OCTOBER 2019 # 59

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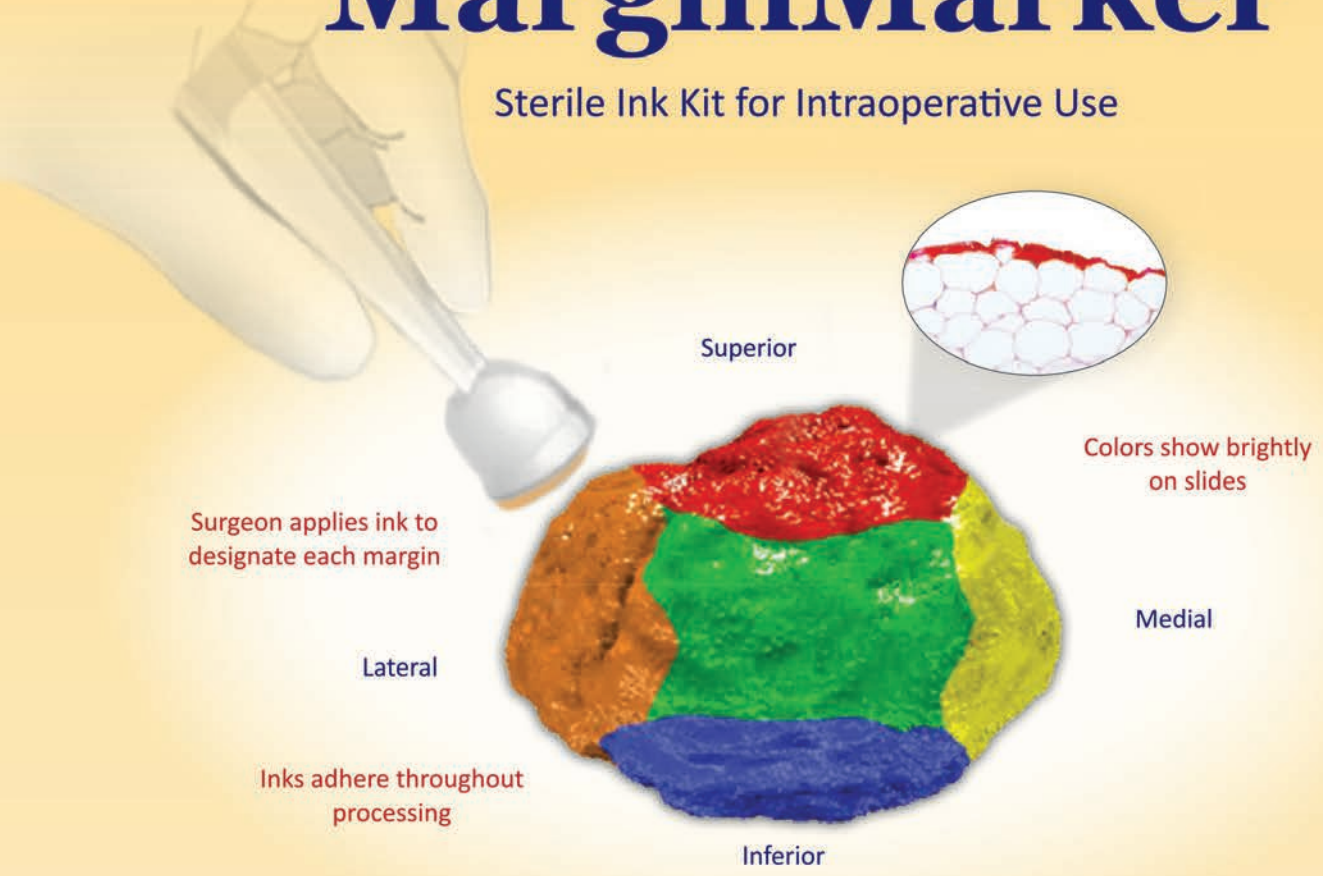

Pathology Receives Specimens With Margins Clearly Marked by the Surgeon

In cancer surgery, the single most important predictor of local recurrence is the tissue margins.[1] Research shows discordance rates as high as 52% in the identification of specimen margins.[2,3] Re-excision rates exceed 20% in breast surgery.[4] Use of Vector Surgical's MarginMarker can result in:

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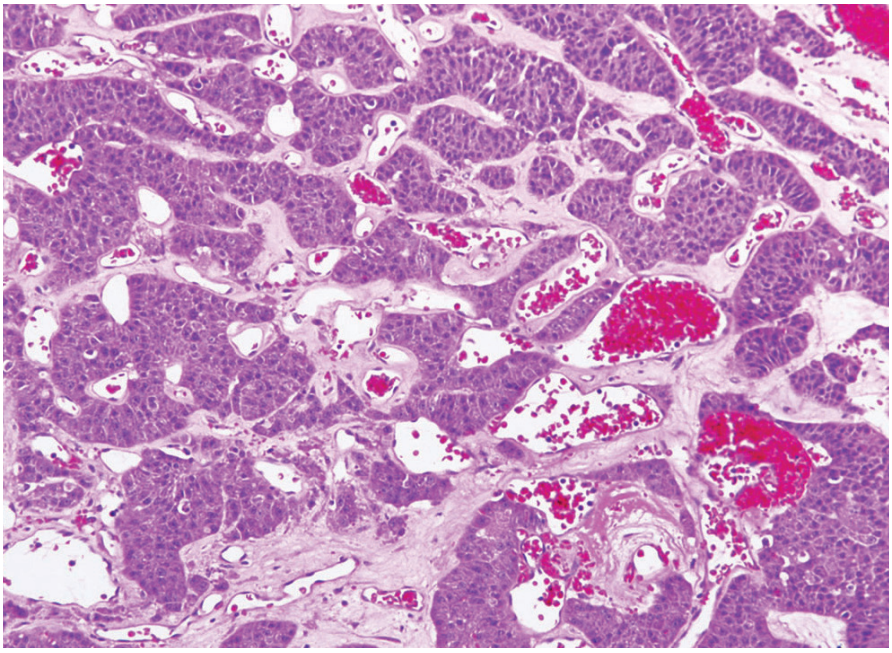
References: (1) Dooley, W.C. and Parker, J. "Understanding the Mechanisms Creating False Positive Lumpectomy Margins." *American Journal of Surgery* 190 (2005): 606-608. (2) Britton, P.D.; Sonoda, L.I.; Yamamoto, A.K.; Koo, B.; Soh, E.; and Goud, A. "Breast Surgical Specimen Radiographs: How Reliable 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.; Schmitt, 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.

Case of the Month

Pancreatic tumors of this type occur most often in which syndrome?

- *A* Multiple endocrine neoplasia type I
- *B* Multiple endocrine neoplasia type II
- *C* von Hippel-Lindau syndrome
- *D* Neurofibromatosis type I



Answer to last issue's Case of the Month…

A. Calretinin
B. Estrogenic changes

This case corresponds to ovarian thecoma. Macroscopically, these tumors are solid and present yellow cut surfaces. Cysts, hemorrhage, necrosis, and focal calcification may occur (1). Microscopically, they are composed of sheets and nodules of round cells with ill-defined borders, including moderate to abundant eosinophilic to pale cytoplasm. Nuclei are round to slightly spindled with little or no atypia. A fibromatous component often forms septa that separate nodules, and hyaline plaques with calcification may be seen (2). The neoplastic cells are positive for calretinin, inhibin, CD56, and WT1. They are often also positive for estrogen and progesterone receptor (1).

Submitted by Luis Humberto Cruz Contreras, Hospital Materno-Infantil, Irapuato, Mexico..

To register your guess, please go to http://tp.txp.to/1019/case-of-the-month
We will reveal the answer in next month's issue!

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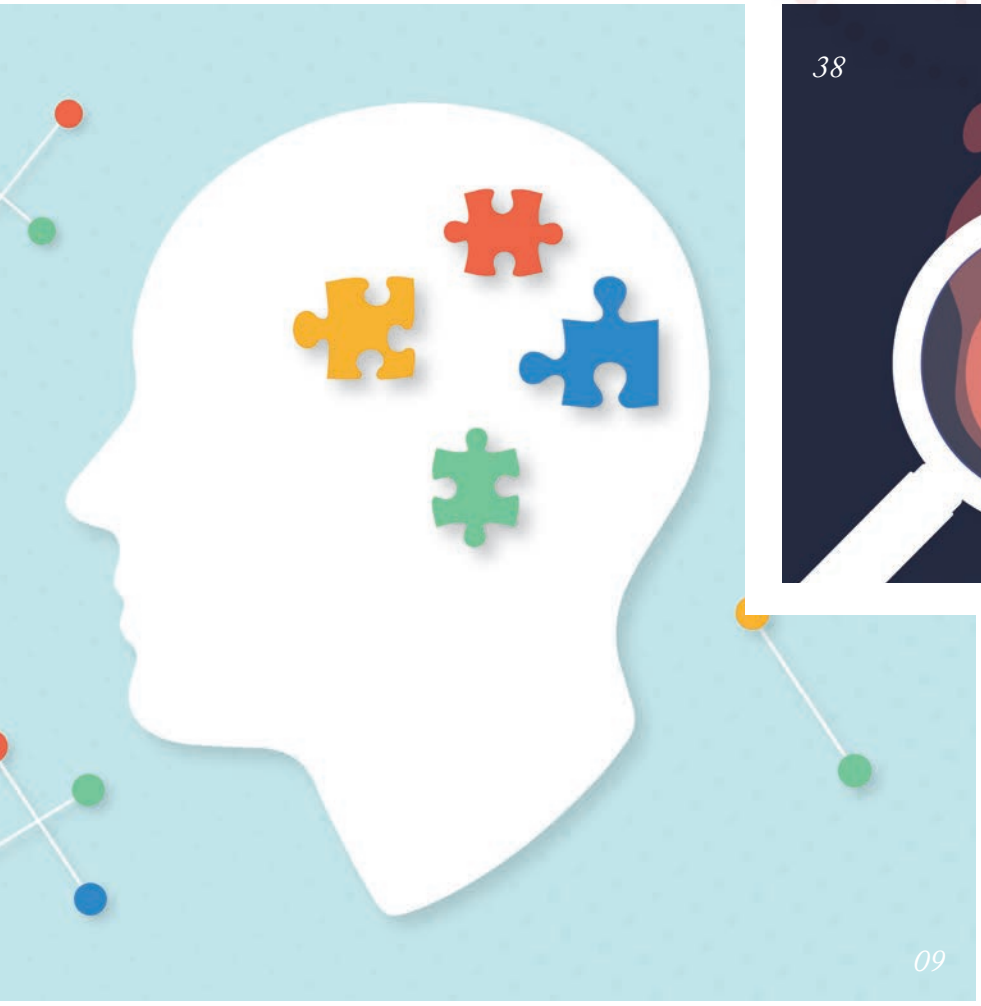

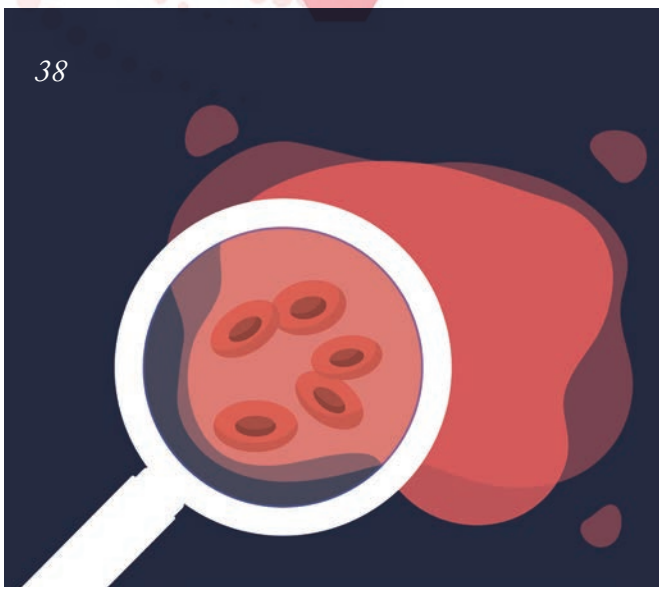

On The Cover

This shot of biobanking in action illustrates the process and highlights its value.

