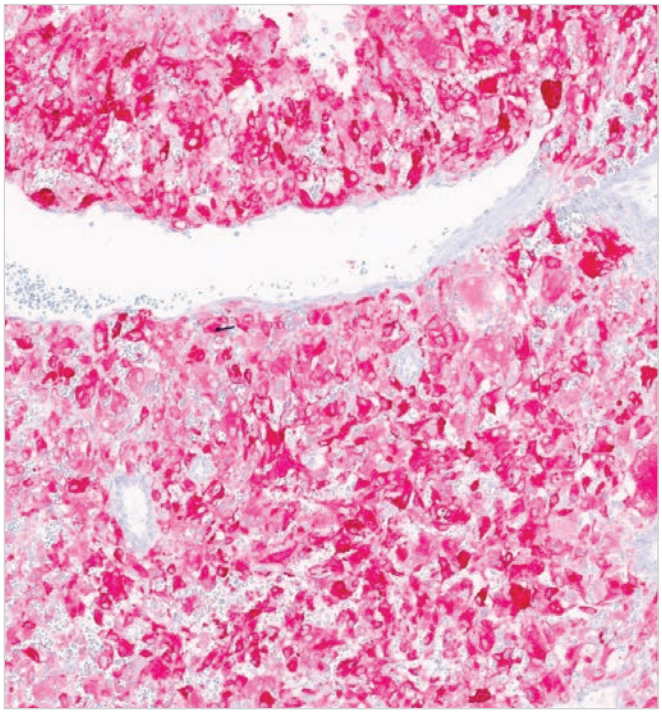
The framework looks for pathologic amyloid plaques (A), tangles (T), and neurodegeneration (N) in patients' cerebrospinal fluid. New research reveals that adding another marker, neurofilament light chain, allows diagnosticians to distinguish between Alzheimer's disease and frontotemporal degeneration (1), two conditions that share a variety of symptoms and can be difficult to differentiate.

"Alzheimer's is a diverse disease, and it is common for other conditions to also be present in the brain," said lead investigator Katheryn A.Q. Cousins (2). "The ATN framework may provide a more complete look at a person's diagnosis and give us a much richer understanding of not only Alzheimer's disease, but other co-occurring neurodegenerative conditions. However, to accomplish this, additional biomarkers that can detect other neurodegenerative conditions are critically needed."

See references online at: tp.txp.to/expand-frame

CASE OF THE MONTH





A 47-year-old man with a history of lung nodules was found to have an incidental exophytic mass (4.6 cm) arising from the upper pole of the left kidney on a CT scan of the abdomen in 2017; it increased in size to 5.9 cm in 2019 and to 11.6x8.3x5.7 cm in 2020.

What is the diagnosis of this renal neoplasm?

a) Malignant PEComa
b) Clear cell renal cell carcinoma with sarcomatoid transformation
c) Chromophobe renal cell carcinoma
d) Melanoma

Answer to last issue's Case of the Month…
d) Multicentric Castleman disease

Sections demonstrate overall preserved lymph node architecture with prominent interfollicular vascularity and secondary follicles that show involution and variable hyalinization of germinal centers with prominent mantle zones. Plasma cells and plasmablastic cells are increased in the interfollicular region and mantle zones. The plasmablasts show stippled nuclear staining for HHV8 and cytoplasmic staining for IgM.

Multicentric Castleman disease (MCD) is a systemic lymphoproliferative disease that occurs in patients with immunodeficiency or immunodysregulation. It has multiple sites of involvement and patients typically present with diffuse lymphadenopathy, splenomegaly, and systemic symptoms. In HIV-positive patients, MCD is almost always associated with human herpesvirus type 8 (HHV8) infection. Plasmablasts in MCD are characteristically positive for IgM and show lambda light chain restriction. HHV8 LANA1 immunostain is strongly positive; EBER in situ hybridization for Epstein Barr virus is negative. A subset of MCD cases are HHV8-negative.

Submitted by Anna Shestakova, Hematopathology Fellow, University of Michigan, Ann Arbor, Michigan, USA.

To register your guess, please go to http://tp.txp.to/0921/case-of-the-month
We will reveal the answer in next month's issue!

Case of the Month is curated by Anamarija M. Perry, University of Michigan, USA.

In advanced ovarian cancer,

If you're not testing for HRD, you're not seeing the whole picture

1 in 2 women with HRD-positive tumors do not have a *BRCA1/2* mutation[1-4]

Homologous recombination repair deficiency (HRD) testing identifies tumor characteristics—beyond *BRCA1/2* mutation—that make it sensitive to PARP inhibition.[1,5]

Personalized medicine begins with personalized pathology. Discuss establishing a testing protocol for HRD in ovarian cancer with the multidisciplinary team at your institution.[6-8]

Learn more at **testforHRD.com**

BRCA, breast cancer susceptibility gene; PARP, poly ADP-ribose polymerase.

References: 1. Frey MK et al. *Gynecol Oncol Res Pract.* 2017;4:4. **2.** Pennington KP et al. *Clin Cancer Res.* 2013;20(3):764-775. **3.** Konstantinopoulos PA et al. *Cancer Discov.* 2015;5(11):1137-1154. **4.** Ledermann JA et al. *Eur J Cancer.* 2016;60:49-58. **5.** Watkins JA et al. *Breast Cancer Res.* 2014;16(3):211. **6.** Cheema PK et al. *J Oncol Pract.* 2017;13(2):e130-e138. **7.** Hoskins PJ et al. *CA Cancer J Clin.* 2017;67(6):493-506. **8.** Sundin T. *Med Lab Manag.* 2019;8(11):6.

Precision Medicine & Nurse Education

AstraZeneca

In My View

Experts from across the world share a single strongly held opinion or key idea.

Check-In Time

The urgent need to resume routine cancer screening in a post-pandemic world

By Matt McManus, Vice President and General Manager at Asuragen, Austin, Texas, USA

Beyond its direct toll on public health, the COVID-19 pandemic has also resulted in other healthcare challenges – perhaps none more noticeable than in routine cancer screening over the past year. For at-risk individuals, this could have devastating consequences. I believe that pathologists have a clear role to play in getting cancer testing back on track to reduce the chances of negative outcomes from prolonged delays. In the last year, there has been a major drop in the number of patients seeking testing for cancer diagnosis and recurrence monitoring. A survey of more than 4,000 US adults run by the American Society of Clinical Oncology found that 24 percent of adults had delayed or canceled routine cancer screening tests due to COVID-19 (1). Separately, the Epic Health Research Network reported in May 2020 that preventive cancer screenings in the US had plummeted, with 86 percent fewer colon cancer screenings and 94 percent fewer breast and cervical cancer screenings than in prior years (2).

Aside from concerns about COVID-19 exposure, access to regular screening may have been challenging for some patients because many commercial laboratories and hospitals were appropriately focused on performing large-scale COVID-19 testing. This was a necessary shift, but one that limited some labs' capacity to provide other forms of needed testing. Many hospitals went to great efforts to put in place measures designed to protect patients and pave the way for other types of testing – such as facilitating off-site blood draws – but these measures were not always sufficient to reassure patients that it was safe to keep up with cancer screening.

This trend is concerning. Regular cancer screening is critical for early detection, treatment, and long-term monitoring. In the case of chronic myeloid leukemia (CML), for instance, patient care can be more effectively managed in the chronic stages with routine monitoring tied to oral treatments. However, if a relapse is missed because a patient has not been monitored regularly, dramatic interventions may be required. Monitoring cancer through frequent testing is a key aspect of keeping most CML cases manageable.

For many CML patients, targeted therapies extend survival by a decade or more. Unfortunately, mutations in the cancer mean that a fair number of patients eventually develop resistance to the first treatment – but new generations of targeted therapies can be swapped in to add to progression-free survival. The key is to switch medications before the patient develops widespread resistance. CML monitoring assays measure *BCR-ABL1* to flag cancer progression so physicians can adjust treatment when needed. Such monitoring makes it possible to follow a patient's drug response and pick up on signs of resistance before the patient's prognosis worsens. For patients who achieve remission and can discontinue treatment, long-term monitoring is equally important for detecting the earliest signs of recurrence and getting patients back on an effective targeted therapy in the event of any recurrence – before symptoms occur.

Current guidelines from the National Comprehensive Cancer Network recommend that cancer monitoring should be performed every three months. If a patient's condition is relatively stable and they miss a single monitoring test, there will probably be few significant consequences. But after more than a year of the pandemic, many patients have skipped multiple screenings – and that's something they may not be able to afford. As patients return to their doctors' offices, we may see more relapses and advanced disease than we would typically expect. To contain the human and monetary costs associated with managing late-stage cancer, it is imperative to return to pre-pandemic levels of screening and monitoring for all cancer patients.

With the availability of vaccines for COVID-19, hospitals and clinical laboratories are now moving past "pandemic response mode" and resuming routine testing. As they do, it will be essential to help patients feel comfortable in the clinical environment again. Some of this will happen organically as more of the public gets vaccinated and restrictions on social interaction are further relaxed. In fact, the Epic Health Research Network published an update to its initial findings last summer (3), noting that the number of cancer screenings had already begun to rise (though they still had not returned to pre-pandemic numbers).

The effects of the recent gap in testing may not be evident for some years to come. For now, getting patients back into the clinic for regular screening should be a priority. Pathologists will be critical in helping to achieve this – not least by reassuring the physicians they work with that their labs are open for business and can handle the levels of cancer screening needed.

References available online at: tp.txp.to/can-screening

The Next Microscope

How insourcing NGS can benefit community-based hospitals

Brandon S. Sheffield, Pathologist at William Osler Health System, Brampton, Ontario, Canada

Pathology is one of the oldest fields in medicine, with practices such as autopsy and anatomy dating back to ancient civilizations. The adoption of the microscope in the 19th century truly refined the trade, allowing practitioners to assess disease at the cellular level. Though there was initial resistance to this new technology, it is now a mainstay of laboratory medicine. We are currently embracing a new technology that is enabling the routine evaluation of diseases at a subcellular level – next-generation sequencing (NGS).

NGS uses similar principles to Sanger sequencing at a high throughput, or massively parallel, level. Traditional setups of the technique relied on a lengthy workflow including nucleic acid extraction, library preparation, and bioinformatic analysis. As such, NGS was first adopted by large, subspecialized laboratories that could offer the space, equipment, and personnel to perform these tasks. Novel NGS technology has since become available that condenses the entire workflow to a single instrument that can be operated by a single user, with results in a single day. Though the core technology is unchanged, this streamlined delivery has now brought NGS within reach for community-based practices like the William Osler Health System (Osler).

NGS allows for the simultaneous interrogation of multiple genetic biomarkers. As the number of targeted therapies and actionable biomarkers grows, NGS is an increasingly necessary standard of care in modern oncology. Despite this, there are multiple barriers to NGS access. In particular, the technology is not available in-house at many cancer treatment centers – especially community hospitals. This leads to long delays in obtaining biomarker results – or worse, treatments prescribed based on incomplete testing. With this in mind, we recently installed a new automated NGS platform and validated it for clinical use. Our new system integrates several elements of the NGS workflow, including library preparation and bioinformatic analysis. This simplified setup allows our histotechnologists to operate the instrument – so NGS can be integrated with morphology and immunohistochemistry (IHC) in a single report.

Before investing in comprehensive genomic testing, Osler had already shifted from an outsourced testing model to in-house single biomarker testing. This gave us much faster test results, but we found that, for some cancers, we were running upwards of seven different single-gene tests and still had an incomplete picture. That's why we started to explore comprehensive testing, which offers a more economical alternative in scenarios where multiple single-gene tests are needed.

When insourcing single-gene testing, we established a median turnaround time for lung cancer biomarkers of four days. In turn, 94 percent of patients had complete biomarkers available at the time they first met with an oncologist, compared to 17 percent when testing was sent to an outside center. Modern lung cancer treatment is simply not possible without biomarker data. Having these available at first consult provides dramatic cost savings while simultaneously improving patient outcomes and experiences.

> *"NGS allows for the simultaneous interrogation of multiple genetic biomarkers."*

Switching to comprehensive NGS markedly expanded the number of actionable markers for which we were testing. *MET* exon 14 skipping alterations are a prime example. There is no good single-gene test for these events, so they were not being routinely tested even though well-tolerated oral therapy is available. Since implementing NGS testing, we have identified a large number of these events, enabling improved outcomes and quality of life for these patients with highly effective pill-based therapies.

The transition to comprehensive profiling has carried tremendous benefit for our cancer patients – and our median turnaround time has remained unchanged at four days. This rapid delivery will once again enable

patients to meet with their oncologist for the first time and have biomarker data available for discussion – only now, it's comprehensive.

But precision medicine is not without its barriers – particularly in a publicly funded healthcare system. Despite its immense promise and clinical benefit, many institutions cannot overcome the fiscal hurdle of NGS. Insourcing costs include capital expenditure, reagents, maintenance, storage, supplies, and more. The true cost of delivering NGS data for clinical care is poorly understood, but one thing we do know is that technologists are the most expensive component. Using a fully automated gene sequencing system paired with a minimum number of technologists was the key for us in moving past the financial barriers – and, from there, the additional savings on shipping, accessioning, and stenography bolstered our argument.

Gene sequencing has been around for over half a century, and we are now a decade past the advent of NGS. Now, with fully automated workflows, the technology is reaching its true potential in the healthcare setting. Combining all the necessary steps into a single instrument means the technology can be placed in a clinical pathology lab – enabling comprehensive biomarker testing in a clinically relevant timeframe and with ongoing cost savings.

The pathologist's role is always changing. It's our job to use all available laboratory data to guide patient care to the best of our ability. As pathologists, we have the opportunity – and, I would argue, the duty – to bring the benefits of precision medicine to more patients by leading the adoption of comprehensive molecular profiling in our own facilities and communities. The technology is here; it's up to us to bring it to our patients.

Dear Medical Students

An open letter about pathology

By Matt DeJong, MS4, Stritch School of Medicine Loyola University Chicago in Maywood, Illinois

The sample is ready. I wheel over a chair, adjust the optics, and peer into the glass lens of the microscope. Just a few rooms over, a patient is lying on a table in a room surrounded by spotlights and masked medical personnel. Everyone in that operating room, conscious or not, is waiting for the answer we are about to find – an answer contained on a 75 x 25 mm piece of glass that only a pathologist can interpret. What we report in the next few minutes will direct the actions in the operating room moments from now – and potentially impact medical decisions made for years to come. That answer is found as I scan the slide, seeing the patterns and differences among the thousands of cells. Large cells with pink cytoplasm forming large pearl-shaped swirls and… "Squamous cell carcinoma." The words slip out of my mouth just as the thought forms in my head. My statement is affirmed by the attending and the report is delivered to those waiting in the OR. Just like that a diagnosis is made, an answer is given, and a life is changed forever.

When I began shadowing pathology, I was not prepared for how rapidly I could label someone with a lifelong diagnosis. I did not associate pathology with that kind of weight and responsibility. However, I quickly learned how little I knew about a specialty that is essential to medicine. Therefore, I challenge other medical students to explore a field that attracts intellectually curious minds to provide diagnostic answers in medicine.

What is pathology? Think of a movie and its soundtrack. When watching a movie, you often fail to notice the soundtrack that guides the story along its path. But, if the soundtrack were removed, imagine how much the story would lose. That subtle component, once removed, is obvious in its absence – and the film is incomplete. Similarly, pathology guides much of the medical decision-making in the hospital. It can go unnoticed at times but, without it, the hospital would have a hard time treating its patients. Like a film score, pathology becomes obvious in its absence.