Feature

Reports

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Profession

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the **Pathologist**

ISSUE 59 – OCTOBER 2019

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Distribution:
The Pathologist (ISSN 2055-8228), is published monthly by Texere Publishing Limited, Booths Park 1, Chelford Road, Knutsford, Cheshire, WA16 8GS, UK
Single copy sales £15 (plus postage, cost available on request info@thepathologist.com)
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Burn Brightly – But Don't Burn Out

With the many pressures facing laboratory professionals, it's important to guard against overload

Earlier this week, I realized that I don't have any more international events scheduled for this year. There may only be three months left in it, but still – the break is a welcome change of pace. I love attending events, meeting pathologists and laboratory medicine professionals, and even the sensation of not being quite sure what time zone I'm in – or even what day it is! Nevertheless, I'm sure anyone who travels for education or engagement understands the pleasure of being able to unpack without immediately having to repack for the next trip.

But I'm only writing about pathology and lab medicine. I may be busy, but my chosen profession isn't making headlines for overwork, burnout, and staffing shortages. I'm not debating whether to work evenings and weekends or allow patients to wait additional days for what may be life-changing diagnoses. And my photograph didn't appear in the news next to a paragraph stating that only 3 percent of histopathology departments in the UK last year had enough staff to meet clinical demand.

Medical science is speeding along and treatment options are exploding. Patients are living longer than ever, even after severe or life-limiting diagnoses. Laboratory professionals are facing retirement, often without knowing who will take their place in busy hospitals or remote settings with few local doctors. Together, these and other factors mean that today's pathologists and lab medicine professionals are increasingly at risk of burnout. It sounds obvious – a concern discussed over and over in conferences, journal articles, and right here in The Pathologist – and yet it's still a problem without a good solution.

We can train more people – but that relies on having sufficient space, resources, and interested parties. We can outsource work – but that relies on having affordable, accessible laboratories who can take on the extra burden. We can relegate simple, repetitive tasks to our computers – if we have the software, hardware, and knowledge to do so. There's no one-size-fits-all answer, but the common thread running through all laboratories is this: you need a break.

So if you're heading to a meeting in the next few months, perhaps you'll consider staying for that gala dinner or that free conference breakfast. If you work long hours on evenings and weekends, perhaps you'll find some time to yourself to clear your mind. And if you're in a position to influence decisions, perhaps you can encourage others to do the same – or to find solutions that ensure that no member of the laboratory team has to take on more than they can handle.

Michael Schubert
Editor

Upfront

Reporting on research, innovations, policies and personalities that are shaping pathology today.

Do you want to share some interesting research or an issue that will impact pathology?

Email: edit@thepathologist.com

Putting a Finger on Substance Abuse

A new fingerprint-based testing cartridge quickly and noninvasively screens for multiple drugs

The words "drug testing" perhaps call to mind on-the-spot requests – from "fill the cup" to high-speed blood draws from unconscious patients suspected of an overdose. Few people, upon hearing those words, picture a dignified or noninvasive situation – but that's exactly what a new fingerprint drug screening test hopes to provide.

How does it work? The patient presses a finger to a small cartridge, which collects sweat samples in under one minute and screens them for methadone and buprenorphine in under 10 minutes. The cartridge can also test for other opiates and benzodiazepines. Testing can be conducted anywhere – from the community to the emergency room – without waiting for samples, and without overloading already busy laboratories.

Why test for methadone and buprenorphine? These drugs are commonly used to assist with recovery from addiction, and many providers find a combined screening tool valuable. By providing a tool that can screen for not only these drugs, but also some of the most common substances of abuse, the new cartridge offers a simple and comprehensive solution.

Vicki Markiewicz, Director of Change, Grow, Live – a substance misuse and criminal justice intervention charity – said in a recent press release (1), "As an early adopter of fingerprint-based testing, we already know that the process is easy and dignified. Now, with the addition of the new drug treatment screening test, we can not only test for drugs to establish adherence to treatment regimes, but also ensure safety in prescribing substitute opioids by testing our service users for drugs that may impact on the safety of prescribing regimes."

Reference

1. *Intelligent Fingerprinting, "New fingerprint-based drug test for drug rehabilitation provides a more dignified and convenient way to help clients overcome drug abuse and addiction" (2019). Available at: https://bit.ly/2LKx1Qk. Accessed September 3, 2019.*

Exploring the Epi-signature

Bekim Sadikovic updates us on the progress of DNA methylation as a diagnostic tool

"Do patients with intellectual disabilities have identifiable and specific changes in their epigenomes – and can we find the answer in peripheral blood?" This is the question that Bekim Sadikovic, Head of Molecular Genetics at London Health Sciences Center (LHSC) in Canada, posed in a recent article on the potential diagnostic power of DNA methylation (1). At the time, evidence showed that significant genome-wide DNA methylation alterations could be detected in peripheral blood, providing hope that DNA methylation tests could determine whether a variant of unknown significance is pathogenic. But, 18 months on, how much progress has Sadikovic – and the field as a whole – made?

His latest research applies the technique to DNA samples from patients with neurodevelopmental and congenital anomalies who lacked a definitive diagnosis (1). "Currently, the more sophisticated genetic analyses produce a 30–35 percent diagnostic yield for patients who present with developmental disabilities, leaving many cases unexplained," Sadikovic says. "When we applied genome-wide DNA methylation analysis, we were able to diagnose a significant number of additional patients in whom we identified an underpinning genetic condition based on evidence of an epigenetic defect."

The team have demonstrated that genetic defects can be manifested either as a result of, or in association with, genome-wide DNA methylation patterns. When these methylation defects occur across multiple loci, they are referred to as epi-signatures. "We can compare these epi-signatures to our reference database, which contains data for various conditions, to identify underlying genetic defects," explains Sadikovic.

In the new study, a computational model facilitated the concurrent detection of 14 syndromes with over 99 percent accuracy. Across 965 undiagnosed patients, the model identified 15 subjects with syndromic Mendelian disorders, 12 with imprinting and trinucleotide repeat expansion disorders, and 106 with rare epi-variants. "For the last couple of decades, genomics has been at the forefront of molecular diagnostics. We're reaching a plateau in terms of what we can do using genomics and DNA sequencing – but what we're now doing is moving the technology from research into the clinic."

As the first site in the world to offer this form of genetic testing, Sadikovic hopes that the work at LHSC sets a precedent. "The technology is easily accessible and most labs already have the ability to run genomic testing. The challenge will be interpreting the data. We have already built large reference databases to map epi-genetic signatures – and hopefully these continue to grow as the technology becomes more widespread."

References

1. *Bekim Sadikovic, "The Diagnostic Power of DNA Methylation", The Pathologist (2018). Available at: https://bit.ly/2mmzJ41.*
2. *Aref-Eshghi et al., "Diagnostic utility of genome-wide DNA methylation testing in genetically unsolved individuals with suspected hereditary conditions", Am J Hum Genet, 104, 685, (2019). PMID: 30929737.*

Quick Hits

The latest stories from the world of pathology and laboratory medicine

Too Little, Too Late

Approximately 115,000 cancer patients in England each year are diagnosed too late to ensure the best chance of survival. That's according to Cancer Research UK, who say that almost half of all cancers are diagnosed at stage III or IV. They blame a desperate shortage of National Health Service staff for the figures and call for increased recruitment of pathologists, radiologists, and oncologists to fill the vacancies. The UK government has already pledged to increase the number of cancer patients diagnosed early from 50 to 75 percent by 2028 (1).

A Splash of Color

Pathology is a field packed full of beautiful and intricate images. In fact, pathology is so much like art that the UK's Royal College of Pathologists has launched a coloring book that includes a series of drawings by scientist-turned-artist Lizzie Burns. The resource, called Incredible You, includes all 17 pathology specialties and was designed to support learning and relaxation for all ages. Jo Martin, President of the Royal College, said, "We hope people will use our resources to express their creativity, de-stress, and discover more about our bodies." Incredible You can be accessed for free at http://tp.txp.to/IncredibleYou (2).

Exploring the Unknown Genome

Although the Human Genome Project was completed in 2001, many regions of the genome remain uncharted because they are invisible to most current sequencing technologies. With the help of fiber FISH and Bionano optical mapping, new research has uncovered an unprecedented – and extreme – level of variability in the DNA on chromosome 22 (22q11). The new sequencing approach could uncover links between the amount of DNA in this region and a disposition to 22q11 syndrome (3).

Barrett's Esophagus: An Update

An updated version of the "ASGE guideline on screening and surveillance of Barrett's esophagus" has been published by The American Society for Gastrointestinal Endoscopy. As a precancerous condition, the role of screening and surveillance of Barrett's esophagus plays a crucial part of the new guideline, especially as early detection of esophageal adenocarcinoma provides the best chance of successful treatment. The utility of techniques such as chromoendoscopy, confocal laser endomicroscopy, and endoscopic ultrasound are all discussed in the updated recommendation (4).

The Color of Colon Cancer

A new urine test that can indicate the presence of colon cancer has proved successful in mice. The technology uses ultra-small gold nanoclusters (AuNCs) connected to a protein carrier, which are broken down by matrix metalloproteinases (MMPs) after being injected into the mice. Many cancer types – including colon tumors – produce high levels of MMP enzymes, which act on the nanosensors in the tumor microenvironment. When broken apart, the AuNCs are small enough to be filtered through the kidneys and produce a blue color change in the urine (5).

References

1. *Cancer Research UK, "Lack of government action on NHS staffing undermines ambition to diagnose cancer early" (2019). Available at: https://bit.ly/2m2beIU. Accessed on September 19, 2019.*
2. *The Royal College of Pathologists, "Incredible You – A new colouring-in pathology resource for all ages" (2019). Available at: https://bit.ly/32JO4b5. Accessed on September 19, 2019.*
3. *W Demaerel et al., "The 22q11 low copy repeats are characterized by unprecedented size and structural variability", Genome Res, 29, 1389 (2019). PMID: 31481461.*
4. *ASGE Standards of Practice Committee, "ASGE guideline on screening and surveillance of Barrett's esophagus", Gastrointest Endosc, 90, 335 (2019). PMID: 31439127.*
5. *CN Loynachan et al., "Renal clearable catalytic gold nanoclusters for in vivo disease monitoring", Nat Nanotechnology, 14, 883 (2019). PMID: 31477801.*

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In My View

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

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

Contact the editors at edit@thepathologist.com

The Healthcare Crisis We Are Failing to Address

Only advocacy can solve the forensic pathology recruitment crisis

By Kristen Adams, Assistant Professor and Course Director of General and Systemic Pathology at the University of Mississippi Medical Center, Jackson, USA

Across the USA, a pattern of crisis has emerged. In local newspapers and, occasionally, wider media, you see headlines that read, "Where Have All the Forensic Pathologists Gone?" and "Shortage of Forensic Pathologists!" I think it's interesting that the assumption is that they've gone somewhere – maybe Fiji or Disney World, who knows? The more accurate title should read, "We, as a nation, have failed to adequately prepare and train a forensic pathology force to deal with the aging population and massive opioid epidemic we are currently facing."

I know that my peers in medical school and I cannot be blamed for this crisis. We were told that caring for the living is the first priority; the dead will have as much dignity as we can provide, but we didn't have a moment of training or time to mull over death investigation. It wasn't even an afterthought tucked into the last moment of the last month of the semester. I am sure students at most other medical schools have a similar experience. Your entire foundation of knowledge must be devoted to the living and breathing patients before you – no time wasted for the dead. And so I can't blame the 99.94 percent of physicians who do not choose forensic pathology as their specialty.

The National Resident Match Program data show an all-time record high number of registrants at 44,603 for 2019. Of the 32,194 who matched, less than 2 percent chose pathology as their specialty. When you look at the number of residents who chose forensics, roughly 21 successfully completed a fellowship and passed their board exam in a year. That means that just 0.06 percent of applicants who complete residency go on to choose – and successfully complete – a fellowship in forensics. Truly a

"Just 0.06 percent of applicants who complete residency go on to choose – and successfully complete – a fellowship in forensics."

specialized group. At the moment, there are about 500 board-certified forensic pathologists doing work that should be distributed between double that number. In about five years, we might be able to meet current demands, but that's without taking into account the increasing population size, retirement, loss to the private sector, and other factors that would reduce the current workforce of practicing (and aging) forensic pathologists. Here in Mississippi, we conduct 1,500 autopsies per year. At a minimum, we need five forensic pathologists; six would be ideal for accurate and timely autopsy reporting. We currently have two. I think about those two pathologists performing 750 autopsies each per year – an average of about two a day, including weekends and holidays – and I wonder when they have time to sleep or see their families.

The only real option we have to address this crisis is advocacy. To reach out to undergraduate and medical students early and often. To make them aware of this critical need. To demonstrate that forensic pathology is medicine at its most humanistic, most humble, most honest, and most compassionate. We need to show them that these patients were neglected for far too long and actually needed us the most. The responsibility is on those of us in academic medicine and other positions of advocacy to promote forensics to lawmakers and other groups. To advocate funding increases for the salaries of forensic pathologists, loan forgiveness programs to increase recruitment, and other support for their practices.

To end this crisis, we as pathologists must all share the responsibility of advocacy.

What Would Your Oncologist Think If…

A perspective on current issues in PD-L1 testing

By Roberto Salgado, Department of Pathology, GZA-ZNA Hospitals, Antwerp, Belgium

What would your oncologist colleague think if you, a pathologist, said that you would order one of three different assays for her hormone receptor-positive breast cancer patient depending on whether she planned to treat with an aromatase inhibitor (for example, exemestane), tamoxifen, or fulvestrant? Would she perhaps wonder why each treatment required a different test? After all, are you not asking the same questions about the patient's health regardless of the treatment the oncologist selects?

What would the oncologist think if you then tell her that, for each different assay, you are going to use a different method of scoring? Some scoring systems combine tumor cells with immune cells, whereas others score only tumor cells, and still others only immune cells. You might even add that each assay has a different cutoff for positive or negative results – so a patient assessed with scoring system X is considered positive, but the same patient under scoring system Y is considered negative. And then, on top of that, each assay has a different sensitivity (some three times more sensitive than others) – even though the antibodies used are very similar. In short, depending solely on the assay you use, the patient's results – and therefore treatment – may change.

Imagine that your oncologist contacts you to ask for the expected positive rate for a biomarker in the population she wants to treat in a clinical trial – and your response is, "It all depends on the assay and scoring system you use, so I can't answer." To complicate matters further, it's unlikely that a laboratory would implement different antibodies for the same analyte, so if you have antibodies for assay X, you'll need to outsource assays Y and Z to other institutions. All of this creates delays in treating patients in urgent need – and those delays are only exacerbated when healthcare practitioners disagree with one another on the assay and scoring system to be used.

Now imagine that your oncologist has asked you to perform a PD-L1 assay on a solid tumor within a compassionate use or medical need program, but tells you, "I don't know what assay or scoring system to use." Currently, pembrolizumab is FDA-approved in 10 different cancer types, but in five of those (urothelial, gastric, cervical, head and neck squamous cell carcinoma, and non-small cell lung cancer), its approval is restricted to PD-L1 positive cancers – and the PD-L1 positivity threshold of each cancer is different.

By this point, I'm sure you must be asking, "How can this ever work in our daily pathology practice?" In the European Union (EU), unlike in the US, we aren't obligated to use a companion diagnostic if the oncologist

"Your oncologist might say, 'Companion diagnostics are not meant for the convenience of the pathologist, right?' – and yet, it still falls to us to identify the right patients to undergo each treatment."

wants to treat a patient with a specific treatment that has proven clinical utility in a phase III trial. Reimbursement agencies will point out that a PD-L1 assay should be used, but not specify a vendor or clone. Accordingly, a breast cancer patient in the US may be tested differently than in the EU, potentially with a different result. So how can we develop international guidelines to direct our pathology colleagues and serve our patients by implementing technologies that guide optimal treatment? Your oncologist might say, "Companion diagnostics are not meant for the convenience of the pathologist, right?" – and yet, it still falls to us to identify the right patients to undergo each treatment.

The question that we – pathologists, oncologists, the drug and assay industry, regulators, and patients – need to evaluate thoroughly is this: "Does a positive phase III trial using a particular assay overrule the notion that it may not be implementable in daily practice?" In my opinion, the answer is a clear no, but the solutions are not straightforward. I emphasize that we should not doubt the results of phase III trials that have proven clinical utility – but just because an assay is theoretically useful does not mean it is practically so.

If we don't act now, the problems we currently have with PD-L1 testing will spread to gene expression assays, tumor mutational burden assays, and other future tests – so it's clear that we need a new drug and biomarker development paradigm (1). I propose to introduce a risk management approach for the implementation of biomarkers in clinical trials (2). A risk analysis must be performed before the trial is activated, evaluating the performance of the assay in comparison with other assays – or at least demonstrating reproducibility in a powered study and assessing concordance in a set of samples that is representative of the patient population of interest. Ultimately, a drug is either given or not – a binary decision that can be linked to a binary assay. The cut-point for the binary assay can be tuned depending on whether it is an objective, continuous assay or a subjective assay. If the assay is subjective, the cut-point must be chosen in a manner that can be reproduced around the world without exceptional skill or extensive training.

In daily practice, recurrences are frequently biopsied to retest ER, PR, and HER2 for treatment selection. How should we deal with PD-L1 in this setting? Should PD-L1 be tested in the metastatic biopsy or in the primary tumor? What recommendations should we give to pathologists in their daily practice? How do findings from a clinical trial using checkpoint inhibition extrapolate to a real-life setting if the trial practices are different to those routinely used in the lab? We need to convene with oncologists, pathologists, industry, and regulatory bodies to find answers to these questions.

The proposed risk management strategy stipulates that users must ensure that training yields reliable test results before the assay is introduced into daily practice. Concordance rates should be assessed using a statistically powered number of pathologists (always more than two or three), mimicking a real-life situation. This is a departure from the current practice in which a limited number of pathologists at a central testing site evaluate the biomarker of interest, assuming that this will extrapolate to the full pathology community. Conceptually, we may even question the need to have different companion diagnostics for PD-L1 (given that they all target the same analyte) or even the need to have a PD-L1 companion diagnostic at all (given that we don't have companion diagnostics for all other protein biomarkers we use in our daily practice).

All this is to say that a positive phase III trial should not be taken as a guarantee that the assay used in the trial can be implemented in daily practice. It is our job as pathologists to select the most suitable patients for each treatment using the best assays available. Finding the assays to select those patients is a responsibility that regulatory bodies, academia, and industry all share – and we must form partnerships to identify those assays now for the sake of our current and future patients.

References

1. *R Salgado et al., "Addressing the dichotomy between individual and societal approaches to personalised medicine in oncology", Eur J Cancer, 114, 128 (2019). PMID: 31060925.*
2. *JA Hall et al., "A risk-management approach for effective integration of biomarkers in clinical trials: perspectives of an NCI, NCRI, and EORTC working group", Lancet Oncol, 15, e184 (2014). PMID: 24694642.*

Seeing the Patient Behind the Slide

Remembering our samples' human origins inspires us to provide the best possible care

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

The healthcare system in the US is increasingly moving toward value-based, personalized care. As laboratory scientists, our role in this shift is critical; we are the ones at the forefront of research to better understand the complex elements of diseases, how they develop, and how they can be treated.

As part of this growth, there has been a rise in the use of biobanks – the repositories that collect and store human biological materials to be used for research and discovery. Yes, there are still challenges surrounding biobanking – data security, patient privacy, and research consent, to name a few – but research suggests that the biobanking market will continue to grow rapidly over the next decade, particularly in North America, where chronic disease is prevalent and research opportunities and drug discovery are active.