After my first two years in medical school, my view of pathology was admittedly narrow. I knew that a team of physicians called "pathologists" existed, but was not quite sure what they did. Some I remembered as educators, but mostly I associated the specialty with the slides of "metaplasia" or "atypia" I saw in test questions. Seeing those slides could trigger anxiety; they symbolized a challenging aspect of board examinations. Unfortunately, this fear of histology-based test questions affects the way medical students view pathology. Instead of seeing it as a constant force permeating almost every aspect of medicine, medical students shy away from the field without ever understanding its true nature. In my

opinion, discovering pathology requires a genuine curiosity about clinical decision-making and mentors willing to guide that curiosity. Without these factors, many medical students overlook a foundational specialty because they lack the opportunity to explore it and therefore fail to understand what a pathologist truly does.

My curiosity developed when I was a third-year medical student doing an inpatient oncology rotation. I stumbled upon pathology by asking questions such as, "How are we differentiating these masses as specific cancer types?" or, "How are we finding these treatment-defining gene markers?" They all brought me to the same place: the pathology report. There, I saw the answers to clinically pertinent questions – but I wanted more. Reading a statement did not satisfy my curiosity. While it is necessary to know "what" clinically, I needed to know "how" those answers were determined intellectually. It was then that I truly began discovering how intertwined pathology is throughout all of medicine.

Thankfully, I reached out to an outstanding pathologist and exceptional educator at my institution who took the time to curate a month full of experiences showcasing the world of pathology. I saw surgical pathologists dissect, analyze, and diagnose specimens that ranged from small parathyroid adenomas to watermelon-sized liposarcomas. I participated in brain dissections, searching for signs of infarct or hemorrhage in a patient with an unknown cause of death. I investigated causes of death during autopsies, treated patients directly while shadowing transfusion medicine, and combed through fine needle aspirations in cytology. I toured the microbiology lab, where I saw bacteria that I assumed I would only ever encounter in textbooks – and so much more. The variety within pathology was joyfully overwhelming! I never once considered, before my experiences, how involved the pathologists at my institution are. Every culture swab, CBC, surgical specimen, blood transfusion, and more passes through the skillful hands of a pathologist before being relayed to the patients. In essence, most clinical decision-making would not be possible without the diagnostic or confirmatory information pathologists provide.

Consider a patient with a UTI who is treated empirically with antibiotics, but does not improve. Samples must be taken and analyzed by pathologists, who can then definitively say which bacteria has caused the infection and which antibiotic is best for the patient. What other specialty in medicine provides the skillsets and opportunities to have the final say on a diagnosis and therefore set the foundation for successful workup and treatment?

As well as the field's expansive diversity, I saw a consistency of themes among the pathologists I met. First, they all truly loved what they did. Many attributed their passion to the variety, intellectual challenge, and ability to provide concrete answers within the world of medicine. Additionally, almost all referenced the ability to maintain their desired work-life balance. The positive effect of balance is evident in the low burnout ratings seen among pathologists, especially when compared with specialties such as family medicine and neurology. Second, the pathologists highlighted ongoing opportunities to educate in their daily workflow as another reason they loved their careers. One of the primary daily teaching opportunities is during "patient rounds." Here, the team sits around a table together with a shared microscope that everyone can view simultaneously. As they round on the slides, or "patients," presenters will discuss patient background information (as though we were standing outside the patient room) and correlate it with the pertinent information from the slide. In this way, every pathologist in the room is aware of disease characteristics and engaged in understanding, diagnosing, and treating the patient. From faculty to residents, it seemed that everyone had a deep understanding of disease function and development, making them ideal educators. In fact, I saw a profound continuity of this theme when thinking about my favorite medical educators outside my own school. Husain Sattar, who developed Pathoma, and Edward Goljan, the author and editor of many medical study resources, both happen to be pathologists. Coincidence? I think not. By engendering such a positive learning atmosphere in a specialty teeming with happy, fulfilled physicians, it is not hard to see why pathologists love what they do.

Why do people become physicians? Often, it is because we want to help our fellow humans in a significant way. We want to help them recover from whatever illness they are facing. In many medical students' minds, that means sitting next to them in offices or patient rooms, providing clinical expertise, holding their hands, and treating their ailments. That is the "image" of a doctor we have seen all our lives. But we forget (or do not know) to consider the team of doctors combing through samples and specimens, diagnosing disease, and dictating clinical decision-making. Medical students must learn that diagnosis is no less crucial than the management and intervention methods with which we are so familiar. Without accurate clinical analysis from pathologists, other medical specialties would have a much more difficult time providing care.

The desire to become a better, more complete physician should drive all medical students to explore pathology. No matter what specialty you go into, an intellectual curiosity that spurs the pursuit of clinical answers will do nothing but better your future as a caregiver. Be the pulmonologist who can analyze a fine needle aspiration sample. Be the oncologist who wants to look through the cells of a bone marrow biopsy. Be the surgeon who looks at and discusses the specimen with the surgical pathologists. Or be the pathologist who can analyze, diagnose, and direct a patient's treatment. Whatever you choose, be the physician who seeks out answers on behalf of your patients because you have the curiosity and motivation to do so.

Hanging in the Balance

COVID-19 and pathologists' importance to detainees at the US–Mexico border

By Drew Bernhisel, medical student at Texas Tech University Health Science Center, El Paso, Texas, USA

The United States–Mexico border remains a site of ongoing public health and humanitarian challenges, drawing attention and action from governmental, charitable, and medical organizations. There are tens of thousands of asylum-seekers currently detained by immigration enforcement along the border, an increasing proportion of which are unaccompanied children. I previously wrote about the role pathologists have in meeting the health needs of these vulnerable populations (1) – but, since that time, the COVID-19 pandemic's rapid international spread and deadly disease course has changed the priorities and strategies of the anatomic and clinical pathologists who diagnose and treat such patients. Because of their integral role in these ongoing challenges, pathologists' awareness of – and advocacy for – patients in border detention remains essential.

During a dedicated clinical pathology rotation at my medical school in El Paso, Texas (one of the country's largest border communities), I spent time in the laboratory with pathologists and other lab personnel, reviewing the changes in priorities that were thrust upon us by the onset of the pandemic. Viral antigen tests and PCR analysis rapidly became a massive new management task for labs across the globe, and validation of these and other clinical tests relevant to the spread of the virus became an urgent need. Since testing began in February 2020, in the El Paso Field Office alone, over 600 detainees in custody have tested positive for the virus number. In fact, El Paso had the largest outbreak of COVID-19 of any detention facility in December 2020, likely contributing to the region's spike in cases that winter. The unprecedented nature of the pathogen, combined with the cramped physical conditions of border detention, has created a burdensome public health crisis within a greater humanitarian challenge.

In clinical rotations, I've also had the chance to experience challenges such as lab safety with infectious disease specimens and managing diagnostic tests from isolated groups like senior care centers, incarcerated populations, and people in US border detention. For communities on or near the US–Mexico border, this latter group represents a significant testing requirement and a major contributor to the hospitalized population, highlighting the heightened need for pathologists' diagnostic capabilities.

The advent of widespread vaccination has brought generally lower caseloads nationwide but, during the pandemic's peaks, morgue capacities were overrun, creating severe challenges for forensic pathologists and staff. At the El Paso County medical examiner's office, temporary morgue units and emergency staffing by prison inmates were needed to meet the vastly increased load of deceased individuals and the accompanying need to determine whether or not their deaths were COVID-19-related. Despite being much younger than the US population average, border detainees' all-cause mortality has spiked since the onset of the pandemic, largely driven by COVID-19 deaths.

> *"Pathologists' awareness of – and advocacy for – patients in border detention remains essential."*

Though it is possible that the worst of the pandemic in the United States is past, ongoing work is needed to address the diagnosis, treatment, and prevention of COVID-19 and similar illnesses, especially in vulnerable populations. Recent immigrants and refugees are an important case study of these needs, and recent increases in unaccompanied migrant children in facilities and worries about overcrowding only increase the need for detainee vaccinations and effective care by all medical providers. Pathologists are at the front lines of these developments, and our work and advocacy on behalf of these vulnerable populations benefits us all.

Reference

1. *D Bernhisel, The Pathologist (2021). Available at: https://bit.ly/37wOIN4.*

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Scientific Detectives for Better Patient Care

It's our job to help patients piece together the clues to their own healthcare

By E. Blair Holladay

Over the past decade, we've seen a growing trend of patients feeling empowered in their healthcare journeys. They are more involved than ever – curious about their diagnoses, the tests they undergo, and their treatment plans. They have a wealth of research at their fingertips and their interest in their own health and wellness has been accelerated by the move toward personalized medicine. Patients ask questions and expect answers. Being an active participant in healthcare today requires a high degree of independence.

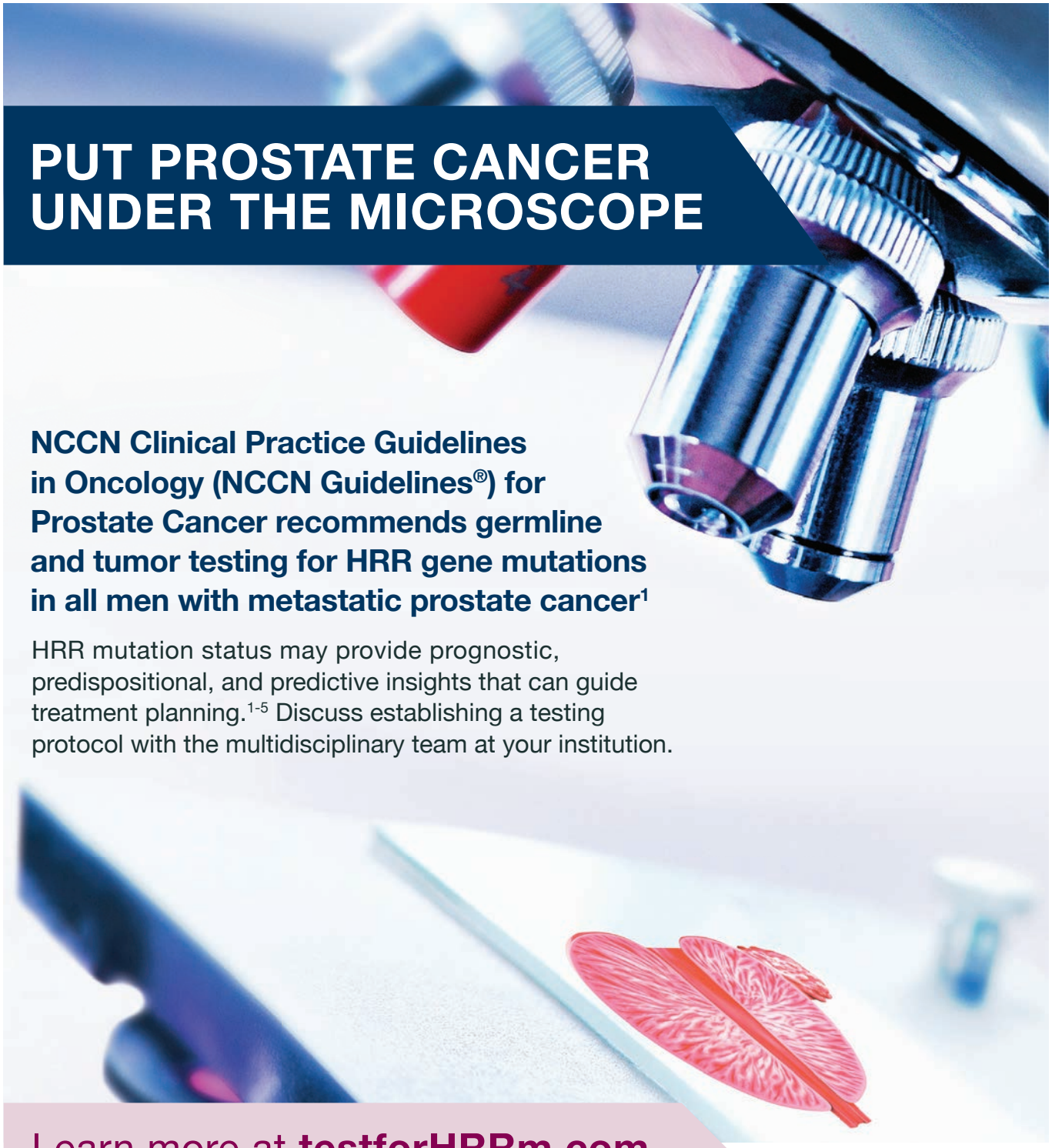
But patients can't go on their journeys alone.

Though they may be able to track their symptoms or investigate treatment options, patients cannot make a diagnosis themselves. They cannot analyze test results to best chart a course of medication in a vacuum. For a patient, true empowerment means collaborating with their care teams. Patients cannot embark on a treatment plan without the knowledge and support of their clinicians. They certainly cannot move to the next step of their healthcare journey without leaning on the expertise of the laboratory.

We know that laboratory professionals touch almost every part of a patient's care journey – from blood draws to diagnoses. Do patients know this, too? Some do, but raising the visibility of the laboratory is part of our duty as pathologists and medical laboratory professionals. We are the scientific detectives of the healthcare world and we are the leaders to whom patients can and should turn when they are faced with a diagnosis that has the potential to upend their lives. We must convey our critical role in their care and help patients understand that the laboratory is the bedrock of their healthcare journey – and this only becomes more important when patients are faced with a diagnosis that they simply do not understand. We have the insight to help them make sense of this step of their journey.

Whether the diagnosis is something we see in the lab every day or a condition we rarely diagnose, what matters most is the patient behind the diagnosis. That is where we, as scientific detectives and caregivers, can share and use our skills, applying what we know and what we continue to learn to best treat the patient. We bring a wealth of solutions to save patients' lives every day, and our role as trusted leaders in our patients' healthcare journeys is something we can, and we must, pursue and embrace.

"Patients can't go on their [healthcare] journeys alone."



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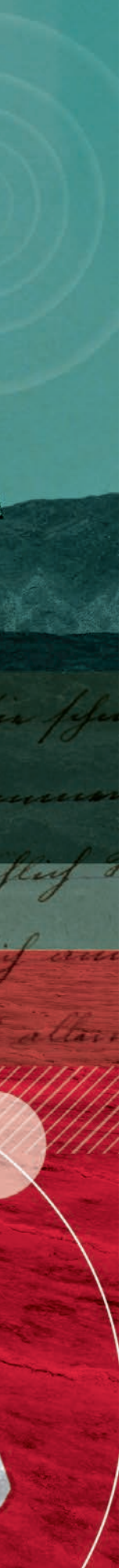
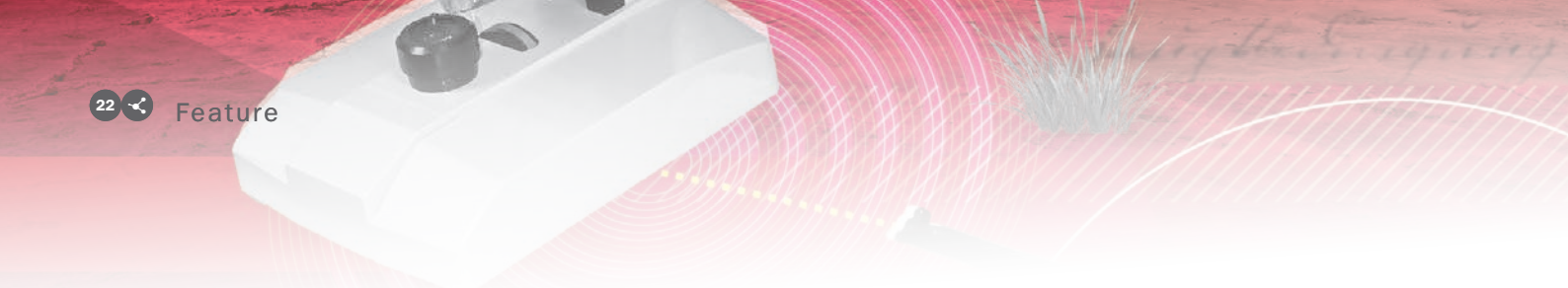


OUR OWN WORST ENEMY

EXAMINING PATHOLOGY'S PLACE – OR LACK THEREOF – IN THE US MEDICAL SCHOOL CURRICULUM

By Olivia Gaskill



"Pathology recruitment numbers are falling," we hear – time and time again. And it's true. Despite an 18.3 percent increase in pathology positions offered by the National Resident Matching Program in the US from 2008 to 2017, positions filled by US medical graduates over the same period declined from 77.7 percent to 50.1 percent (1).