That's good news for laboratory scientists; biorepositories are key to expanding our research and improving patient care. And with the growth of biobanking, laboratory involvement will also grow. We are the interpreters of data, we set the baseline for quality, and we oversee and secure the foundation upon which healthcare is built.

Remember: the laboratory is the cornerstone of healthcare and, as we move toward personalized medicine, it is imperative that "personalized" is the word we keep in focus. Too often, as we go about our daily duties in the laboratory, we forget that the slide under the microscope or the sample in the analyzer isn't simply a specimen. It's a person. The test results you are providing aren't just words on a chart; they're directives about how to handle a life. When you make your diagnosis, you're not making a conclusion about a piece of tissue, or a cluster of cells; it's a conclusion about a human being. And what you diagnose ultimately affects not only the patient's life, but the lives of the people around him or her. That "slide" has a mother, a father, a husband, a wife, three kids, a community of friends that extends well beyond the laboratory.

Laboratory scientists are not known for having a great deal of patient interaction. That could change as personalized medicine becomes more prevalent and multidisciplinary healthcare teams become more collaborative. And as biobanking plays an increasingly prominent role in research, and more scientists engage with these repositories to further scientific knowledge that will contribute to these teams, understanding the effect our discoveries have on humans – our family, our friends, our neighbors – will be integral to our success.

"That 'slide' has a mother, a father, a husband, a wife, three kids, a community of friends that extends well beyond the laboratory."

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BANK WITH US

The more we learn about disease, the more essential biobanks become – so why aren't more people aware of their value?

Biobanking – the act of preserving and storing samples of biomaterial – is an often-overlooked aspect of biomedical research, diagnostics, and prognostics. The samples stored in biobanks can yield valuable insight into disease processes over time, assist with retrospective studies of conditions and their causes, and stand families in good stead when a seemingly isolated instance of disease turns out to have a hereditary culprit. Not only researchers, but also pathologists, laboratory medicine professionals, and patients should value these resources. And yet, many people outside the laboratory aren't even aware of the existence of biobanks, let alone the varied purposes they serve. Fewer still know how biobanks are changing to keep pace with our increasingly computerized world and the growing value of the data that accompanies samples – after all, what is a whole slide image archive if not a digital biobank? And what is a small piece of tissue without its accompanying clinical data?

We spoke to three experts from the Biobanking and Biomolecular Resources Research Infrastructure (BBMRI) – two pathologists involved in research and clinical care, and one trained patient expert – to learn more about what biobanking can do for today's laboratory medicine.

The POWER of PLANNING

Biobanks can meet current and future pathology research needs – if we anticipate those needs

Daphne de Jong

Why is biobanking valuable for medical research?

The fundamental importance of biobanking is that it lets us translate findings from basic research to the patient level. For that, we need patient data that are complete, accessible, and as unbiased as possible. Why? So that by the time we have a pertinent question, we also have the resources to pose that question to a relevant patient cohort. And that is why you must set up a biobanking system that has all the prerequisites in place – so that it's there when you need it and you do not have to start from scratch. In other words, it's important to have the right samples and their accompanying medical information – before a specific question even arises.

Basic research is usually on a small scale, with a limited number of samples. When you translate it into practice, you need a much greater scope, which can be extremely time-consuming to identify and collect if you haven't already prepared for that. And the task only grows as the prerequisites to remain in compliance with privacy and safety legislation increase – so unless we take the necessary steps ahead of time, we won't be able to move forward.

Do biobanks hold value outside a research context?

As pathologists, we have an archive of pathology material. Historically, national regulatory bodies, have not always sufficiently appreciated the importance of those archives – either for our education or for our patients' health. Let's not forget about families, either; the more we learn about hereditary health issues, the more important it is to have those records so that we can go back to the material and perform new tests that can impact clinical care.

In the past, some regulatory bodies made the mistake of saying that, for privacy reasons, archives must be destroyed after a certain number of years. This error of judgement has done massive damage; various pathology labs in the Netherlands, where I live, have actually destroyed part of their archives, doing irreparable harm to their records, especially now that patients' life expectancy is so much longer and our need to go back to previous material has increased.

Is it important for you and your colleagues to have input into biobanking decisions?

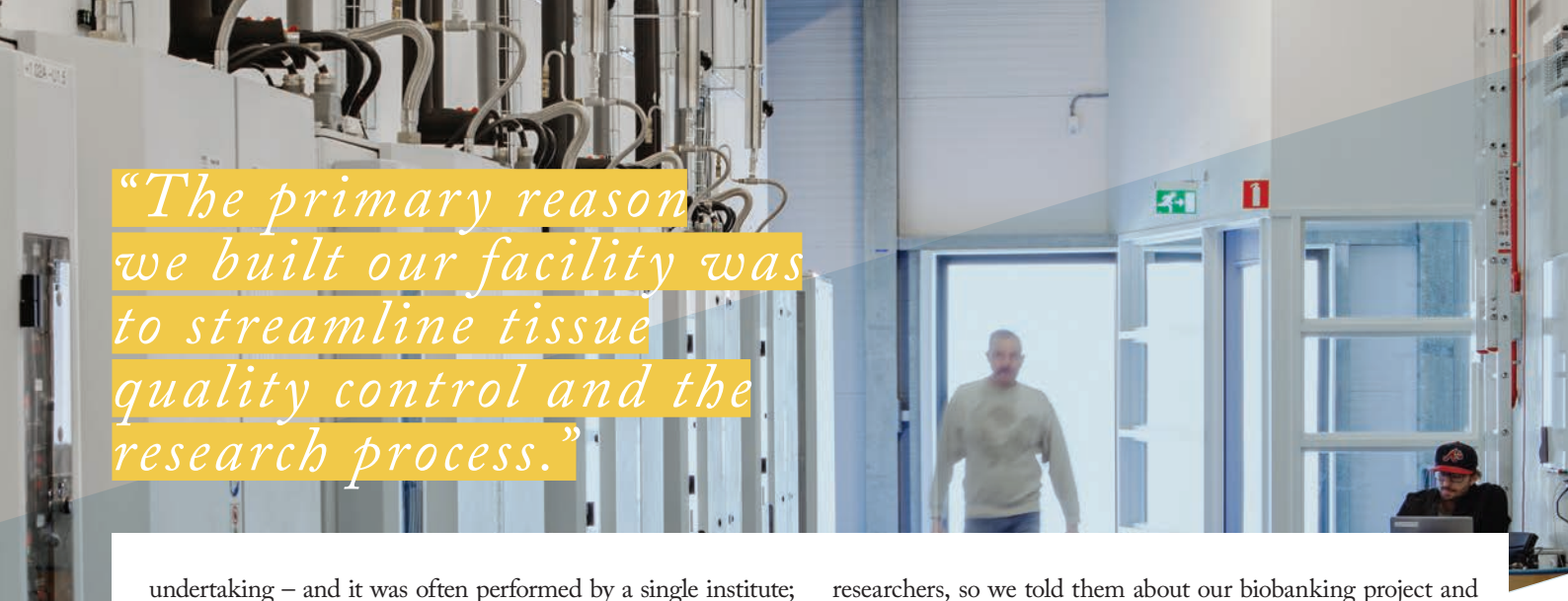
I think it's very important for people in pathology and laboratory medicine to be involved in biobank design, especially to help connect samples from disparate registries. I think it's very powerful that, in the Netherlands, we have been able to link physical biobanks (such as pathology archives) to virtual records (such as cancer registries). Because our work requires us to know the design of both databases, we can associate them – which lets us accomplish things like assembling a population-based cohort of a rare disease, including clinical data, without going back to the patients and without falling afoul of privacy legislation. And that is fantastic. We've only been able to do that because we have had an influence on the design of both databases and have therefore been able to suggest useful features.

Of course, we aren't the only people who should have a say. Biobank design should be a multidisciplinary effort. You need the input of administrators, database experts, ethics experts, and users. Above all, it's important to future-proof biobanks. These archives are supposed to last us for decades, so we must consider not only our current needs, but also what we might ultimately need. Ask yourself, "What will we be doing with this biobank in five or 10 years' time?"

How did your biobank come about?

My colleagues in pathology and laboratory medicine and I came up with the idea for a pathology facility to support our work for the national hematology clinical trial organization Hemato-Oncologie voor Volwassenen Nederland (HOVON). We set the project up on a research basis and received support from related institutions; they lent us their experience and support and helped us to understand both their needs and our own. It was particularly helpful that one organization had previously dealt with database building and biobanking as one package, so they were well-versed in coordinating the two. We couldn't have done this work without our advisors.

Why did we do it? The pathologists and hematologists involved in HOVON wanted to do clinicopathological side studies on clinical trials. From the early 1990s onward, we had always collected material after the conclusion of each trial. For some trials, it took us two years of full-time work to collect the blocks, review, biobank, and then ultimately conduct a side study. It was an expensive, time-consuming



"The primary reason we built our facility was to streamline tissue quality control and the research process."

undertaking – and it was often performed by a single institute; sometimes even by a single person. There was no sustainability and the material was not available to others, so other researchers had to start from scratch each time. The primary reason we built our facility was to streamline tissue quality control and the research process.

It works fantastically well! Within three months of concluding a large trial in central nervous system lymphomas, we had the entire review completed, including all the biomarkers needed for a primary publication – something that might previously have taken years. That demonstrated to us that our new approach works. We are now already working on the a clinicopathological side study, so it's clear how much faster things are moving than with our old system.

How can others promote biobanking?

Pathologists and laboratory medical professionals must show clinicians that we can really deliver. Our HOVON pathology facility is a showcase for that, so I hope our colleagues will use our success as proof of concept.

When we started building our biobank, I said to the board at HOVON, "My aim is that, by the time we finish, you won't remember a time when this facility didn't exist. You won't even be able to imagine that there was a time when you couldn't rely on it for your work." And indeed, four years later, the president of the board said, "You were right. We can't imagine not having this facility – it's an integral part of what we do." It helps to have a wide support network; not just pathologists, but clinicians, basic and translational scientists, and administrators too.

For non-pathologist laboratory staff, I think it helps them to know why they're doing the work of biobanking. For us, that has meant hosting meetings on biobanking – or traveling to laboratories to attend meetings – and explaining what biobanking is, how it's used, and why it's valuable. I've noticed that understanding the importance of their work really motivates people to do it well.

In the Netherlands, we have a very active patient support organization, called HEMATON, which is very involved in policy. They have a research advisory group for clinicians and researchers, so we told them about our biobanking project and asked for their thoughts. They immediately adopted the idea and began promoting it for us! They even educate patients entering clinical trials and encourage them to consent to the use of their material for research purposes. The patients get very enthusiastic – we have almost a 100 percent rate of consent – thanks to our close relationship with patient organizations; something I recommend for others moving into the world of biobanking.