We can see the statistics falling, but why is pathology as a profession getting lost – and, perhaps more importantly, where in the pipeline are things going wrong? Previously, we've considered the perception of pathology and whether or not it deters students from the profession; we've explored the impact of social media on residency matching programs; we've even highlighted tips for teaching pathology to get students interested.

But should we spend more time assessing pathology's place in the medical school curriculum? We spoke to two professors of pathology – Gurmukh Singh and Louis Maximilian Buja – and two medical students – Amber Berumen and Lauren Miller – to gain their perspective on where pathology could do better in its recruitment efforts.

Fixing what's not broken

Let's start at the beginning; over the past few years, almost every medical school in the US has gone through a curriculum reform, moving from discipline-based teaching to an integrated approach. Since the Flexnerian reform of medical schools in 1910 – a complete overhaul of US medical training based on the principles set out by Abraham Flexner (2,3) – physiology and biochemistry have been foundations of students' training and are seen as an essential part of their journey to becoming doctors.

Fast forward to the present day, however, and pathology has faded into the background – with nothing setting it apart from other specialties. "In some medical school curricula, it's no longer an identifiable specialty unless there's something that prompts you or you've had prior exposure to pathology – or if you go out of your way to get an elective in the lab," says Gurmukh Singh, Vice Chair of Pathology at Augusta University. When he started working in the US in 1978 at the University of Pittsburgh, pathology had a year-long module within the curriculum. "It was taught in the traditional way of lectures and afternoon laboratories in which we examined the gross and microscopic features and held case discussions. People were exposed to pathology as a discipline and they had the opportunity to meet a number of pathologists."

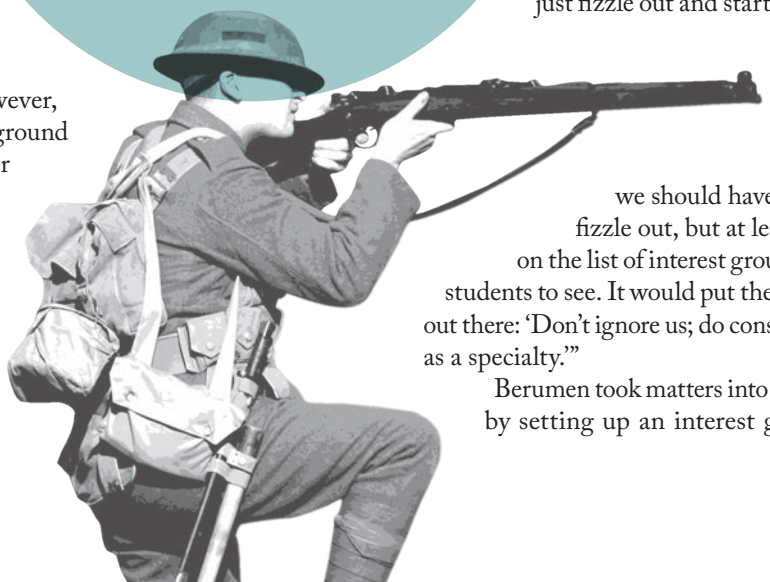
"Back before the reform, our pathology department had both formal lectures and a weekly laboratory session that gave students the opportunity to examine histologic slides," says Louis Maximilian Buja, Professor of Pathology at the University of Texas. In the process of the reform, his institution lost a formal pathology course and laboratory session and now has only pathology lectures that are interdigitated throughout the first two years. "The presentation of pathology as a distinct entity – which has both a basic science and a clinical component – has been diminished by this integrated curriculum approach."

Though Buja believes such an approach will negatively impact pathology, he's also concerned about the broader effect. "I believe it's diminishing the importance of basic biomedical science, because students are not getting the solid grounding they need to be astute clinicians later in their careers."

> "THE PRESENTATION OF PATHOLOGY AS A DISTINCT ENTITY [...] HAS BEEN DIMINISHED BY THIS INTEGRATED CURRICULUM APPROACH."

With this lack of exposure, pathologists are already treading the line of invisibility to medical students. Amber Berumen, a final-year medical student at the time of writing, highlights that her school didn't have a Pathology Interest Group – with faculty members advising that she shouldn't even start one. "I was told the interest group would just fizzle out and starting it wouldn't be worth the time," says Berumen. "That's not the attitude we should have. Yes, it might fizzle out, but at least it would be on the list of interest groups for medical students to see. It would put the right message out there: 'Don't ignore us; do consider pathology as a specialty.'"

Berumen took matters into her own hands by setting up an interest group, but the



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pandemic had other plans. " COVID-19 made it difficult to do many of the in-person activities we had hoped to do with the group, but luckily, a group of students have taken on leadership positions for next year, so the Pathology Interest Group at my former medical school will continue. I'm happy about that, because I'm keenly aware of the need to be involved with medical students' education. My goal is to make myself visible to medical students when I'm a pathology resident – showing them what I do – and that I exist!"

Shrouded in mystery

The lab is something of a mystery to students, invariably leading to common misconceptions – from both students and the wider clinical care team. "The biggest misconception I've heard is that the entire field is forensic pathology. When I tell people I want to specialize in pathology, they usually respond with, 'Oh, so you like dead people?'" says Lauren Miller, a fourth-year medical student at the Medical College of Wisconsin.

With the rise of CSI-style shows over the past few decades, a phenomenon known as the "CSI effect" has mystified the role of the laboratory in forensic investigation – and what laboratory professionals actually do. Researchers have traced this term back to a 2002 Time magazine article that discussed how public perception of forensic science may be influenced by its portrayal in popular crime scene shows (4). Pathology seems to have fallen into this trap, too. Medical students with no previous exposure to or interest in the field may think the lab is only for medical examiners and forensic pathologists. And, with no distinct pathology course in their education, there's little opportunity to prove otherwise.

"Most students are surprised when they take the elective and realize what we do and how we contribute to patient care – but, if they don't come, they can't have that revelation," says Singh. "And that's understandable, given that most people who go into medicine have the idea of working with and treating patients and, of course, it's not very often that we pathologists

LOUIS MAXIMILIAN BUJA

If you could change one thing about the current curriculum, what would it be?

The current curriculum has an integrated organ system approach, which has its merits; however, the various biomedical scientific disciplines have lost their identity. I would specifically identify content as pathology, physiology, and so on in each organ system block.

Which specialities do a good job of bringing attention to themselves?

In contrast to the integrated approach for the basic sciences, the clinical disciplines have retained their identities as specific clinical clerkships. This advantages specialties such as internal medicine or surgery in students' minds.

Have any countries got it right?

I only know that the US has produced a curriculum that unfortunately de-emphasizes pathology and other basic disciplines. The movement to a pass/fail system promotes mediocrity.

How would you like to see pathology represented in the curriculum in 10 years?

Rightfully recognized as the basis for the understanding and practice of scientific medicine.

deal directly with patients."

This void in appreciation can lead to students' thinking that test results come out of a black box without understanding how important pathologists and laboratory professionals are. Miller sees her peers largely overlooking the role of the lab, usually until they are exposed to it themselves. "Many students seem to have no idea that the laboratory is a busy, highly efficient department full of people running specimens and providing lab results in real time," she says. "They are often shocked at how much work goes on behind the scenes to provide diagnostic services to patients."

The final diagnosis

Though pathologists' role in the patient pathway is critical – a fact well known to members of the profession – the lack of patient contact may deter medical students from choosing a life in the lab. "Pathologists are the doctor's doctor; we provide information and the diagnosis to other physicians rather than dealing with patients directly. Clinicians, not patients, are our direct clients," says Singh, who highlights that this can make the profession unattractive to those who thrive on patient contact.

For some students, the lack of direct patient contact may be the deciding factor in choosing pathology. Singh recalls the role his personality played in his choice of specialty. "I'm an introvert and prefer to deal with science on my own rather than deal with other people too much. Some might say, 'Isn't that contradictory to being a doctor? You have to be around people,'" he laughs. "I understand the paradox, but that contributed to my going into pathology."

Singh's story seems to resonate with Berumen's own experience. "When I first started medical school, I was actively struggling with my social anxiety. Because of that, I initially thought I would specialize in radiology or pathology because I wouldn't have face time with patients," she says. "But, once I started clinical rotations and got direct exposure to patients, I found that I really loved my interactions with my patients and I cared for them deeply. The loss of direct patient care was something that made choosing pathology much harder."

One could argue that pathologists do deal with patients; after all, their decisions directly impact patient care. Buja became particularly fascinated with this side of the field. "To realize that the type of analysis we do with the gross and histologic examination yields information that is critically important to understanding the patient's clinical problems – that solidified my interest in a career in pathology."

Though his introverted personality laid the foundations for his pathway to pathology, Singh had a similar realization to Buja's in understanding the importance of pathologists in patient care. "I read The Final Diagnosis, by Arthur Healy, and

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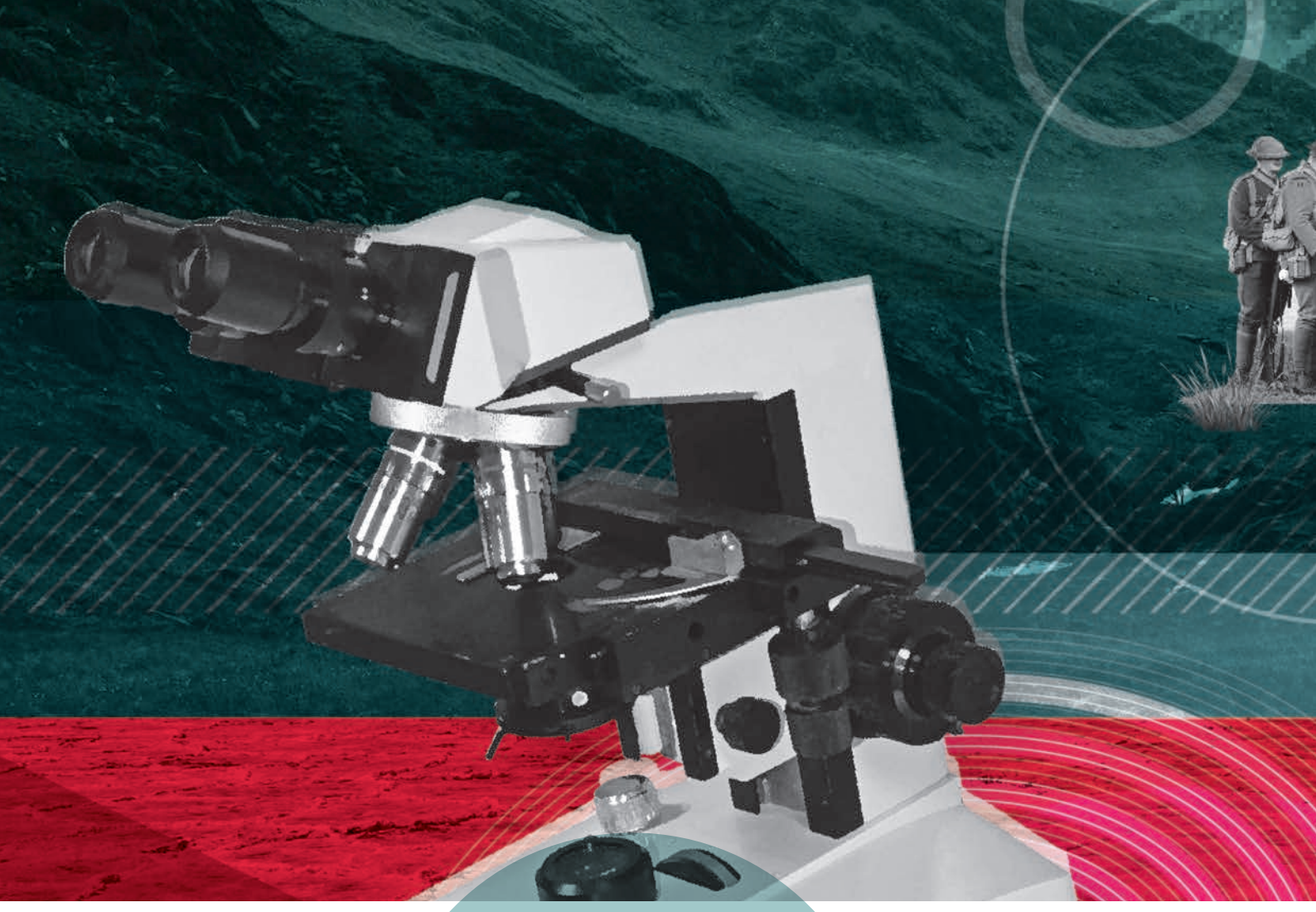

it scared the daylights out of me; a pathologist hadn't kept up to date with developments in science – and, because of that, a baby didn't make it." He recalls, "It reminded me that we make the final diagnosis and provide that information to other physicians. If that work is of interest, then pathology would be attractive to you, but that doesn't necessarily get communicated very well in medical school."

Cited as a defining factor for some choosing to pursue pathology and laboratory medicine, personality may not even enter into others' decisions. "Pathology and laboratory medicine was always on my radar as a potential specialty, especially because I continued to work in the lab during my first two years of medical school," Miller says. "I still kept an open mind as I went through my training in case I fell in love with a different specialty, but nothing else came close to how much I love pathology. Choosing pathology was really inevitable for me – regardless of where I was in my training, I was always being called back to the lab."

"CHOOSING PATHOLOGY WAS REALLY INEVITABLE FOR ME [...] I WAS ALWAYS BEING CALLED BACK TO THE LAB."

Who goes into pathology?

"Of course, from my attendings, I heard the stereotypical, 'But one of your strengths is working with patients – why would you go into pathology?'" says Berumen. "Another situation was when I was asked to introduce myself and state what specialty I was going into. When I said pathology, it really threw them off. They would say things like, 'Who goes into pathology?'"

Such responses may come from clinicians who don't fully understand either the day-to-day work of laboratory medicine professionals or the role they play in the patient pathway. And, though medical students are the ones most affected by these misconceptions, they are not in a position to change

the situation. Rather, qualified pathologists and laboratory colleagues have the power to increase their visibility to other clinicians in the curriculum. Buja, in particular, has been working on this since the start of his career.

"After I graduated from medical school, I was fortunate enough to get a position in a cardiovascular pathology lab at the National Institutes of Health (NIH), which solidified my decision to pursue pathology. However, I had to wait a year to get to the NIH. In the meantime, I undertook a clinical internship taking care of patients directly – which gave me excellent background to understand clinicians' needs and how to help them," says Buja. The relationship between pathologists and patient-facing clinicians can be disjointed and lack effective communication – but could the "walk in another's shoes" approach improve this relationship (and clinicians' perceptions of pathology) from the start?

"There are some professors who might ask their students, 'Why do you want to do pathology?' And that's why it's important for me to proactively interact with my clinical colleagues," says Buja. "I have to make sure they have respect for me as a knowledgeable member of the clinical care team." He hopes that building this rapport with fellow clinicians will increase their awareness of pathologists' role in the patient care pipeline. "I'm constantly providing our cardiologists and heart surgeons with important information to help them care for their patients. Hopefully, the respect I've gained translates to their general impression of the field – this should be important for all professors of pathology."

Clinicians from other specialties may not fully understand the role of the lab and its viability as a specialty for students – but Berumen says the field stands out for its collaborative opportunities and friendly nature. "I definitely see more collaboration in pathology, which is something that is really apparent once you do a pathology rotation or two. After having direct exposure with pathologists and other lab professionals, I know that these are the kinds of people I want to work with."

According to Berumen, while at medical school, students were invited by pathology attendings to speak up if they disagreed with the diagnosis of slides. Pathologists who invite these learning opportunities can set themselves – and the lab – apart. "Pathologists have been more open to my disagreeing with a diagnosis – and they won't just say, 'You're wrong,' because I think there's an understanding in pathology that everything is subject to interpretation and everything needs some sort of context," says Berumen.

GURMUKH SINGH

If you could change one thing about the current curriculum, what would it be?

Formal lectures are being replaced by case studies. Pathology may have to insert itself into anatomy and physiology, as well as clinical medicine, by developing engaging case studies with a prominent role for laboratory diagnostics – this would help to highlight the role of pathology in patient care. Showing pathology's role in diagnostic workup will also be important.

Which specialities do a good job of bringing attention to themselves?

Surgeons probably do, which is likely related to their more outgoing personalities. Pathologists are mostly introverts.

Have any countries got it right?

I have experienced pathology in India and the US; neither is a role model in promoting pathology.

How would you like to see pathology represented in the curriculum in 10 years?

A paradigm shift would require pathologists seeing all patients prior to surgery and explaining the role of tissue examination for diagnosis. It would be even better for the pathologist to see the patient post-surgery to explain the results of their blood tests and tissue examination.

Leading by example

Professors and incoming pathology residents have managed to see through the fog of misconceptions, which makes them ideal guides to show medical students the reality. For example, simply making students aware of the opportunities available behind laboratory doors may spark interest. "Students should know how diverse the field actually is. Each medical specialty has a corresponding pathology service and you can choose a unique and personalized career path," says Miller.

Singh echoes this sentiment. "I try to emphasize to my students that pathology is vast and there's something for everyone. All they have to do is pick an organ system of interest and there will be a multitude of clinical material to work with." However, if medical students aren't exposed to pathologists in the first place, this message may need to come directly from peers or residents. "You need one ambassador for pathology in each medical school to reach out to students and invite them into the lab," Singh continues. "That representative needs to be enthusiastic and dedicated and make increasing pathology's visibility part of their mission."

At Augusta University's Medical College of Georgia, where Singh teaches, a "disproportionately high number" of medical students go into pathology. He believes this is in part due to the head of the Pathology Interest Group at the institution. "They meet with the students and invite them to take electives – and that's how we've maintained an interest in pathology as a discipline."

Singh's institution is not the only one to run such initiatives; McGovern Medical School at the University of Texas Health Science Center in Houston has trainees and pathology residents who are interested in promoting the field. They've also started a Pathology Interest Group for students. Like Singh, Buja believes it is a strong way to promote the profession. "From what I can tell, it's a very positive experience for medical students to interact with people closer to their age and level."

But don't just take their word for it – Berumen's first interaction with the lab is a prime example of a single moment sparking an interest that can last a lifetime. "During my internal medicine rotation, my attending sent the internal medicines and myself to the pathology lab to check on the results for a peripheral smear slide they had ordered. The pathologist showed us the slide – and I was fascinated," she says. "One of the residents turned to me and said, 'I haven't seen you this excited about anything before. Why don't you look into pathology?' It was a weird moment where everything just clicked – that single moment inspired me to look into the field as a potential specialty, get more involved,

and seek out pathologists with whom to connect."

Pandemic effects

We can't talk about the role of medical school in pathology recruitment without touching upon the COVID-19 pandemic. Before March 2020, you might have been lucky to find a member of the public who knew what a polymerase chain reaction was – but, even within the clinical care pipeline, pathology's visibility has increased.

"We pathologists, as laboratorians, have done fantastic work to develop COVID-19 testing and roll it out," says Buja, who asserts that our understanding of the disease wouldn't be where it is today were it not for the work of pathologists around the world. "It's a strong indication of how important the profession actually is."

Miller agrees. "The pandemic has certainly demonstrated how much of a role pathology plays in disease diagnosis and the discovery of pathological processes. The laboratory has become much more visible through testing development and helping to understand how the virus affects the body on a cellular and molecular level." She hopes the increased visibility could spark interest and potentially increase the number of applicants.

If so, we should see a new wave of medical students considering pathology as their chosen specialty; however, Singh worries that the effect may be fleeting. "We did get initial interest at the start of the pandemic because people learned that diagnostic laboratories develop and perform the tests – but it was directed more at medical technologists than at pathologists," he says. "At our institution, one pathologist spearheaded and took control of the testing for the whole state, but that was transient – people are already over it."

Berumen flags another effect of the pandemic that we may not even have considered: the lack of students going onto campus or into labs. "As a medical student, getting exposure to pathology is something best experienced in person," she says. "There are so many aspects of pathology that require you to be hands-on, such as grossing specimens and performing autopsies."

Only time will tell whether the COVID-19 pandemic has had an effect – positive or negative – on pathology's recruitment efforts. But one thing is for sure: it has made the discipline visible to an otherwise unknowing public – and possibly an unknowing clinical care team.

Redefining pathology's place

It seems clear that pathology needs a much greater presence in the medical school curriculum. But how do you prove its importance in medical education? How do you present the message that pathologists are an essential and integral part of the clinical care team?

> "WE NEED A DISTINCT PLACE IN THE MEDICAL CURRICULUM THAT SHOWS PATHOLOGY AS A CLINICAL SPECIALTY."

One might proactively lobby the curriculum committee for courses to include designated pathology blocks (as opposed to the fragmented approach typically seen throughout the first two years), but would that be enough? Should the curriculum give students more opportunities to spend time inside the lab – or even make it mandatory? "Lectures really didn't cover how diagnostic tests are performed and analyzed, so students need to be taught what common tests mean, how to use them clinically, and what goes on in the 'black box' of the lab," says Miller. "It would also be helpful to advertise opportunities for students to come into the clinical lab or the histology lab and see what happens – especially those who may never take a pathology rotation."

Such opportunities would grant students the exposure to pathologists that is so desperately needed – and, if they still decide that the lab isn't for them, at least they are making an informed decision. Berumen reminds us of her first encounter with the lab: "One slide changed everything." How many similar revelations are lying dormant in medical students who never experience what goes on behind the doors of the lab? "We need a distinct place in the medical curriculum that shows pathology as a clinical specialty – and how pathologists practice medicine," muses Berumen. "Or even that we do practice! There are some who don't realize or

LAUREN J. MILLER

If you could change one thing about the current curriculum, what would it be?

I would love to see pathology as a required rotation for medical students. All physicians, regardless of specialty, use the laboratory for diagnosis and patient monitoring. Having a better understanding of laboratory operations and testing methodology would facilitate patient care and allow for more efficient use of healthcare resources.

Which specialities do a good job of bringing attention to themselves?

Internal medicine and emergency medicine are particularly vocal and in the spotlight. Both specialties have the benefit of being well-known by patients, but both also have strong, active, and visible specialty organizations that are involved in advocacy and education. Additionally, both specialties use social media very well – making them accessible to patients outside a typical healthcare setting.

Have any countries got it right?

All countries have their own challenges because each country's curriculum is unique. There's no one "best" system of study, though other countries seem to have better exposure to pathology pipelines and more direct routes to practice than the US. However, in working with trainees from other countries, we have found that many of the same myths about pathology exist, regardless of training format.

How would you like to see pathology represented in the curriculum in 10 years?

It would be wonderful if the connection between pathology, disease diagnosis, and the practice of patient-facing medicine was distinctly highlighted in the curriculum. It seems that many students see pathology as buzzwords and pictures to memorize for licensing exams without understanding how they will rely on biopsy and laboratory results while caring for patients. Most physicians do not need to be experts in analyzing specimens, but everyone benefits when we can communicate more efficiently by understanding what each member of the healthcare team contributes to patient care.

acknowledge this latter point."

Clearly, it's not just pathology's place in the curriculum that needs an overhaul. To draw in students, the field must become more attractive – and that means common misconceptions must be dispelled. As practicing pathologists, Singh and Buja can certainly attest to the lighter side of the profession. "There's certainly a good work-life balance. The call schedule is not onerous, so you still have evenings and weekends for any activities or family interests you may have," says Singh. "The compensation is competitive, too – pathologists are not the highest or the lowest paid, but we don't tend to complain about the pay, so that's not putting students off." Pathologists should showcase these benefits so that students are aware of the opportunity to balance work and personal lives without sacrificing their role in patient care or enhancing medical knowledge.

"We need to show students how fundamental pathology is to medicine and how rewarding it is as a career. Practitioners in the field have less burnout than other disciplines," says Buja. "Pathologists are happy people and we get a lot of fulfilment from what we do. It's a wonderful way to study the fundamentals of medicine and contribute to patient care."

Medical schools undergoing curricular reform are standing at the edge of an opportunity to raise the profile of pathology – showcasing the value of the lab and its role in patient care and outcomes. Seize that chance and the future is bright. "It feels like there's a new wave of pathologists who acknowledge that we need to be more visible," Berumen says. "There's a real movement to change how we are perceived."

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References

1. *RP Jajosky et al., "Fewer seniors from United States allopathic medical schools are filling pathology residency positions in the Main Residency Match, 2008-2017," Hum Pathol, 73, 26, (2018). PMID: 29180247.*
2. *A Flexner, Medical Education in the United States and Canada. Science and Health Publications: 1910.*
3. *TP Duffy, "The Flexner Report--100 years later," Yale J Biol Med, 84, 268 (2011). PMID: 21966046.*
4. *SA Cole, R Dioso-Villa, "Should Judges Worry About the 'CSI Effect'?" Court Review, 47, 16.*

Transforming Immuno-Oncology Research

Harnessing the potential of spatial biology to predict melanoma patient outcomes

An interview with Paolo A. Ascierto

Can you give me an overview of spatial biology?

Spatial biology is the study of tissue within a 2D or 3D context. In particular, we're interested in investigating the interaction between different cells within the tumor microenvironment (TME). Such interaction is important not only from a histological point of view but also from a molecular perspective, because it is possible to look at the distance of specific cells, understand the expression of some molecules, and search for the presence of cytokines. For immuno-oncologists, such knowledge is crucial to understanding the process that underlies the immune response to treatment.

How can spatial biology support immuno-oncology research?

We have seen exceptional growth in the field over the years – and now we're looking toward uncovering a set of biomarkers to gain a better understanding of primary resistance. At the moment, we don't have many biomarkers; PD-1 expression is used in lung cancer, but it is useless in cancers such as melanoma. There has been an increased interest in tumor mutational burden, but it's not used in clinical practice. We have also seen the importance of microsatellite instability markers. With the Immunoscore, we're seeing that the immunocontext is just as important.

Clinicians want to know which patients will respond to high-cost treatments before prescribing them but, without any biomarkers, that's difficult. I believe that specific knowledge of the spatial relationship between immune system cells and tumor cells will be important for finding prognostic biomarkers – not only because spatial biology can give us a better understanding of the mechanism of resistance, but also because it might be important for finding additional drug compounds that could help patients.

Spatial biology is a growing field. How could it affect the future of melanoma research?

At the moment, there is no biomarker for melanoma patients. We don't know which patients might benefit from anti-PD-1 or anti-PD-L1 therapy (or a combination). Using the TME immunocontext, spatial biology can help clinicians identify those patients who may be more likely to respond to a particular treatment.

We can also treat patients with second-line therapies; if a patient fails first-line treatment, we can look at the progression of the TME and see if there's something that will help us to select the next best treatment.

What could shorten spatial biology's journey into the mainstream?

By using new technologies in the early phase of development, we are more likely to find a biomarker that is correlated with treatment. Introducing spatial biology in phase I trials is important because we can investigate direct correlation without complications and, sometimes, with increased safety. If we can find a biomarker that could be useful for clinical application, then the technique could be used in phase III trials to confirm what we have seen in the earlier phases of development.

What are the main barriers to spatial biology adoption?

Validation and reproducibility of analysis are two critical points. We need to collect data on a large cohort of patients to validate the technology. After that, I am convinced that more widespread adoption is just a matter of time. Once we have the data, it is relatively easy to move forward with validation and demonstrate spatial biology as a reproducible technology.

What needs to be done to encourage the adoption of spatial biology?

I believe it's important for researchers who are experienced in spatial biology to model leadership in the field and increase training of the next generation. It's also important to organize a network to enable a group of experts to discuss data, slides, samples, the meaning of the interactions between cells, and their correlation with outcomes. Furthermore, there is a significant need for collaboration between industry and academia. A focus on this latter point, in particular, would help speed up the process of translational research and, in turn, clinical application.

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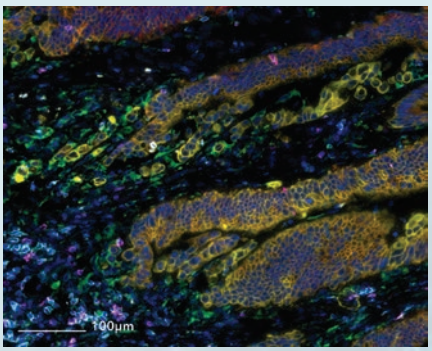


Colorectal cancer 6-plex: CD8 (magenta), CD4 (cyan), CK (yellow), CD3 (white), vimentin (green), E-cadherin (red).

In Practice

Technologies and techniques
Quality and compliance
Workflow

Meet the Humble Mast Cell

Identifying the risks and understanding the mechanisms behind allergic reactions to the COVID-19 vaccine

By Lakiea Wright

COVID-19 vaccine allergic reactions have put mast cells in the clinical research spotlight like never before. That's not only because they play a key role in the human immune response, but also because mast cells are at the root of allergic reactions, including anaphylaxis – making them a prime target of inquiry as we try to understand why, in rare cases, COVID-19 vaccines trigger an allergic response.

Mast cells are found in connective tissue throughout our bodies. In mast cell disorders, these cells are overly active and may proliferate, resulting in a variety of harmful – and potentially life-threatening – effects. The inappropriate activation of mast cells is considered a clinical disorder falling into one of two broad categories: mastocytosis or mast cell activation syndrome (MCAS) (1).

Mastocytosis is defined as an abnormal accumulation of mast cells in one or more organ systems. In addition to putting a patient at greater risk of anaphylaxis, this systemic disorder can also have adverse effects on other organ systems, including the skin, cardiovascular system,

"Mast cells are at the root of allergic reactions, including anaphylaxis – making them a prime target of inquiry."

gastrointestinal tract, and bone marrow. The diagnostic criteria for mastocytosis are well-established and the condition is usually identified through tissue biopsy in combination with genetic and/or blood tests. Serum tryptase is a common test that can be ordered as part of the initial workup, but the diagnostic criteria for mastocytosis are described below in more detail.

The World Health Organization (WHO) has a consensus on definitions of various forms of mastocytosis and on diagnostic criteria (2). The major criteria include histological or immunohistochemical alterations, such as mast cell aggregates containing more than 15 mast cells in bone marrow sections. The minor criteria include cytological alterations (for example, greater than 25 percent of mast cells morphologically abnormal), detection of c-KIT mutations on codon 816, and immunophenotypic alterations. These last may include expression of CD25 (with or without CD2) in mast cells from bone marrow, peripheral blood, or other organs, and total serum tryptase levels persistently >20 ng/mL (not applicable if there is a comorbid blood disorder or evidence of acute mast cell release). The diagnosis of systemic mastocytosis requires at least one major criterion plus one minor criterion or at least three minor criteria.