What input do you have into routine biobanking?

I'm a hematopathologist; I diagnose and do research on lymphoma. The HOVON pathology facility and biobank is completely separate from our pathology lab; it's part of HOVON, so governance is in the hands of the HOVON board and any researcher in the Netherlands can submit a proposal to use that material. The system to efficiently collect and bank biopsy samples runs smoothly because we, the users, are the people who have designed it: what we want to biobank, how we want to bank it, who owns the material, how we deal with practical, ethical, and legislative issues – all of the considerations taken into account when starting a new biobank from scratch.

In our case, it was particularly valuable to have the possibility of linking various biobanks and databases to one another. For example, we were studying a rare form of lymphoma that occurs in women with breast implants, known as breast implant-associated anaplastic large-cell lymphoma. As you can imagine, it's difficult to locate these very rare patients for an informative study – but we were able to retrospectively identify every case since 1990 in the Netherlands and also associate with a registry of breast implants! That allowed us to retrieve data on the breast implants with which the disease is associated, as well as a high percentage of the biomaterial – so we have a unique clinicopathological series with valuable biomaterial, thanks to having anticipated our need for such studies. It pays to "future-proof!"

Daphne de Jong is Professor of Pathology at Amsterdam University Medical Center, Amsterdam, the Netherlands. She is also Coordinator of the HOVON Pathology Facility and Biobank.

"Once a disease manifests itself, you can go back to the archive of samples to take a closer look at previous cases and controls."

PROSPECTING *the* GOLDMINE

Biobanking has a lot to offer, but requires careful explanation and education

Folkert van Kemenade

What makes biobanking so valuable?

Biobanking is vital for translational research (for instance, investigating biomarkers) and epidemiological research (to better understand disease). You can bank with healthy volunteers, the approach taken by the Lifelines biobank in the Netherlands. But you can also use existing biobanks, which give you follow-up for free: once a disease manifests itself, you can go back to the archive of samples to take a closer look at previous cases and controls. Prospective longitudinal biobanking delivers insight into things like polygenic risk scores, interactions between genes (and other disease factors), whole exomes or even genomes, and so forth. Existing biobanks, with samples collected for diagnostic reasons, are ideal for observational studies – and, in my opinion, the only feasible way to study rare diseases.

For diagnostics, biobanking has a different significance to its value for prospective, cohort-based studies. In diagnostics, one needs to maintain files and samples – if possible; you can't bank all blood samples, for instance, but you can preserve tissue. Diagnostic tissue forms a bio-archive with a dual purpose: not only the diagnostic process for which it was collected, but also research. In pathology, I call this the "tissue bonus." Any patient's archived tissue can double as diagnostic file that can be reconsulted (and re-tested) while, at the same time, serving as a basis for biomarker discovery. Just make sure that you adhere to due diligence for sample anonymization!

What kind of input should pathologists have into biobanking?

The pathology residual tissue bank typically tends to be one the biggest collections; in ours, we have more than 1,000,000 FFPE samples. We pathologists and laboratory medicine professionals are keen on economy of scale and well-accustomed to fair governance and responsible use. We can, in other words, provide valuable help with general decisions. Our hospital biobank has a central council, and we have a delegate on that council who represents our interests and helps develop solutions when conflicts arise.

All of the pathology residual banks in my country are linked to one database, known as PALGA (Pathologisch-Anatomisch Landelijk Geautomatiseerd Archief). That's extremely useful for studying rare diseases (among other things). My colleagues and I conducted a nationwide study to test the hypothesis that two rare conditions were related. We ended up collecting 32 blocks assembled over many years from a range of laboratories – although our hypothesis was unfortunately incorrect. Or take the breast implant-associated anaplastic large-cell lymphoma study my colleague Daphne de Jong is conducting; thanks to biobanking, she can collect blocks from all over the country. But even studies of this size are small compared to the possibilities for more common diseases. I was recently involved in a nationwide procurement of 18,000 blocks from residual archives in different labs. My colleagues and I helped pick the blocks, the researcher assembled tissue microarrays (a herculean task), and we helped return the blocks to the archive. Large-scale, residual, biobank-based research is possible! Incidentally, in the slipstream of that project, we were able to provide help for the HOVON project Daphne mentioned earlier. The next step to ensure accessibility is to streamline the organization and make sure to return information to stakeholders. Scientific results should be shared with all stakeholders, including patients.

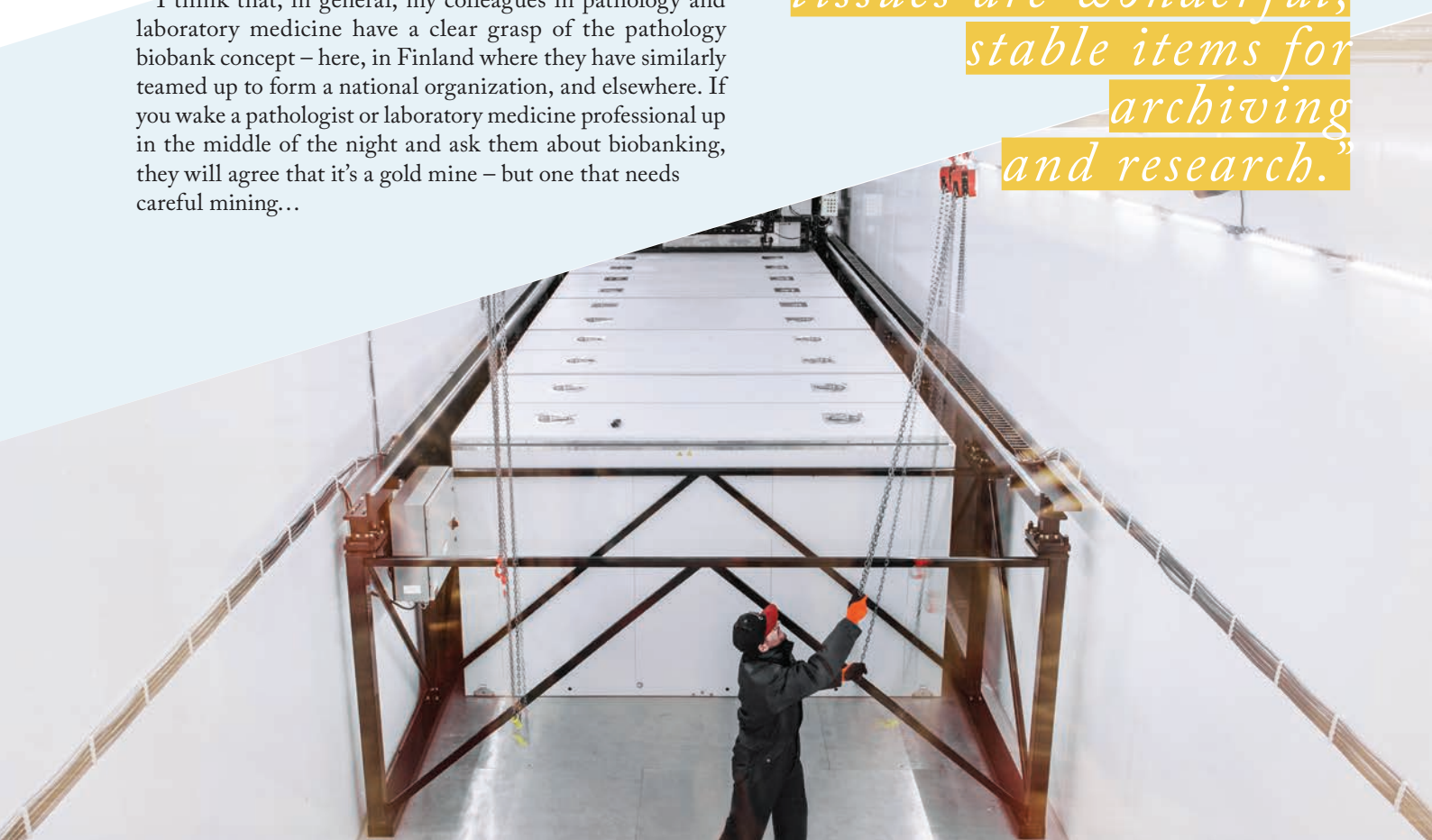
I think that, in general, my colleagues in pathology and laboratory medicine have a clear grasp of the pathology biobank concept – here, in Finland where they have similarly teamed up to form a national organization, and elsewhere. If you wake a pathologist or laboratory medicine professional up in the middle of the night and ask them about biobanking, they will agree that it's a gold mine – but one that needs careful mining…

What would you like others to know about the pathologist's role in biobanking?

The concept of a tissue block should be better-known outside the pathology silo. We must also explain the diagnostic process – over and over again until everyone (physicians, researchers, patients, the public) understands that we store samples for patient care, and that we combine care with research. Outside pathology, it is often believed that we store everything (which we don't) or that we can culture cells back to life from blocks (which we can't). Blocks of archived tissues are wonderful, stable items for archiving and research. They can yield DNA sequence information; they are relatively low-cost (unlike stored digital data); they cannot crash; they cannot be hacked; and they have great battery life!

Folkert van Kemenade is Chair and Professor of Pathology at Erasmus University Medical Center, Rotterdam, the Netherlands. He is also part of the BBMRI-NL Scientific Executive Committee.

"Blocks of archived tissues are wonderful, stable items for archiving and research."



A Winning COLLABORATION

To tackle rare diseases, biobanking partnerships between patients and pathologists are vital

Marleen Kaatee

Why is biobanking valuable for patients?

Biobanking is an essential part of medical research. Many diseases – especially rare diseases – do not yet have a cure, so those of us who experience them are eager to do what we can to help with research.

Personally, I find that that patients in general know very little about biobanks. We may be missing opportunities to have patients to consent to banking their samples simply because they don't know what a biobank is or what the implications of contributing their samples might be. The simple fact is that, with enough samples, I truly believe that biobanking might hold the key to cures.

As a patient expert, I did a course on biobanking and saw the range of possibilities – especially with the rise of digital biobanking (wherein users can access computerized whole slide images, rather than obtaining the glass slides themselves). There are so many opportunities, and as research progresses, it could be that our samples point scientists in the direction of a cure. That's especially true with the amount of high-quality international collaboration taking place right now. Organizations like BBMRI-ERIC, a European research infrastructure for biobanking, encourage researchers to share both samples and data. I think that, if we link all of that information together, we'll have a pool of easily accessible data ready for researchers and healthcare providers to examine for answers.

As we say in the Netherlands, "unknown is unloved." That's why I really hope that we – patients and professionals together – can bring the public up to speed on the hows, whats, and whys of biobanking. It's especially important for patients who, like me, have a rare disease. If there's no cure, hope is the best you have – so if you understand that biobanking can help research move forward faster, you feel empowered to help yourself and others by signing the consent form. You finally feel like you're contributing to a solution.