When patients do not meet the clinical criteria for mastocytosis, but still experience chronic allergic reactions to triggers such as food, prescription drugs, or insect stings, they may be diagnosed with MCAS. Although the allergic responses can vary in severity, the symptoms of MCAS typically include hives, swelling, low blood pressure, difficulty breathing, and diarrhea.

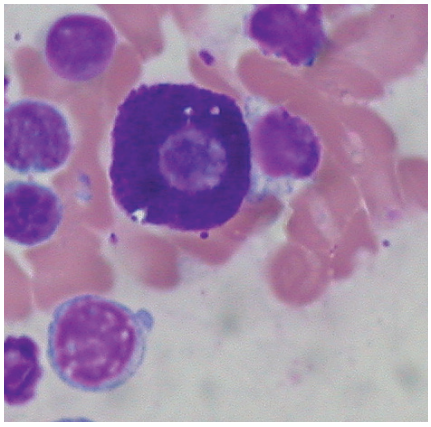
Because there have been cases of allergic reactions – including anaphylaxis – following COVID-19 vaccinations, many are now wondering whether mast cell hyperactivity or mast cell disorders are playing a role. If so, what are the risk factors for allergic reactions to COVID-19 vaccination? Are patients diagnosed with mastocytosis or MCAS at higher risk? And should they take precautions before or after immunization?

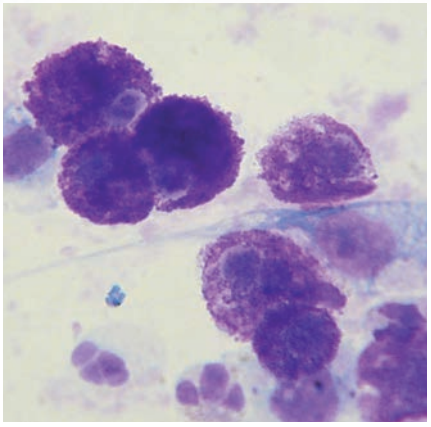
First, it is important to keep in mind that allergic reactions to the COVID-19 vaccine are quite rare. As more and more people are vaccinated, the statistics continue to evolve – but, as of this writing, allergic reactions occur at 11.1 cases per million Pfizer-BioNTech vaccine doses (3), 2.5 cases per 1 million Moderna vaccine doses (4), and fewer than 0.5 cases per 1 million Janssen vaccine doses (5). According to the UK's Medicines & Healthcare products Regulatory Agency, out of 19.5 million administered doses of the Oxford-AstraZeneca vaccine, also known as Vaxzevria, only 455 (0.002%) were associated with an anaphylaxis-related adverse reaction (6). There has also been conflicting data regarding risk factors for allergic reactions to COVID-19 vaccines. Some patients who have reactions appear to have a history of eczema, asthma, and allergies, whereas others do not (7).

Lakiea Wright

Because allergic reactions to COVID-19 vaccines are so rare and the data surrounding them so uncertain, the risk factors and mechanisms of these reactions require further investigation. According to the Centers for Disease Control and Prevention, health care providers who suspect a patient is having an allergic reaction may consider obtaining a serum tryptase test. Because tryptase is released during anaphylaxis, clinicians should aim to collect tryptase between 30 and 90 minutes after the start of the reaction, but patients can be tested for elevated tryptase up to six hours after the start of a reaction (8).

Tryptase is an enzyme released by activated mast cells during normal and abnormal immune responses – meaning that serum tryptase concentrations can indicate mast cell activity (9). If mast cells are activated appropriately, there is a brief rise in serum tryptase concentration; however, patients with mast cell disorders show a high level





Credit: Ed Uthman (CC BY 2.0).

"The COVID-19 vaccine is not contraindicated in individuals with mastocytosis or severe allergies unless they have a known allergy to a vaccine ingredient."

of serum tryptase over a prolonged period and a higher count of mast cells in the body. These higher levels of tryptase trigger an inflammatory response, including symptoms such as flushing, rapid decreases in blood pressure, and even anaphylactic shock. An anaphylactic reaction like this must be treated immediately with epinephrine (adrenaline); without immediate treatment, anaphylaxis can be fatal.

The suspected allergen in the COVID-19 vaccine is polyethylene glycol (PEG) or polysorbate, but additional studies are needed to confirm the culprit allergen(s) and the mechanisms involved in the allergic response. To that end, the National Institutes of Health is currently conducting a multi-site national clinical trial, the Systemic Allergic Reactions to SARS-CoV-2 Vaccination study, to further evaluate COVID-19 vaccine allergic reactions and risk factors (10). The study team will enroll 3,400 participants, of whom 60 percent will have either a history of severe allergic reactions or a diagnosis of a mast cell disorder and 40 percent will not. The findings of this phase II trial will provide more clarity around the risks and benefits of receiving the COVID-19 vaccines, particularly for people who have allergies or have been diagnosed with a mast cell disorder. However, based on current recommendations, the COVID-19 vaccine is not contraindicated in individuals with mastocytosis or severe allergies unless they have a known allergy to a vaccine ingredient (11).

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References

1. *P Valent et al., "Definitions, criteria and global classification of mast cell disorders with special reference to mast cell activation syndromes: a consensus proposal," Int Arch Allergy Immunol, 157, 215 (2012). PMID: 22041891.*
2. *P Valent et al., "Mastocytosis: 2016 updated WHO classification and novel emerging treatment concepts," Blood, 129, 1420 (2017). PMID: 28031180.*
3. *CDC, "Allergic Reactions Including Anaphylaxis After Receipt of the First Dose of Pfizer-BioNTech COVID-19 Vaccine — United States, December 14–23, 2020" (2020). Available at: https://bit.ly/3bXJkFo.*
4. *CDC, "Allergic Reactions Including Anaphylaxis After Receipt of the First Dose of Moderna COVID-19 Vaccine — United States, December 21, 2020–January 10, 2021" (2021). Available at: https://bit.ly/3bVA2tu.*
5. *CDC, "Safety Monitoring of the Janssen (Johnson & Johnson) COVID-19 Vaccine — United States, March–April 2021" (2021). Available at: https://bit.ly/3frE3Yx.*
6. *MHRA, "Coronavirus vaccine - weekly summary of Yellow Card reporting" (2021). Available at: https://bit.ly/3xFUW8o.*
7. *M Sokolowska et al., "EAACI statement on the diagnosis, management and prevention of severe allergic reactions to COVID-19 vaccines," Allergy, [Online ahead of print] (2021). PMID: 33452689.*
8. *CDC, "Lab Tests to Collect Shortly After Severe Allergic Reaction/Anaphylaxis Following COVID-19 Vaccination" (2020). Available at: https://bit.ly/3un1vKJ.*
9. *LB Schwartz, "Diagnostic value of tryptase in anaphylaxis and mastocytosis," Immunol Allergy Clin North Am, 26, 451 (2006). PMID: 16931288.*
10. *NIAID, "COVID19 SARS Vaccinations: Systemic Allergic Reactions to SARS-CoV-2 Vaccinations (SARS)" (2021). Available at: https://bit.ly/34l5c9r.*
11. *P Bonadonna et al., "COVID-19 Vaccination in Mastocytosis: Recommendations of the European Competence Network on Mastocytosis (ECNM) and American Initiative in Mast Cell Diseases (AIM)," J Allergy Clin Immunol Pract, 9, 2139 (2021). PMID: 33831618.*

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Studying Brains on a Lab Bench

Monitoring epileptic and amyotrophic lateral sclerosis neural networks in vitro with microelectrode array technology

By James Ross

Studying the brain can be an intimidating prospect, even for neuroscientists. This mysterious organ is composed of a complex network of tens of billions of cells – all transporting electrical and chemical messages over vast distances in milliseconds. Understandably, measuring this activity has not been easy – but, in the last decade, electrophysiology research has become more accessible, thanks to the introduction of microelectrode array (MEA) technology.

MEAs allow neuroscientists to study broad network activity on the lab bench, recording information that was previously out of reach. Historically, scientists who sought to measure the electrical activity of neurons could only look at one or a handful of cells at a time, failing to capture the immense richness of network activity in the brain and beyond. This limited their ability to create complex models of neurological disease in vitro. Furthermore, traditional electrophysiological techniques can take up to a year to master – meaning that most labs cannot readily study neural activity at this level. That's where MEAs come in; the technology is accessible to most neuroscientists seeking to study the electrical properties of their cells.

Studying neural circuits at the bench

In many ways, neurology lags behind other medical specialties in understanding the nuances of disease and effective treatment. For example, around 65 million people have a form of epilepsy – but there are currently no biomarkers that can predict what treatment is most likely to work for a given individual (1). Rather, physicians must rely on trial and error to find an effective therapeutic. For around one-third of these patients, their prescribed treatment does not work – demonstrating that we still have a lot more to learn about how the brain works in patients with epilepsy (2). Meanwhile, one in 50,000 people develop amyotrophic lateral sclerosis (ALS) but, with no definitive diagnostic criteria or approved treatments, patients face an uphill battle against the disease (3).

To understand and treat diseases such as epilepsy and ALS, scientists need to study neural network activity under various conditions. The standard approach to measuring this activity in vitro is a complex method called whole-cell patch clamp electrophysiology. With this technique, the scientist inserts a micropipette into a neuron in a slice of brain tissue or in culture to measure voltage or current. This technique's limitations are clear: it's invasive, because the pipette needs to perforate the cell membrane; cell activity can only be recorded over a period of minutes; and only a handful of manipulations can be reliably tested before the cell dies. Though the method is suitable for understanding the electrical properties of single neurons or

"Unlike more technically challenging methods, [MEA technology] is accessible to any scientist who uses cell cultures."

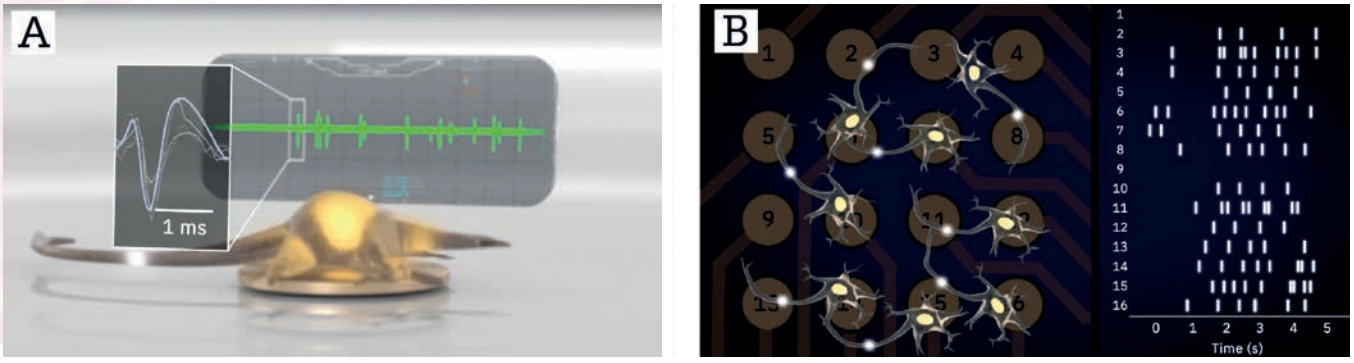

Figure 1. A) Image of a neuron growing over an MEA electrode. Voltage activity from the neuron is recorded (green) and individual neural signals or action potentials (white) are automatically tracked. B) In an MEA culture dish, neurons form a network over the electrodes. The location and time of every recorded action potential is assigned a tick mark. The relationship between tick marks reveals deep insight into how the neurons interact.

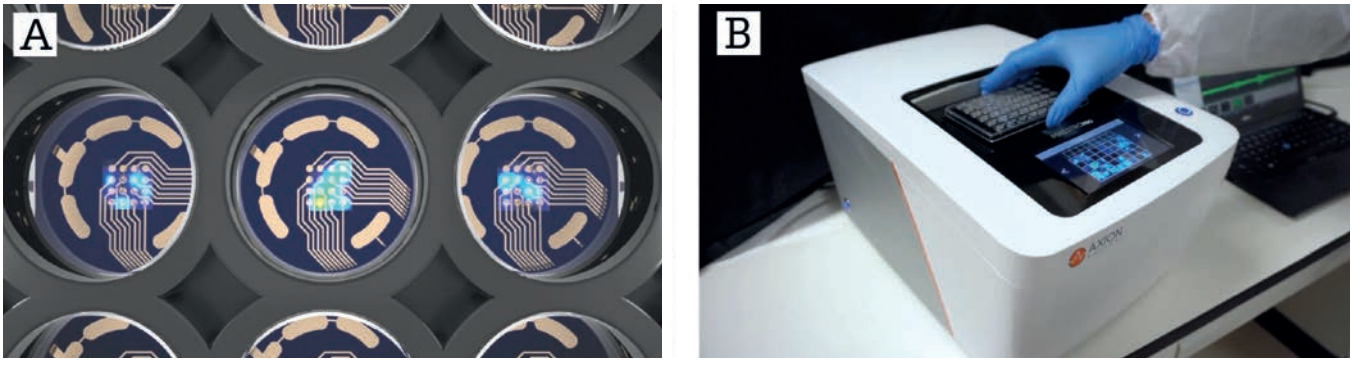

Figure 2. A) Multi-well solutions allow scientists to simultaneously track up to 96 "brain-in-a-dish" cultures – accelerating scientific discovery and changing the way scientists ask questions. B) A 48-well MEA plate being docked into an MEA system.

ion channels – especially in response to stimulation – and the impact of genetic and pharmacological manipulations on this activity, it cannot measure network activity over long periods. Ultimately, this limits the kinds of questions that a neuroscientist can ask – and answer.

Moving forward with MEA

MEA technology represents a complementary approach to studying the brain in vitro. In essence, an MEA is a grid of tightly spaced microelectrodes embedded in a substrate or culture surface. Traditionally, MEAs have been used to record network activity on the brain's surface in living animals – a challenging technique to implement. But MEAs can also be embedded into the wells of a multi-well plate, making extracellular recordings of cultured neurons much simpler.

Neurons and other electrically active cells can be cultured over the electrodes, creating a cohesive network (see Figure 1). Researchers can then noninvasively record the electrical activity of this network over weeks and months. The multi-well plate format allows them to grow replicates of the culture and simultaneously test multiple genetic, pharmacological, and environmental manipulations on the network (see Figure 2). Unlike more technically challenging methods, this technique is accessible to any scientist who uses cell cultures. This includes neuroscientists like Evangelos Kiskinis, assistant professor of neurology at the Northwestern University Feinberg School of Medicine, who studies epilepsy and ALS using induced pluripotent stem cells (iPSCs).

Epilepsy in a dish

To study epilepsy effectively, Kiskinis wanted to create a model system that mirrors the disease's pathophysiology. Previously, scientists have relied on non-human cells or animal models to study the disease – but these fail to capture the patient-specific nuances of epilepsy, relevant diagnostic biomarkers, and druggable targets. Kiskinis solved this problem by developing patient-specific models of epilepsy in vitro using patients' own stem cells and differentiating them into neurons. By using MEAs to study their electrical patterns and responses to treatment, Kiskinis hoped to identify predictive biomarkers that could help physicians determine the most effective drug for each patient.