It takes an average of five years for a person with a rare disease to get the correct diagnosis (1), so anything that can help make that diagnosis earlier saves patients and families a lot of trouble. If we can get researchers and clinicians familiar and comfortable with biobanking – especially digital biobanking – then everyone will have more samples for comparison, which gives us a better chance of finding the right diagnosis fast.

Patients who aren't offered the option of biobanking can take the initiative to encourage it, too. Ask your doctors and researchers, "Have you looked into biobanking? Do you know about the incredible collaborations that are taking place?" Right now, a lot of biobanks are static sample archives, whereas they should be active, evolving, collaborative ecosystems – and it's up to us to drive that. The more people know about biobanking, the better and more powerful it will become.

Should patients have input into general decisions on biobanking?

I joined the BBMRI-ERIC Stakeholder Forum because I think biobanking is overlooked by the general public. It doesn't get the attention I think it should get. When the average person thinks about research, they picture microscopes and laboratories and tubes – but biobanking is an essential part of that research.

Patients have a lot of power to drive change. For instance, I've learned that there isn't any standardization for saving samples within specific rare disease areas. As a patient advocate, I can encourage research and clinical professionals to address that issue and to work together to resolve it. It's not always easy for researchers to share data, but patient requests carry a lot of weight. We can also bring additional information to the table. Of course, biobankers know more about sample storage and preservation than we do – but we have insider information about our own conditions. What a scientist or doctor might see under a microscope, we live with 24/7. For instance, a group of patients with primary sclerosing cholangitis, a rare liver disease, discovered that many feel much better with specific, often non-obvious, diets. Perhaps we can advise researchers to take that into account when collecting samples. They get more information and we get more insight into our bodies – a win-win!

Have you personally had the opportunity to weigh in on biobanking?

I shared my interest in biobanking with one of my hepatologists. At the next annual conference on my disease, they – for the first time – invited a pathologist to speak. After all, some of us had been contributing samples for years – and a liver biopsy is no walk in the park; it's a real commitment – so we were eager to know what happened to our tissue and how it had benefited disease research. It was a great success; the hepatologist and the pathologist gave a presentation together and patients were very appreciative.

It's also interesting for people to actually see tissue samples from different disease stages. It really helps clarify the difference between severity levels, why it's important to identify and adhere to the right treatment, why patients shouldn't miss tests or checkups… Primary sclerosing cholangitis, for instance, causes inflammation, stricture, and sclerosis of the bile ducts. Seeing actual bile ducts – and the difference between a healthy and a diseased duct – helps you to understand what is happening in your body and why you might need procedures such as stent placements.

I found that such concrete representations had three positive outcomes: educating patients about their conditions, encouraging them to consent to biobanking, and prompting them to consider their lifestyle and its potential contribution to disease. A liver biopsy can reveal a lot of secrets!

How did you get involved in biobanking?

I initiated the process myself. I consented to banking a few liver biopsies – but that was when I was still what we call a "naïve patient," one who is just starting to learn about the world of chronic illness. Just last week, I got a letter asking to use my samples for a new research project, and of course I agreed. Many people don't know that can happen – that they can have a liver biopsy and then, five years later, someone may ask to use their samples for unrelated research. If you aren't aware of biobanking, you might not be pleased to find that your samples are "out there" and available to strangers. But if you've been educated, you know that it's all part of studying the condition – part of finding better diagnostic tests, better treatments, and maybe, one day, a cure – and you might be thrilled to help.

I think patients should be offered the opportunity to consent to research, not only on their own diseases, but also for comparative purposes. That way, you may be helping both yourself and others. There should be a warm relationship between healthcare providers, researchers, and the patient community, because each of us can contribute in ways that benefit all of us.

In June 2018, I took a summer course on Fundamentals of Biobanking and Cohort Research at University Medical Center Groningen, the Netherlands. After our introduction to biobanking, we were taught in depth about clinical biobanking and sample analysis, ICT and infrastructure, regulation, participation, and practical considerations such as standardization and essential biobanking tools. We also received a tour of the Lifelines biobank

"It would be great if more pathologists proactively asked patient advocates to get involved with their work – especially as it pertains to research."

in the north of the Netherlands. They have collected samples for quite a few years; long enough that they can work on prospective studies using samples from healthy people, as well as retrospective studies from individuals with specific conditions. To see the way they operate – all of the machines, the freezers at -80°C, the size of their building – was amazing. Even better was to hear the passion with which the people there spoke about their work, and the care and concern with which each sample was treated. Imagine how I felt when I saw how my samples could contribute to the greater good.

In all honesty, before becoming a patient, I had never heard of biobanks. I've now mentioned to the Ministry of Health that it should be a part of the high school curriculum, so that everyone is aware of these institutions and what they do. Eventually, you or a family member may need a biobank. It's not common to know about biobanks but it's certainly not uncommon to need one!

What should "the experts" know about the patient's role in biobanking and in medical research?

It's good to look into your patients' diseases and try to gain an understanding of them, because that will help you form a good relationship with your patients. For rare diseases, especially those without a cure, your interest in the disease – and the patient – offers hope. The more extensive your knowledge of that condition, the more likely you are to understand the intricacies of the patient's needs, and that will come across to the patient.

Speaking as a patient expert, it would be great if more pathologists proactively asked patient advocates to get involved with their work – especially as it pertains to research. A lot of patient advocates are well-trained, and they have information (ranging from anecdotes to scientific surveys of the community) that could help highlight unmet needs and research priorities. It's also nice when the pathologist gets out of the laboratory, so that people know who they are. They often seem to be the people behind the scenes, but the more they interact with patients and the public, the more motivated those people will be to assist with their work.

Patient advocates can also pass on questions from pathologists and laboratory medical professionals to the community. I haven't spoken to many pathologists, but the ones I have met were all very enthusiastic; they are proud of their biobanks and I've been invited to visit! I recommend having some basic information about biobanking available for patients and working with patient advocates to open up opportunities for research. Perhaps we can help you find answers faster; perhaps we can help you investigate in more depth; there are a zillion possibilities. The more proactively you approach the patient community, the more we can (hopefully) help. All you need is to ask – we are eager to answer!

Marleen Kaatee is the founding President of PSC Patients Europe and a fellow of the EUPATI Patient Expert Training Course. She is also a member of the BBMRI-NL Patient and Public Advisory Council.

Reference

1. *Global Commission to End the Diagnostic Odyssey for Children With a Rare Disease, "Ending the Diagnostic Odyssey for Children With a Rare Disease: Global Commission Year One Report" (2019). Available at: https://bit.ly/2Uf3yQR. Accessed August 30, 2019.*

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The Next Generation of Clinical Variant Profiling

How in-depth analysis of gene variants can drive the adoption of precision cancer medicine

By Anoop Grewal

By enabling the sequencing of millions of DNA bases rapidly and simultaneously, the emergence of next-generation sequencing (NGS) has played a major role in the shift toward precision medicine. The technique's ability to home in on specific target regions makes the diagnosis of challenging tumors easier than ever by uncovering significant treatment targets and prognostic biomarkers.

But the amount of patient-specific data that NGS generates and the ever-growing body of knowledge in which it has to be interpreted has proven as much of a challenge for healthcare facilities as an opportunity. Although extensive genomic data has spurred advances in cancer genomics and paved the way to more accurate and personalized patient management, many clinical labs aren't prepared to deal with the demands of huge, complex datasets in a rapidly advancing field.

As sequencing technology becomes more affordable and accessible, a growing number of clinical labs are bringing NGS testing in-house. The flexibility and immediacy of in-house NGS analysis make this approach an attractive option; however, to use NGS effectively, the clinical lab must stay on top of an onslaught of information coming from internal datasets, external databases, guidelines, drug approval agencies and peer-reviewed literature. It's a task that historically would have required hordes of clinical scientists to review and synthesize information for each variant encountered – which is why many labs have previously chosen to outsource variant interpretation and why now, even as the technology comes within reach of more laboratories to bring in-house, the prospect may still seem daunting.

Obstacles to overcome

Right from the start of the tertiary analysis workflow, variant filtering and annotation requires referencing variant results against multiple data sources to identify the subset corresponding to pathogenic variants. Then, for each variant, users must determine clinical significance, reviewing applicable drug labels and medical guidelines and even identifying clinical trials for which the patient may be eligible. Finally, the process requires a clinical report that an oncologist can use to determine the best route forward, putting the onus on the clinical scientist and lab director to produce a comprehensible report that could include mutations that the oncologist has never heard of.

Increases in sample volumes and panel sizes are expanding the possibilities of NGS, but also contribute to the time-consuming task of interpretation. Because the datasets to be analyzed and reported are so large and complex, they need to be referenced against a vast corpus of evolving evidence – something that could limit the routine use of NGS in the clinic. Last, but not least, the challenge of keeping database knowledge up to date in a constantly changing field and synthesizing large amounts of medical data into meaningful reports can prove a significant hurdle for the lab workflow.

These obstacles are challenging but, thanks to cutting-edge technology, they are no longer insurmountable. Clinical NGS reporting solutions can help labs interpret the clinical significance of mutations, enabling oncologists to understand what the results mean for the patient's treatment options, for prognosis, and even in some cases, for a more refined diagnosis. NGS reporting solutions take responsibility for much of the "heavy lifting" involved in collecting and organizing relevant clinical information across a variety of data sources, including the most recent literature surrounding variants in particular cancers. That information is then analyzed extensively to provide healthcare professionals with clinically actionable interpretations for each patient.

The data toolbox

Clinical NGS reporting solutions claim to offer many different tools – machine learning-derived predictions, curated content, and more. But with so many options, how can a laboratory decide which is most suitable for their needs? The answer lies in understanding exactly what each of these tools can provide.

One of the most useful tools available is a "knowledge base" – an evidence-based source of scientific and medical research that feeds into the platform at its foundation. Although some solutions can aggregate publicly available information, this may fall short of a lab's needs if it is left to the lab to synthesize that information in a concise, report-ready form with the intended reader – the oncologist – in mind. The most thorough knowledge bases that include the synthesized information can cover a range of tumor types and provide detailed, highly curated information on tens of thousands of unique variants. How is such a knowledge base created? The process begins with information-gathering – from public databases and medical literature – and evaluating the evidence to identify what's relevant. This involves assessing given variants in a specific cancer context and creating a tier system with respect to clinical significance based on Association for Molecular Pathology (AMP) guidelines (1). The evidence is then organized, weighed, and summarized by

the curation team before it is available for inclusion in a clinical report.

And the possibilities don't end there – more sophisticated knowledge bases go beyond the literature and incorporate clinical trials resources to identify clinical trial opportunities based on a patient's genomic alterations. They can match patients to therapies by searching approved drug labels, medical guidelines, and clinical trial outcomes.

Variant combinations

A growing concern in targeted treatment is the implications of variant combinations. Most solutions provide descriptions tied to individual variants drawn from the knowledge base and list any appropriate therapies. But what if a second variant is present that renders some of those therapies ineffective? Patient outcomes differ based on the unique combination of variants they possess, so it's important to identify, resistance mutations, for instance, that could hinder the efficacy of a treatment.

For example, the *EGFR* L858R mutation in isolation will likely yield four appropriate therapies available. Doctors may select any one of those treatments for the patient based on availability and other factors. However, a patient who also has the *EGFR* T790M mutation will be resistant to three of those four therapies, leaving just one suitable option: osimertinib. In other cases, where a combination doesn't include a resistance mutation, subpopulations defined by such combinations of variants may be characterized with respect to prognosis and likelihood of response to specific therapies. A system that can concisely display information about variant combinations provides clinicians with a fuller picture of considerations, allowing them to choose the best patient management options from the outset.

Another important feature of any NGS reporting solution is the clinical report itself. A simple, informative report – one that the oncologist can easily refer to when reviewing a patient's management options – is invaluable. Without the technology, a large amount of time can be spent copying variant information from a research-oriented tool to a report template and manually entering information to support the clinical significance of variants. The most effective NGS reporting solutions generate professionally branded reports that represent the primary results prominently and incorporate the relevant clinically interpretive summaries automatically. More generic reporting solutions may not be able to present NGS data appropriately, let alone pull relevant interpretations from a knowledge base.

Some reporting solutions aggregate and annotate variants, but leave the user to read and interpret their significance manually; more advanced systems synthesize the most relevant information for oncologists into a concise summary detailing each variant in the appropriate cancer context. Reports that clearly state the number of clinically significant variants, present them in order of clinical significance, provide actionable variant information, and enable inclusion of a case-specific executive summary save labs time and clearly set out the best patient management pathway.

A simple solution

When we consider the complete workflow of such an advanced clinical NGS reporting solution, its effectiveness in summarizing the results of a tumor genetic analysis becomes clear. After a tumor sample is sequenced by NGS, imagine the long list of resulting variants: potentially, single nucleotide variants, small insertions and deletions, copy number variations, and gene fusions. The variants are then filtered based on their quality and presence in databases, leaving those that are more likely to be cancer drivers. At this stage, every variant deemed important is referenced against a second source of information – the highly curated knowledge base that represents existing information about each variant.

At this stage, the most advanced reporting solutions even reference variants against applicable drug labels and professional guidelines (i.e., those appropriate to the region where the lab resides) and order them according to the level of evidence supporting their clinical significance. Recruiting clinical trials for which the patient may be eligible can also be applied in a geography-specific manner. Finally, the reporting solution will compile all of this automatically generated information into a simple clinical report. This includes information on the variants that are likely driving the cancer, as well as the latest evidence for effective treatment options – all while taking into account variant combinations that may affect susceptibility.

As NGS becomes increasingly common in clinical labs, there is an ever more pressing need for effective reporting solutions that access the most clinically relevant information about variants and offer suitable treatment and other patient management options. Clinical NGS reporting solutions offer a number of features, all of which users must consider thoroughly in line with their lab's needs. Ultimately, the ability to rapidly and affordably transform large amounts of variant data into actionable insights based on the most current knowledge will drive the widespread adoption of precision medicine.

Anoop Grewal is International Product Manager, Advanced Analytics at Roche Sequencing Solutions, Pleasanton, USA.

Reference

1. *MM Li et al., "Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists", J Mol Diagn, 19, 4 (2017). PMID: 27993330.*

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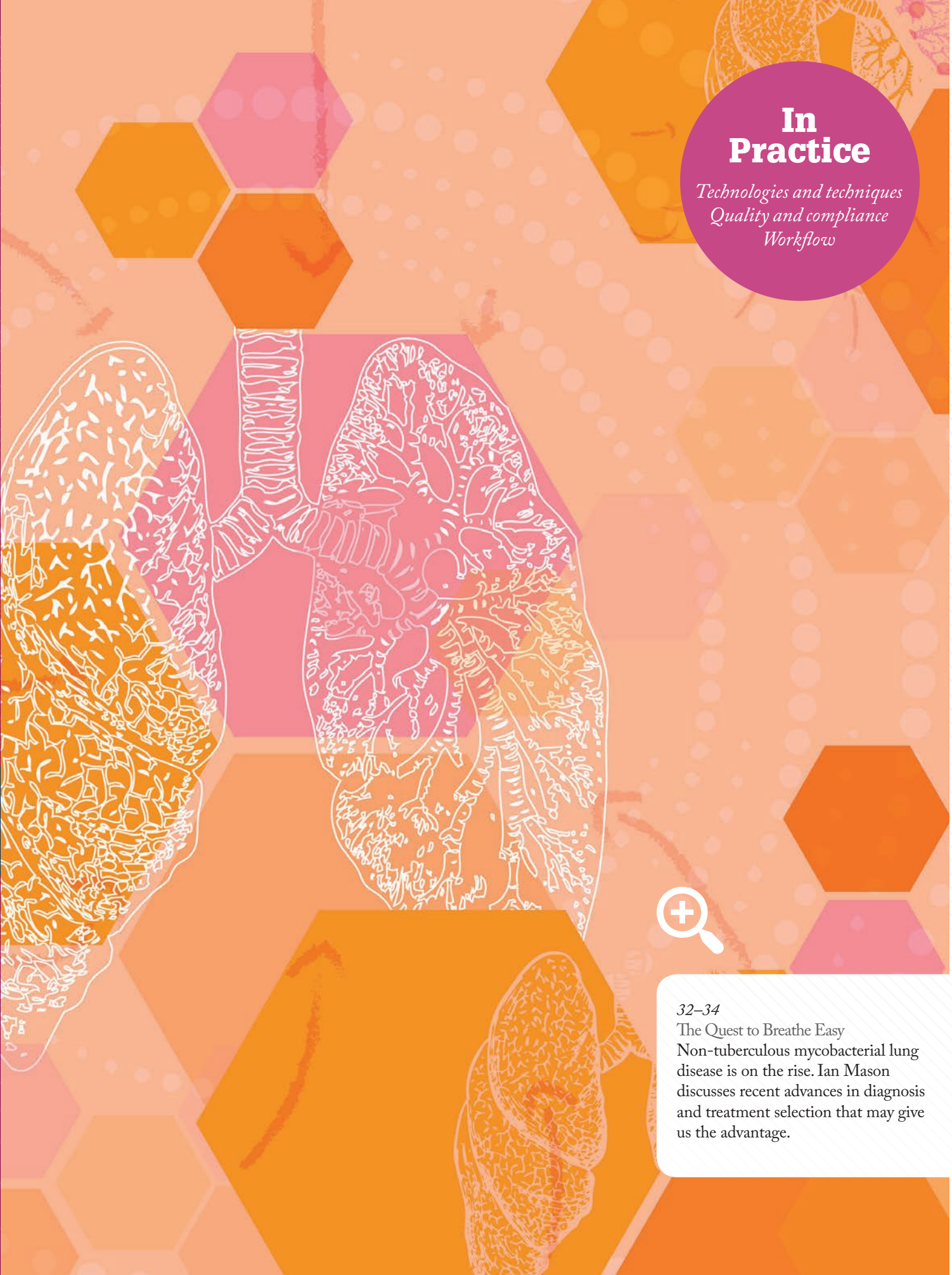


The Quest to Breathe Easy

How science is meeting the challenge of non-tuberculous mycobacterial lung disease

By Ian Mason

Pulmonary infections caused by non-tuberculous mycobacteria (NTM), such as *Mycobacterium avium* complex (MAC), have significantly increased in recent years – and so has research into such diseases. The degree of attention focused on these mycobacteria, especially at the recent European Congress of Clinical Microbiology and Infectious Diseases, reflects the high level of interest in disease caused by NTM – a diverse group of opportunistic bacterial pathogens with a wide spectrum of virulence. Patients more susceptible to NTM infection include those with chronic respiratory disease, such as bronchiectasis, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and idiopathic pulmonary fibrosis (1).

"NTM are intrinsically resistant to most classes of antibiotics. They require very long periods of antibiotic treatment using three, four, or five antibiotics at the same time – with treatment durations of up to one and a half to two years. Just imagine the associated toxicity – this can be a nightmare for everyone involved," said Jakko van Ingen, head of the mycobacteriology laboratory at Radboud University Medical Center, Nijmegen, the Netherlands (2). He pointed out that NTM can pose a real problem when they cause chronic pulmonary infections in humans. "Cure rates are really poor, as low as 40 percent for M. abscessus and up to 70 percent for MAC disease. Even if you cure the patient once, there is a good chance you will see them again because the recurrence rate is around 40 percent." He suggests that, for patients who are particularly susceptible to NTM, new treatment approaches may be warranted. "Current treatment regimens are not based on pharmacokinetic and pharmacodynamic science but rather on case series – on tradition. For all of these reasons we started our research group to use science to find better regimens with better outcomes."

Using a "hollow fiber" in vitro model, his group has shown that many treatment methods currently in use are inadequate – some because they fail to sterilize, others because they are only effective at intolerably high doses. As a result, van Ingen's group and many others are looking at new medications and combination therapies, as well as potentially giving old drugs new life as NTM treatments.

At a Glance

- *Lung infections caused by non-tuberculous mycobacteria are on the rise globally*
- *New research has revealed challenges including antimicrobial resistance, gene variants, and refractory disease*
- *It's important to identify causative pathogens before selecting treatment – but this sometimes presents a challenge of its own*
- *Drug susceptibility testing is variable and requires good external quality assurance*

A global challenge

But even the best treatment for NTM disease is of no use without good diagnostics. Emmanuelle Cambau of Lariboisiere Hospital's National Reference Centre for Mycobacteria in Paris, France, stressed the importance of correctly identifying the species and subspecies of NTM responsible for pulmonary infection and of testing for resistance mutations – particularly in the *erm(41)* and *rrl* genes – before starting a macrolide. She emphasized the need for more research to establish the difference between colonization and infection, and between relapse and reinfection. Cambau also highlighted the need for effective new antibiotics to combat infection.

> *"Identifying the causative organism and its genetic characteristics are a key part of providing appropriate care."*

Charles Daley of the University of Colorado, Denver, USA, said that treatment of NTM lung disease (NTM-LD) should be based on three factors: patient, organism, and goals of treatment. MAC lung disease treatment should include a macrolide-containing three-drug regimen administered for 12 months beyond culture conversion. Aminoglycosides may be added for cavitary or macrolide-resistant disease, and inhaled liposomal amikacin (currently only approved in the US) may be added in treatment-refractory cases. Treatment of *M. abscessus* LD should include at least three active drugs with inclusion of a macrolide when a non-functional *erm(41)* gene is present or when gene status is unknown. These conditional treatment options make it clear that identifying the causative organism and its genetic characteristics are a key part of providing appropriate care.