In his research, Kiskinis examined *KCNQ2*-associated epilepsy. In building his model, he aimed to determine whether iPSC-derived neurons harboring mutations in the *KCNQ2* gene reflected the characteristic firing pattern seen in children with the

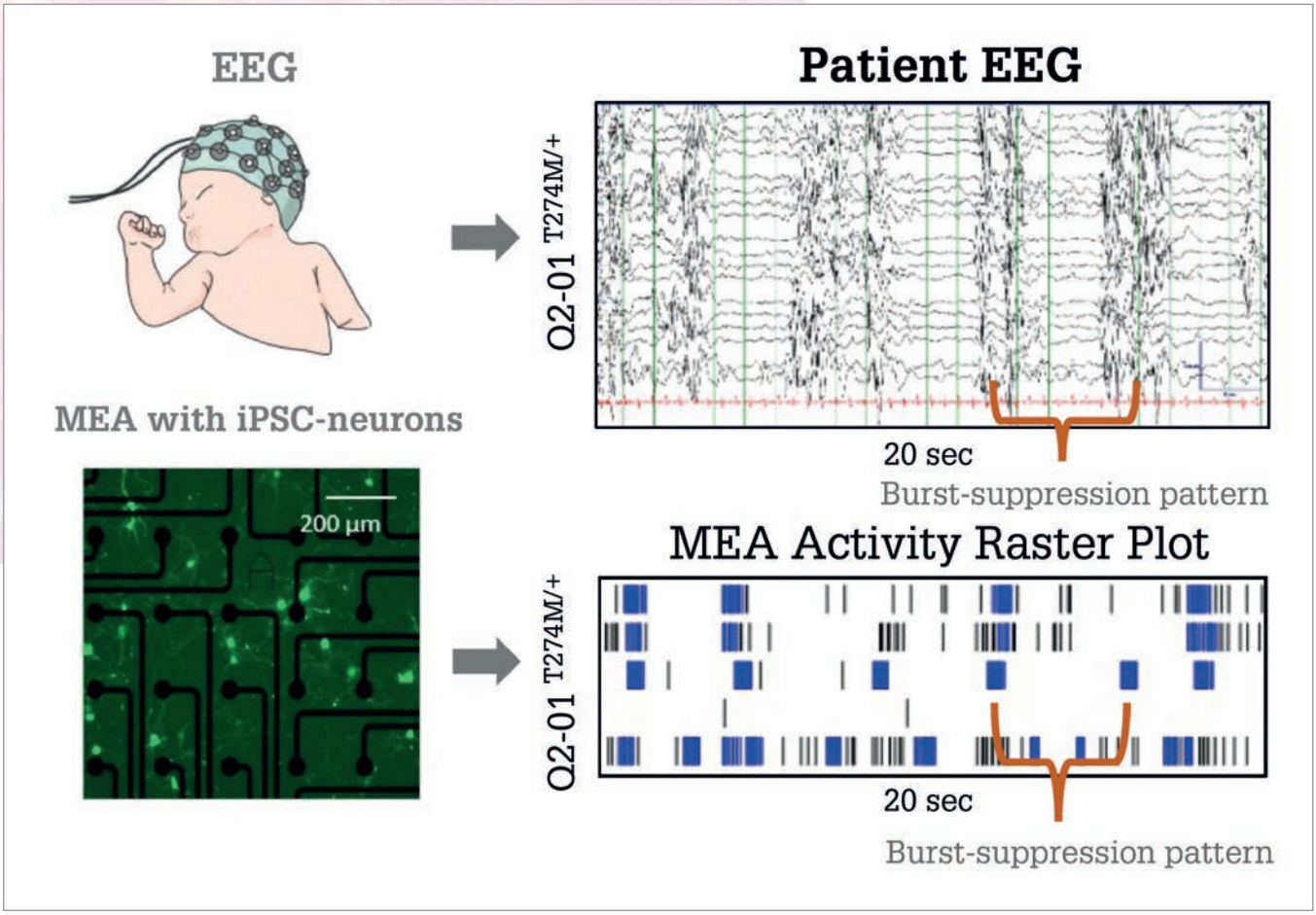

Figure 3. "Epilepsy in a dish." Patient EEG data (top row) compared with patient-derived iPSC-neuronal data (bottom row). Mutations in the *KCNQ2* gene induce a burst-suppression firing pattern in both Patient 01's clinical EEG (top right) and iPSC-derived neural culture (bottom right). Data provided by the Kiskinis Lab.

disease. Children with *KCNQ2*-associated epilepsy exhibit a characteristic EEG pattern known as burst-suppression activity – highly synchronized firing ("bursting") followed by intermittent periods of low activity. To assess this pattern, Kiskinis and his team cultured patients' neurons on MEAs in a multi-well plate and recorded spontaneous firing over several weeks in culture. They found that these neurons exhibited a burst-suppression firing pattern highly reminiscent of the patient's EEG (see Figure 3) – effectively recapitulating "epilepsy in a dish" (4). This model system provided a phenotypic platform for assessing potential therapeutic options.

Antiepileptics for ALS

In another branch of his work, Kiskinis used MEAs to identify an antiepileptic drug that might help slow the progression of ALS. First, his team used MEAs to study the nature of iPSC-derived neurons from ALS patients that featured the *SOD1*^{A4V} mutation (an "ALS in a dish" model) – finding that these neurons were hyperexcitable (5). He used other wells on his plate to block inhibitory neurons, but found no effect, indicating that they were not involved. He also grew neurons with a corrected *SOD1* gene and discovered that the cells' aberrant electrophysiology returned to normal.

The antiepileptic drug ezogabine is known to reduce neuron excitability, but Kiskinis was eager to uncover whether the drug had the same effect in ALS patients. Based on his previous work with MEAs, he was able to advance the drug straight into a phase II clinical trial (6), in which he found that neuronal excitability may be a useful biomarker for ALS drug discovery. Now, he and his team want to investigate whether ezogabine can slow ALS progression.

It doesn't end there

MEAs have also enabled researchers to predict treatments for inherited erythromelalgia (IEM) – a rare neurological disorder also known as "Burning Man" syndrome. Like epilepsy and ALS, treating IEM is not straightforward – existing

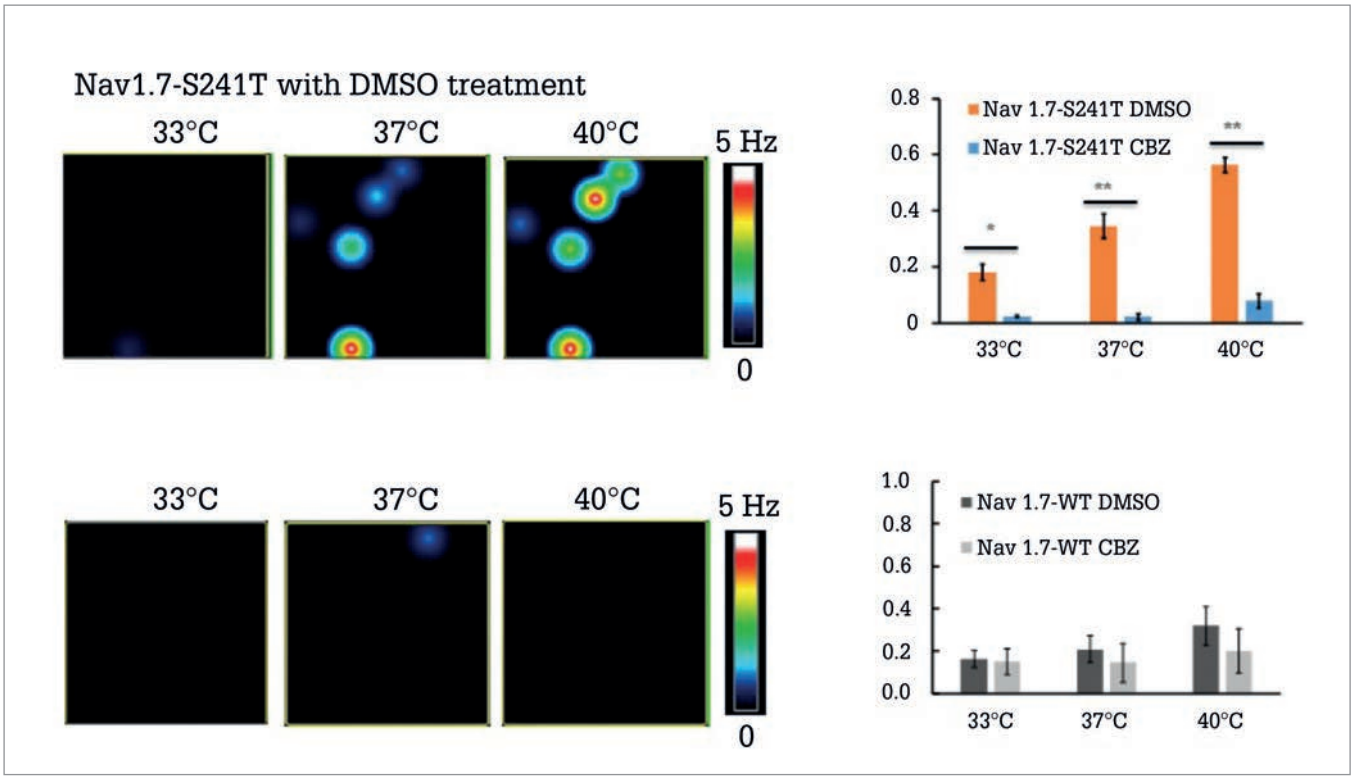

Figure 4. "Pain in a dish." Neurons expressing the Nav1.7-S241T mutation fire significantly more action potentials than wild-type (WT) neurons, and this effect is exacerbated with increasing temperatures (top). When these neurons were treated with carbamazepine (CBZ), the firing frequency was greatly reduced (bottom) – suggesting CBZ as a potential treatment for IEM patients with S241T mutations. Data provided by the Yang Lab (8).

painkillers do not relieve the constant, burning pain patients feel in their hands and feet. The disease is caused by a mutation in a sodium channel gene that leads to hyperactive pain-sensing neurons. Yang Yang, assistant professor of medicinal chemistry and molecular pharmacology at Purdue University, has identified over two dozen mutations in this gene, suggesting that a one-size-fits-all approach to treatment would not be suitable.

Through trial and error in the clinic, neurologists have determined that an anticonvulsant drug called carbamazepine reduces symptoms in IEM patients with a specific mutation, Nav1.7-V400M. Yang and his team used MEAs to investigate whether other mutations also respond to the drug (7) and, using their multiplexing capability, they were able to test carbamazepine on pain-sensing neurons that harbored several different mutations, including the Nav1.7-S241T mutation, located adjacent to Nav1.7-V400M.

Because IEM is triggered by warmth, Yang studied the impact of carbamazepine on the relationship between temperature and neuron excitability (see Figure 4). As expected, a temperature-dependent increase in activity was evident in his cultures for each IEM mutation, but the drug reversed this effect for the Nav1.7-S241T mutation. In a subsequent clinical trial, Yang found that patients with this mutation reported reduced levels of pain after taking the drug.

MEAs cannot replace patch clamp electrophysiology – but they empower neuroscientists to ask and answer different questions. They enable all neuroscientists, even those who do not specialize in electrophysiology, to study the intrinsic properties of neural circuits and efficiently monitor their response to manipulation. As evidenced by the research described here, MEAs can help create complex models of neurological disease and facilitate the discovery of new biomarkers and treatments for diseases that have confounded scientists for far too long.

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See references online at: tp.txp.to/study-brains

A Place for Everything

Getting started with spatial biology

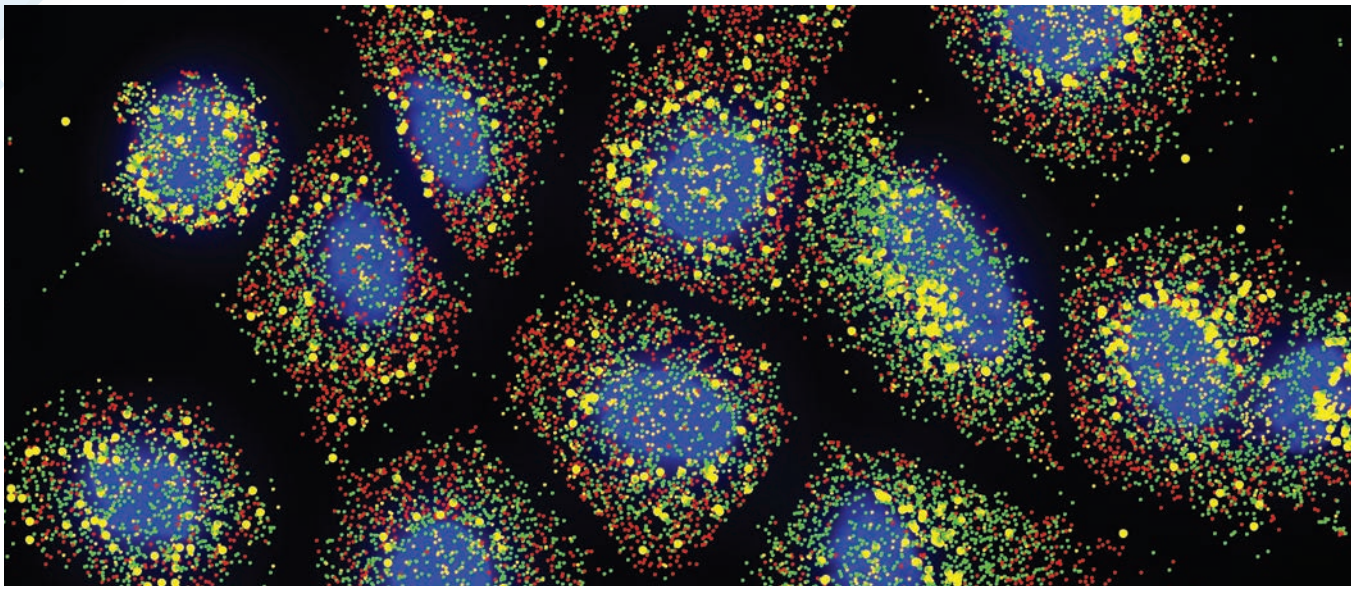
By Nachiket Kashikar

Over the past few years, spatial biology tools have taken the life science research community by storm. Among pathologists – who regularly analyze tissue sections and have a keen understanding of the importance of spatial context in diagnostics – this new suite of technologies may not seem revolutionary. But spatial biology promises to transform the practice of pathology just as much as it has the experiments performed in research labs. These tools aim to bring together the best of both worlds, delivering deep molecular profiles in situ from a tissue section.

Imagine the information you might normally glean from a histology slide – and then consider the value of adding a layer of deep insight into the expression of dozens of genes or proteins at single-cell and even subcellular resolution to reveal critical information about cellular and molecular interactions. Already, spatial biology has driven impressive discoveries about the role of immune cells in the tumor microenvironment, the distribution of viral particles in cases of infectious disease, and the progression of neurodegenerative diseases. Though these insights began in research labs, they are already being translated to the practice of pathology.

How does it work?

Spatial biology emerged from diverse technological advances in next-generation



SARS-CoV-2 transcripts of the nucleocapsid protein (yellow) are predominantly found close to the cell nucleus. Interestingly, some cells show polar localization of SARS-CoV-2 transcripts. Transcripts of 80 other genes (marked in different colors) are distributed throughout the cell. Spatial biology technology enabled scientists to compare infected and non-infected cells to understand subcellular gene regulation and how the infection affects neighboring cells over time. Image courtesy of Medical University of Graz, Austria; captured using the Resolve Biosciences Molecular Cartography™ technology.

sequencing, molecular biology, optics and imaging, proteomics, and bioinformatics. The ability to generate data at higher resolution allowed scientists to finally ask a question that could not previously be answered: how does all of this gene and protein expression play out in the body? There had always been a recognition that spatial context mattered in biology – but, until recently, molecular biology tools required processing samples with methods that made it impossible to map expression data of multiple analytes back to its original location.

Current spatial biology tools build on single-cell analysis, adding important information about natural tissue architecture for the closest view yet to in situ biology. Platforms tend to focus on spatial transcriptomics or spatial proteomics, though some tools can interrogate both. Multiplexing is a key feature of these tools, enabling scientists to measure and spatially resolve tens or hundreds of genes and proteins at the same time from the same sample.

These tools often use in situ hybridization – fluorescently tagging genes in a sample, imaging, removing the tags, and adding new tags to query more genes in an iterative cycle. Some approaches tag genes or proteins with location-embedded barcodes, running the sample through a gene or protein quantification process and mapping the results back to their original location in the sample with those barcodes.

In the pathology lab

Although spatial biology tools are still a strong focus in research labs, they have already shown substantial downstream utility for pathology laboratories.

For example, some of the earliest spatial biology experiments occurred in oncology, where they helped researchers identify clear signatures based on the tumor microenvironment that can accurately predict cancer progression or whether a certain cancer is likely to respond to a specific immunotherapy (1, 2). As these spatial signatures are validated for clinical use, they will offer substantial value by establishing more accurate prognoses or treatment plans for patients.

In the infectious disease arena, some pathology labs are already using spatial biology tools to map out how infections target specific organs or tissues, monitor the distribution of viral or bacterial material in the body, and pinpoint secondary and tertiary events. This approach was quickly applied to patient samples collected in the COVID-19 pandemic when it became known that SARS-CoV-2 had the ability to spread well beyond the lungs and establish a foothold in other organs and tissues (3). Spatial biology offers a deeper and more quantitative view of disease propagation than ever before.

In the future, I expect the copious research being done in autoimmune diseases to translate to pathology as well. Scientists hope that characterizing interactions between immune cells and other types of cells will provide new insights into disease progression — information that could ultimately enable more accurate diagnoses and prognoses for patients.

Implementing spatial biology

Although it's never easy for pathologists to implement platforms designed for research labs, many spatial biology systems have been designed specifically to fit into pathology workflows. If you

are interested in adding spatial biology to your lab's capabilities, here are some considerations to help you get started.

- *Genes or proteins?* Pathologists often stain tissue sections for specific proteins known to be useful biomarkers of disease. Spatial biology opens the doors to gene expression analysis as well – and, because most platforms currently query one or the other, it will likely be necessary to choose which is better suited to your needs. Although proteins are usually considered the most phenotypically relevant, gene expression offers an earlier view of biological events that may be even more useful.
- *Multiplex capacity.* Different applications will require different levels of multiplexing. In pathology labs, the massive multiplexing needed for true biological discovery projects is probably overkill. But more targeted spatial biology tools have a broad range of multiplexing – from a handful of proteins to dozens, or from a few dozen genes to hundreds. On this front, it's important to consider not just what you need today, but also what you anticipate needing in the coming years.
- *Sensitivity.* Applications in which pathologists may need to quantify extremely rare transcripts or proteins are best suited for highly sensitive tools capable of detecting and quantifying even a single analyte in a cell or sample.
- *Sample preservation.* Unfortunately, some spatial biology tools still require the processing of an entire sample, destroying it in the analysis workflow. For pathology labs, where experts often want to go back and query the same sample with different techniques, a platform that preserves the original sample is important for the ability to probe different analytes.
- *Resolution.* Generally, technologies that directly image a sample can provide higher resolution than those that map data back to a source via barcode. If your target applications would benefit from cellular or subcellular resolution, a technology based on in situ hybridization may be a better fit for your lab.
- *Logistics.* Two other factors important for any clinical lab are turnaround time and ease of use. Ultimately, spatial biology tools must deliver results fast enough to have an impact on patient care; their operation must also be straightforward to be incorporated into a clinical lab workflow.

Early adopters of spatial biology in pathology laboratories are already demonstrating the utility of these tools for the entire clinical lab community. From infectious disease to oncology and beyond, spatial technologies offer a deeper, more quantitative view of key genes or proteins to improve diagnosis, prognosis, and treatment selection – ultimately helping pathologists deliver even better patient care.

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See references online at: tp.txp.to/spatial-bio

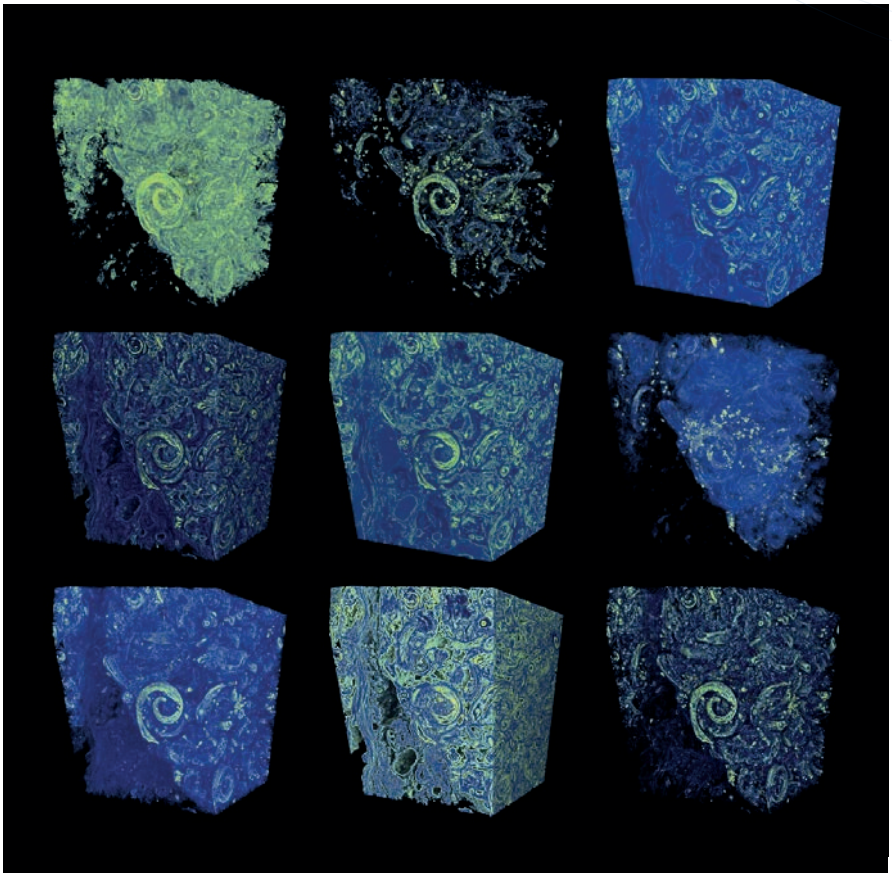
A Worm's-Eye View

A new approach – based on MALD-MSI and micro-CT – aims to bring unprecedented levels of detail to the study of host-microbe interactions, starting with the earthworm

By Lauren Robertson

Charles Darwin was perhaps the most famous proponent of the importance of the humble earthworm – and they've more recently been touted as the most influential species in the history of our world... Unarguably, earthworms play a vital role in our ecosystem – increasing nutrient availability, improving drainage, and contributing to more stable soil structure.

Despite our fascination and nods of approval, methodological challenges have prevented researchers getting a good understanding of the internal workings of an earthworm – including the microbes that colonize it and the associated metabolites they produce. Enter Benedikt Geier and his team at the Max Planck Institute for Marine Microbiology, who set out to accurately image the fundamental interactions between small symbiotic animals and associated microbes in an ecosystem (1). Their recent paper introduces chemo-histo-tomography (or CHEMHIST) – a method for visualizing the metabolic interactions in small animal symbioses, which is based on MALDI-MS imaging and micro-computed X-ray tomography (micro-CT).



Their unique approach outperforms similar methods (developed for medical research in mice) by up to two orders of magnitude. We spoke to Benedikt to find out more.

Benedikt Geier uses MALD-MSI to study the earthworm in unprecedented detail.

What inspired you to conduct this research?

Our team has been working with MS to visually explore how metabolites are distributed in tissues colonized by microbial symbionts. What our research (very quickly) showed us was that we can only understand the chemical images if we know the underlying histology of the host. In other words, we need to know which organs, tissues, and cells of the host animal are associated with symbiotic partners (microbes and parasites) to then assign chemical signals to individual partners.

With this in mind, we set out to create an approach that allowed us to see all this information at once: the host animal's anatomy in 3D, the associated microbes and parasites that live in the tissues, and most importantly, the metabolites they produce.

Why is your research important?

Chemical interactions have allowed bacteria to enter symbioses with organisms across all domains of life. These interactions are critical for the health of individuals as well as whole ecosystems – from deep-sea habitats to our own bodies. The problem is, we know next to nothing about the distribution of metabolites and other small molecules in these associations. CHEMHIST addresses this knowledge gap by linking the identity of tissues and individual cells in complex tissues to their metabolome – independent of mutualistic or pathogenic associations.

We used an environmental sample – the earthworm – to showcase our technique, because it allowed us to provide a glimpse of the complexity that exists in even the simplest of organisms. Though we have a newfound appreciation for earthworms, our focus is on combining high-resolution MALDI-MSI and micro-CT to enable correlative chemical imaging on a new level. In fact, it was particularly important for us to design an approach that was culture-independent – not only to study symbioses between elusive animals and their microbes, but also to come up with a workflow that would enable the study of human/medical tissue samples. Our approach could also offer new insights into the anatomy and metabolomes of samples that focus on medical studies, such as tissue punctures of pathogen infections.

What challenges did you need to overcome?

One major challenge was looking for the associated partners in the host tissues, which can be distributed throughout the whole animal. And that's not easy when you consider that a small animal host may be measured in 10s of millimeters, whereas bacteria are around one micrometer; the resulting four orders of magnitude in size difference is comparable to looking for a Tic Tac mint in an aircraft carrier!

Therefore, we created a multi-scale 3D imaging approach: first, we screen the animal at spatial resolutions just large enough to detect where bacteria or parasites are hidden in the animal tissue, and then we follow up on specific regions with high-resolution MALDI-MSI.

Another challenge was to visualize the broad array of targets from the same animal host: microanatomy to understand the anatomic architecture of the host animal, fluorescence labeling to detect the microbes hidden in the animal's tissues, and ultimately MS imaging to study what each of the partners produce. Because each molecular imaging tool requires different processing steps of the sample that are not necessarily compatible with each other, we had to find a new way of combining all of them at once.

You also integrated metagenomics into the imaging workflow – why?

By analyzing DNA, we include two very important aspects that reach beyond knowing the taxonomy of the associated partners: Firstly, once we know who is there, we can design specific fluorescence probes for FISH to label each species of microbe individually and reveal their community composition within the host tissues. Secondly, because metagenomics sequencing provides information on

the genomic potential of the associated partners, we can check "who can do what." In other words, we can learn which metabolites each partner is capable of producing; by screening the genomes of the associated partners, we can provide essential background information on the metabolite distributions that we record with MALDI-MSI. We described this approach in a paper building up to this publication (2).

Could you tell us about your selection process for appropriate analytical techniques?

MALDI-MSI is extremely powerful because it can deliver both the chemical composition of a sample (like a normal MS) and the spatial distribution of the compounds (like a molecular microscope). On top of that, MALDI-MSI is label-free and allows us to directly observe an animal in its natural habitat, shock-freeze it, and image its body chemistry. As mentioned above, the trickiest part is to understand the chemical images MALDI-MSI produces.

Small symbiotic animals often have complex anatomical structures and even specialized organs or cells that house the symbiotic bacteria – looking in 2D makes it difficult to recognize every structure. This is why we wanted to integrate MALDI-MSI with an approach that would allow us to resolve the detailed 3D anatomy of these tiny host animals at a (nearly) cellular resolution. Although there are fluorescence microscopy techniques capable of 3D imaging, they require tissue clearing approaches for the fluorescence light to illuminate the sample. On the other hand, X-rays pass through any type of biological sample, so we chose micro-CT (an X-ray 3D imaging approach) to resolve and understand the anatomy behind the chemical images that we recorded with MALDI-MSI.

Can you give us specific examples as to how the combination of techniques facilitates discovery?

In the MALDI imaging data, we always saw certain molecules that appeared as large blobs towards the end of the earthworm. We could not explain these chemical signals simply from the 2D tissues sections that we recorded. And then we looked at the micro-CT data, which revealed that the blobs were actually cross sections through cysts that contained parasitic nematodes, which had their own specific chemical composition – possibly related to an immune response of the earthworm against these nematodes. This one example showed us that 3D imaging is not only important to understand anatomic and cellular features that might change throughout the animal, but also sheds light on metabolites that indicate certain functions from immune responses (Figure 3 in the paper) to digestive processes along the gut (Figure 1 in the paper).

Where will your research take you next?

The diverse set of applications that we envision for CHEMHIST is reflected in the different paths that we have decided to pursue. I am looking forward to taking up my new position as a postdoc at Stanford University, California, where I will transfer my knowledge in correlative chemical 3D imaging to organoid-infection models to better understand human pathogenesis through biomedical research. Manuel Liebeke, one of the key members of our team, is Head of the Metabolic Interactions Group at the MPI for Marine Microbiology in Bremen, Germany, and just recently received a new MALDI imaging setup for faster and higher resolution imaging, which he will use to further push the boundaries of imaging symbiotic interactions. In particular, he will be using a specific MALDI-MSI workflow that enables imaging of glycans – cell surface molecules that are involved in the metabolic interactions at the host-microbe interface.

We are convinced that correlative chemical imaging – as presented in our CHEMHIST study – will lead to more work on host-microbe interactions. In terms of a technical outlook, the increasing speeds, spatial resolutions, and sensitivities of imaging setups will allow scientists in the future to not only create a multimodal 3D atlas of one animal, but maybe even a whole time series of different infection or life stages. CHEMHIST is designed as a framework that future scientists can adapt to their needs for studying any symbiotic/host-microbe system.

How would you like to see your research applied in the future?

We would love to see CHEMHIST applied as broadly as possible! For example, to specific medical questions where scientists want to know what metabolites the pathogens, parasites, or maybe even beneficial microbes (for example, in the gut microbiome) produce within their host niche.

One aspect in particular that we wanted our study to highlight is the value of discovery-driven research; for example, observing a system from a different angle and generating hypotheses on the interactions taking place in front of one's eyes. When Darwin studied the Galapagos Islands, he would first observe and describe what he saw before trying to explain it. In a similar way, seeing the distribution of metabolites and the animal and bacterial cells that produce them embedded in the anatomic 3D "world" of the host's body is like discovering a micrometer-scale ecosystem. New imaging methods enable new observations – similar to discovering an unknown island – which allows us to first observe nature and from there create hypotheses that we can test, discuss, and begin to explain. As Thomas Bosch said in his commentary on our work (3), "How excited Charles Darwin would have been if he had found out that the earthworm's behavior might be the result of such complex multiorganismic interactions."

See references online at: tp.txp.to/worms

Profession

Your career
Your business
Your life

The Pathologist's Counterpart

A glimpse into the world of macroscopic technicians and cytotechnicians

By Hatice Beşeren

What is "macroscopy?" It is a diagnostic examination of a sample obtained by surgery or autopsy that uses only the naked eye – what some may know as "gross examination."

The structural changes diseases cause in organs are diverse. Growth, shrinkage, loss of texture, changes in color, consistency, and content… Despite their differences, we often refer to all such changes as "lesions" – and different lesions can coexist in the same disease. Therefore, an accurate diagnosis can only be obtained by evaluating the lesions one by one and investigating the relationship between them – a task performed not only by pathologists, but also by macroscopy technicians (a job title similar to that of the pathologists' assistant). Thus, identifying and correlating these lesions leads to macroscopic diagnosis – laying a foundation upon which microscopic evaluation can build.

A day in the life

There is no pathologist in the institution where I currently work – meaning that I spend my entire day attending to macroscopy. I handle the macroscopic examination and sampling of all specimens, then compile my results into a macroscopy report in my own name. The samples I take are prepared by histotechnicians and sent to pathologists in another center.

Once a week, I attend fine needle aspiration cytology in radiology. I quickly paint the fluid taken from the patient with a syringe using the Diff-Quik method. Is there evidence of cell competence? I evaluate the preparation under a microscope and report to the radiologist. This allows us to make the final diagnostic process easier for the pathologist and helps us to avoid taking unnecessary biopsies.

On weekends, I scan the vaginal smears that come on weekdays and write notes to our pathologist, marking the areas I consider suspicious.

I also work with Haldun Umudum, Head of Pathology at Ufuk Üniversitesi, Ankara, Turkey, to conduct frozen sections via digital pathology. I work over 1,000 kilometers away in Kars, so we are pioneering the use of remote frozen sections in Turkey. I perform the macroscopic examination locally, then send my sample preparations to Ankara digitally for further investigation.

When macroscopy technicians and cytotechnicians are well trained, they can provide vital laboratory services – especially in situations where pathologists are not readily available. I feel like I provide a bridge between macroscopy and microscopy, especially when I have the privilege of participating in academic studies. I love my job!

Talking about training

In North America, macroscopy education is given as a two-year program after completion of an undergraduate degree. The country's first undergraduate program opened at Duke University in 1969 and the American Association of Pathologists' Assistants (AAPA) was founded in 1972. Since 2005, all pathologists' assistants must complete a certification examination conducted jointly between the AAPA and the American Society for Clinical Pathology (ASCP). Certification lasts for three years and must then be renewed via participation in a certification maintenance program.

"I took macroscopy and cytology courses from pathologists at the institution where I work."

In my country, macroscopy technicians and cytotechnicians are not trained separately; the two-year pathology technician program involves both macroscopy and cytology courses, with additional practical training provided by pathologists. This training is not standardized; it takes place under the initiative of the specialist physician and reflects the cases seen at the institution where the technician works. In fact, there is no defined job description,

certification program, or training standard for technicians trained by experts (as opposed to those who complete a certificate). It's unknown how many such technicians are currently in practice – nor can we be certain of their training or skill levels, which vary based on the decisions and expectations of the pathologists who train them.

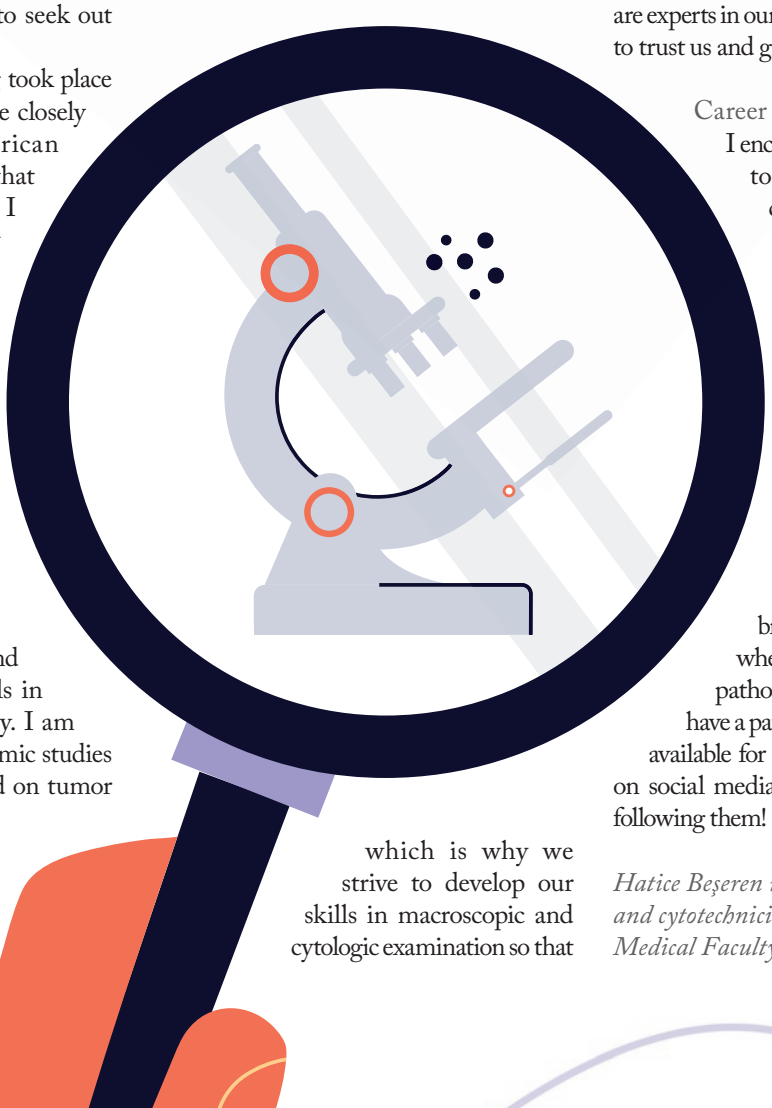
But improvements are on the horizon. A standardization committee has begun work on a certification program for macroscopy technicians that will hopefully soon become official in our country. In addition, Turkish pathologists on social media have explained the work of macroscopy technicians and cytotechnicians to others and expressed their support – which has increased interest in this career path and even inspired my colleagues to seek out additional learning.

Although all of my training took place in Turkey, my career has more closely resembled that of an American pathologist's assistant than that of my Turkish colleagues. I took macroscopy and cytology courses from pathologists at the institution where I work. One of my teachers, Hüseyin Üstün, told me and my classmates that if we wanted American-style training, he would train us meticulously in accordance with these programs. Later, by doing a master's degree in pathology, I established a connection between macroscopy and microscopy and was able to progress my skills in both macroscopy and cytology. I am currently continuing my academic studies in a doctoral program focused on tumor biology and immunology.

Not everyone who aspires to be a macroscopy and cytotechnician will have access to a formal training program. Those who lack such access should seek advice and direction from their pathologist colleagues. A word of advice: never consider your education complete. Read; research; ask questions. Always strive to learn new things.

Macroscopy mysteries

I've found that some pathologists are biased against macroscopy technicians and cytotechnicians – but I think that we ease their workloads. As a well-trained team, we can accomplish great things. As laboratory medicine professionals, we understand that surgical margin evaluation and sampling are vital in resections and in some excisional biopsies – and that error is unacceptable – which is why we strive to develop our skills in macroscopic and cytologic examination so that pathologists can rely on our support.



Many countries have no schools or certification programs at all for macroscopy technician and cytotechnicians. As laboratory workloads increase and disease diagnosis becomes more complex, I think roles like ours will become increasingly necessary to maintain efficiency and diagnostic quality in the lab.

Most people are surprised at the scope of our duties. In fact, when I tell pathologists who are new to my department that I perform macroscopic examinations, I can see their worry. I know they're unaccustomed to relying on the support of a non-pathologist – but it makes me happy to see those prejudices quickly disappear after working with me. Pathologists need to understand that macroscopy technicians and cytotechnicians are experts in our field. In short, I want them to trust us and give us a chance.

Career advice

I encourage potential candidates to think carefully before choosing this career – because it's not a job I recommend to those who don't love the work. You must be constantly open to learning new information. Forget the stereotypes you've heard about the laboratory and those who work in it. Work hard to improve yourself – and, if you have questions, don't be afraid to bring them to your colleagues, whether pathologists or non-pathologist laboratorians. Don't have a pathologist or senior colleague available for questions? There are many on social media – you can learn a lot by following them!

Hatice Beşeren is a macroscopic technician and cytotechnician at Kafkas University Medical Faculty Hospital, Kars, Turkey.

Interrogating Metabolism

Sitting Down With… Timothy Garrett,
Director of Experimental Pathology and Associate Professor,
Department of Pathology, Immunology and Laboratory Medicine,
University of Florida, USA.

Can you tell us about your current role?

Since 2019, I have been an associate professor at the University of Florida, where I am also Director of high-throughput metabolomics for the Southeast Center for Integrated Metabolomics (SECIM) and Director of Experimental Pathology. My interests cover both research and clinical work in several areas, including cancer, rare diseases, and diabetes. My lab comprises 12 scientists, including graduate students and post-docs grappling with complex problems and new fields of research. I often think my job is just to facilitate their work! We are asking fundamental questions: what drives human metabolism? Why does it sometimes fail? How does it change throughout the course of a disease? And how can we better characterize disease so that we can make a diagnosis earlier – or more accurately? By understanding how health may be disrupted, we will help find better treatments.

Much of your work is focused around MALDI MS. When did you become aware of this technique?

My interest in MALDI MS began as an undergraduate in Jonathan Amster's lab at the University of Georgia, working on the characterization of bacterial proteins. MALDI fascinated me: such a simple technique, and yet it generates so much information from such tiny samples. Not only did I fall in love with the technique, I also fell in love with the instruments themselves – how to operate them, fix them, and tinker with them to make them better. Right now, we are pushing MALDI to its limits in metabolomics and lipidomics; we are analyzing populations of metabolites and lipids, and applying informatics to determine which ones are important in disease.

How does your research connect with clinical labs?

It may be easiest to demonstrate this with an example. Recently, a clinical pathologist asked for help with a female patient who had symptoms similar to the lysosomal storage disorder, Fabry disease. Interestingly, this is an X-linked condition typically found in males. To investigate her lipid metabolism for defects, we needed to develop a new diagnostic approach, with careful attention to experimental design. It paid off – we found a defect in a non-obvious enzymatic pathway, completely different to those defects typically seen in male Fabry patients. We can't actually declare the patient to have Fabry disease, because our technique is not yet validated as a diagnostic. Our work does, however, suggest ways of better managing these patients, and, as it is MS-based, it is easy for labs to adopt. Ultimately, it may lead to significant improvements in our ability to diagnose and develop new therapies that target the enzymatic defect we identified.

What most satisfies you about your work?

I'm most proud of having built a resource – the SECIM center – that helps address difficult clinical questions both locally and nationally. And it's immensely satisfying to see our work fundamentally affecting patient care. For example, our method of assessing the immune system of transplant recipients, specifically pediatric kidney transplant patients, allows us to predict organ rejection before development of clinical symptoms. And that can improve healthcare management of these children after validation studies are completed. We're always proud to see our systems solving clinical problems.

Is your technology applicable to COVID-19 research?

Yes. We are developing an MS test that both rapidly diagnoses COVID-19 and also identifies the causative strain. This ability to identify multiple variants – or multiple viruses – in a single assay is a real advantage of MS; PCR, by contrast, is designed to detect only single analytes. We are also collaborating with partners on a multi-omics analysis of the effects of different COVID therapies. The aim is to understand how drugs might affect SARS-CoV-2 infections and prevent the spread of this disease.

What impact has the pandemic had on analytical science?

COVID-19 highlighted the importance of analytical science for diagnosing, monitoring, and tracking infections – and reminded us of its critical impact on public health. But it also taught us that large-scale testing is expensive and slow to implement. It crucially exposed our heavy reliance on reagents – for PCR tests, for example – and we should not forget how limitations in reagent supply forced us to be selective regarding which patients we would test. In a pandemic, these resource constraints are not national, but global. The development of MS-based tests, or diversification of analytical systems in general, will help us address reagent constraints in the future. Having the staff and technology to run mass screening programs is of little use if you have run out of reagents! Overall, then, the pandemic has emphasized the need for investment in faster, cheaper, and simpler analytical technology.

What are your plans for the future?

For me, it's exciting to see how MS can solve problems associated with virus detection – and not just SARS-CoV-2. I want to know how we can harness the power of MS to understand symptoms and to understand when and why one person might be sicker than another. Now, we are looking at how to make testing faster – using techniques like paper spray ionization we could get a diagnosis in 30 seconds, with no sample preparation. And subsequently – by running the same paper sample through MS, we could gain a full metabolite profile. Combining technologies that permit both rapid diagnosis and also deep metabolomics analysis is very promising. For all these reasons, I am very passionate about continuing virus research in my group – but seeing MS reach its full potential will take time!

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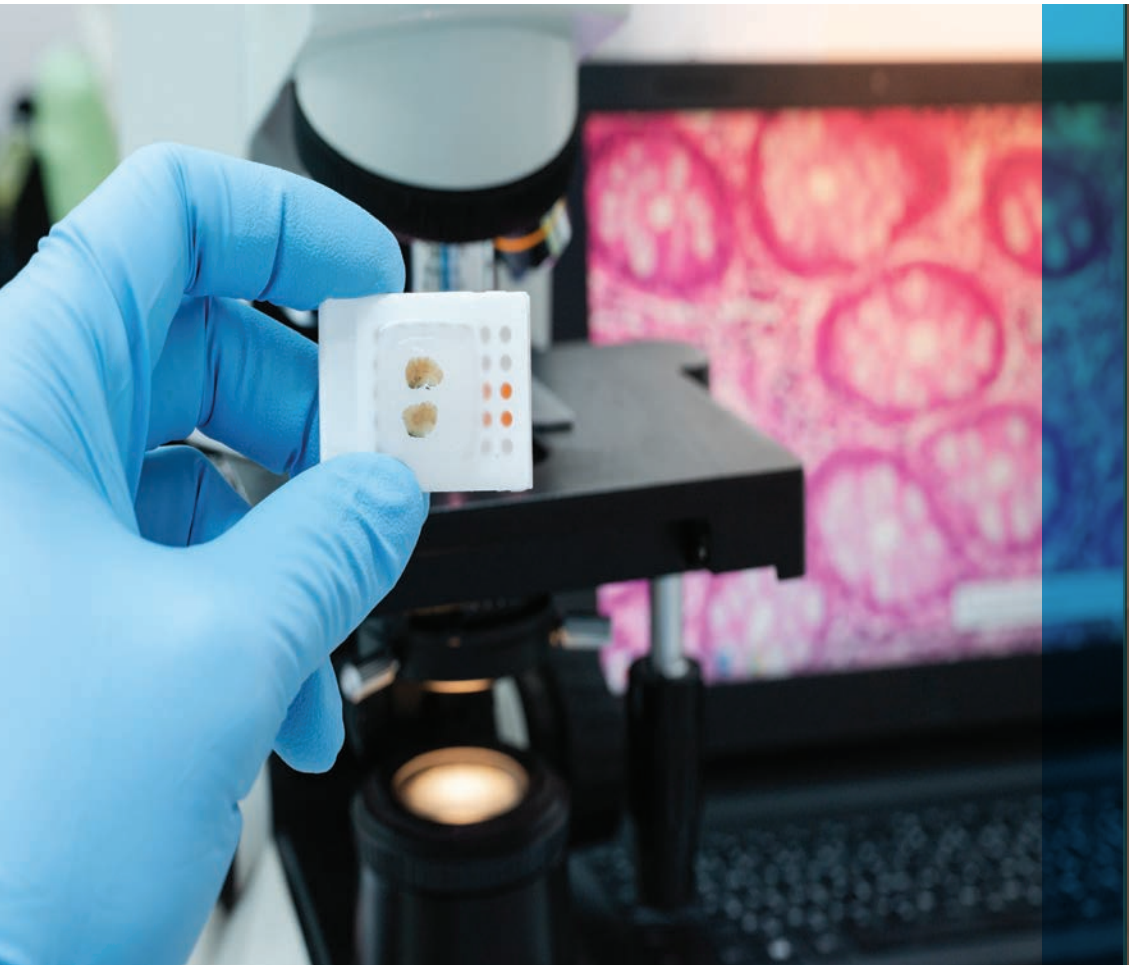

Register for Ki-67 assay F2F Education

Bryce Portier, M.D., Ph.D. Medical Director & Head of Medical Affairs at Agilent will be leading a LIVE educational seminar on Ki-67

Monday, **September 27, 2021** at the Hyatt Regency Chicago.

Choose a time that works with your schedule:

Session 1: 8:00 AM - Meeting Room, Aisle 900

Session 2: 10:00 AM - Meeting Room, Aisle 900

Session 3: 12:00 PM - Meeting Room, Aisle 900

Session 4: 2:00 PM - Meeting Room, Aisle 900

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