Reviewing some challenging or difficult cases, Miguel Santin of Bellvitge University Hospital, Barcelona, Spain, recommended an observational period

for progression before starting therapy in patients with NTM-LD (nodular/bronchiectatic disease). He also advocated surgery as a reliable alternative for patients with refractory NTM-LD – but reinforced the importance of therapy following current guidelines. Prior to making a treatment decision, an observational period and appropriate testing can identify which infections are most likely to require a non-standard course of action.

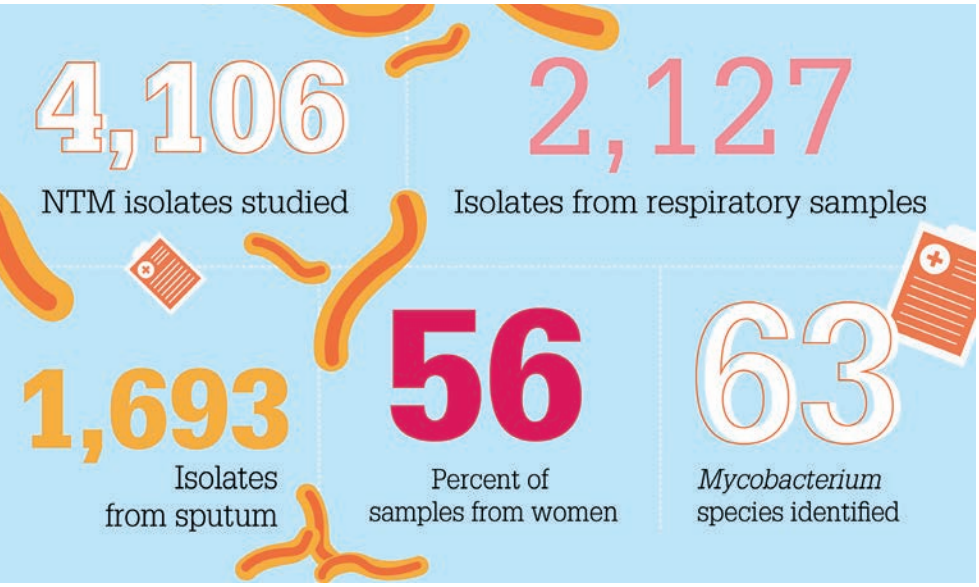
The changing epidemiology of NTM

On behalf of a network of clinical microbiology laboratories from 21 Madrid hospitals (Grupo de Estudio de Micobacterias-SMMC), Jaime Esteban-Moreno reported the results of a five-year, multi-center study of NTM epidemiology. Collectively, the hospitals service a population of more than six million people, and were therefore able to include a total of 6,306 mycobacterial isolates (4,106 NTM and 2,200 tuberculosis) in their research. Although tuberculosis numbers remained stable throughout the study, NTM isolates were not – after a period of stability from 2013 to 2016, they increased from 2016 to 2017 (3). Of the total NTM isolates, MAC represented almost half; rapidly growing mycobacteria were the second-most common, followed by *M. lentiflavum*. Altogether, the researchers identified 63 different species.

Esteban-Moreno and his colleagues plan further studies to establish the clinical significance of the increase in NTM isolates. "We need to know more about the epidemiology because there is no mandatory reporting of NTM infections in Spain. Our take-home message is that NTM isolates are increasing in frequency – now, we need to know more about the patients to find out whether these represent colonization or true clinical infections." He believes that several factors may be responsible for the identified increase, including a growth in the number of susceptible patients with chronic lung disease or environmental factors.

Effective and inexpensive NTM identification

In the five-year study, the researchers identified NTM isolates using commercial molecular biology systems – namely, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). But they're not the only group that sees the potential of MALDI-TOF MS. Katrien Vandebroek and her colleagues at Ziekenhuis Oost-Limburg, Genk, Belgium, found the technique to be an effective and inexpensive tool for the identification of NTM species (4). To reach this conclusion, they grew eight strains of the bacteria on Löwenstein-Jensen agar and used MALDI-TOF MS to identify them; all eight were correctly identified. They also achieved correct identification in 89.8 percent of Mycobacterial Growth Indicator Tube (MGIT) clones. Identification of positive MGITs from clinical samples proved more difficult; however, using a higher MGIT volume for protein extraction improved performance to 92



percent. Some hurdles still remain, of course. Not every species is equally easy to identify; in Vandebroek's study, the *M. chimaera–intracellulare* group proved most difficult. Coinfections were also noted to interfere with MALDI-TOF MS identification. Nevertheless, the technique's accessibility and utility offers promise for future research and diagnosis.

A call for quality assurance

The reliability and reproducibility of drug susceptibility testing (DST) of NTM isolates needs to be improved, according to Vladyslav Nikolayevskyy, the author of a recent study on the subject (5). Nikolayevskyy, of Public Health England, London, UK, presented a multi-center pilot study of a novel external quality assurance (EQA) scheme for NTM susceptibility testing. The study employed a structured questionnaire, followed by a pilot EQA round using identical panels of 10 well-characterized *M. avium* and *M. abscessus* abscessus isolates.

The panels were sent to 22 participating laboratories to be tested for susceptibility to clarithromycin, moxifloxacin, amikacin, linezolid, and doxycycline. EQA results were received from 16 laboratories using the broth microdilution method. Essential agreement ranged from 78.8 (amikacin) to 96.2 percent (linezolid) for *M. avium*, and from 76.0 (amikacin) to 100 percent (doxycycline) for *M. abscessus*. Categorical agreement ranged from 56.8 (moxifloxacin) to 100 percent (clarithromycin) for *M. avium*, and from 53.6 (linezolid) to 100 percent (doxycycline) for *M. abscessus*. These results show that inter-laboratory reproducibility for NTM phenotypic DST is insufficient, highlighting the need for expanding EQA schemes to clinically relevant NTM.

"The take-home message is that, as things currently stand, the reproducibility can be considered suboptimal," said Nikolayevskyy. "This is very important for laboratory accreditation – every laboratory needs to demonstrate that they are proficient and that they participate in external quality assessment schemes [...] There has been a lot of interest [in these results] not only from the UK, but also from the EU, continental Europe, and globally. Drug susceptibility testing is being increasingly used because the prevalence of NTM is increasing globally."

Although rapid advances are being made in the understanding, diagnosis, and treatment of NTM disease, there are still clear gaps in our ability to tackle this increasing threat. With this research and more, those working to combat the disease hope that, soon, every patient will receive a rapid and accurate diagnosis – and a treatment tailored to their specific pathogen.

Ian Mason writes about medicine and science for both medical and lay publications. He is based in Surrey, UK.

References

1. *CS Haworth et al., "British Thoracic Society guidelines for the management of non-tuberculous mycobacterial pulmonary disease (NTM-PD)", Thorax, 72, ii1 (2017). PMID: 29054853.*
2. *J van Ingen, "Kill 'em all! The quest for an antibiotic regimen that cures nontuberculous mycobacterial disease". Presented at the 29th European Congress of Clinical Microbiology and Infectious Diseases; 13–16 April, 2019; Amsterdam, Netherlands.*
3. *K Vandebroek et al., "MALDI-TOF MS: a performant, easy and inexpensive tool for the identification of non-tuberculous mycobacteria". Presented at the 29th European Congress of Clinical Microbiology and Infectious Diseases; 13–16 April, 2019; Amsterdam, Netherlands. Abstract #P0432.*
4. *M Kaelin et al., "Diversity of non-tuberculous mycobacteria in heating/cooling devices: results from prospective surveillance". Presented at the 29th European Congress of Clinical Microbiology and Infectious Diseases; 13–16 April, 2019; Amsterdam, Netherlands. Abstract #P2637.*
5. *V Nikolayevskyy et al., "Novel external quality assurance scheme for drug susceptibility testing of non-tuberculous mycobacteria: a multi-centre pilot study". Presented at the 29th European Congress of Clinical Microbiology and Infectious Diseases; 13–16 April, 2019; Amsterdam, Netherlands. Abstract #P0983.*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Outpacing Resistance

The laboratory needs accurate, reproducible methods for selecting cancer treatments and spotting resistance early

By Lisa Jensen-Long

Drug developers have created cancer therapies that can target specific cancer types, often defined down to a single genetic mutation or protein biomarker. These can be incredibly effective against the right cancer – but matching the right therapy to the right patient continues to present a challenge. Single markers and data points cannot fully identify the broad dynamics of cancer and, for a significant number of patients, these precision treatments often fall short in their promise to deliver a positive outcome.

At a Glance

- *New cancer treatments are either directed toward genetic variations in individual patients or toward harnessing the patient's immune system*
- *Many patients don't benefit from these therapies because scientists do not yet understand cancer biology sufficiently well to know whether a particular therapy will work*
- *Most current diagnostic tools are not simple, reproducible, or accurate enough to enable physicians to consistently develop optimal treatment regimens*
- *Droplet digital PCR assays can efficiently and reliably measure cell-free circulating tumor DNA (ctDNA) in liquid biopsies, enabling clinicians to make therapy decisions rapidly and in real time*

Surgery to remove tumors is often the first-line treatment for patients, but one in five patients experience complications during the surgery that can diminish its benefits (1). Additionally, patients may undergo chemotherapy – a standby of cancer treatment since the end of World War II – to kill remaining disease in the body. However, chemotherapy is unlikely to succeed in patients with late-stage cancer (2).

Oncologists try to tailor treatments based on the results of genetic tests performed on tumor samples, but tumors are heterogeneous; nine times out of 10, cancer evolves to resist the effects of chemotherapy (2). Tissue biopsies only capture cells from one part of a tumor – so cells in other parts may have different genetic profiles, including ones that are resistant to the oncologist's chosen treatment. Subsequently, cancer cells that resist chemotherapy survive to proliferate and mutate.

Furthermore, because tumor biopsies are invasive, they are often performed only once. As a result, oncologists routinely make treatment decisions based on a single snapshot in time, so it is difficult to predict if and when patients will start displaying signs of resistance, making timely and appropriate cancer treatment difficult. Fighting cancer can be reduced to a strategy of trial and error as tumors evolve and adapt to different drug therapies.

Another factor that affects the accuracy of tissue biopsy results is the choice of protein biomarker used. One of the most important markers used in precision cancer treatments – PD-L1 – is also one of the least reliable. Tests for expression of this biomarker, a common target of immunotherapy, are not standardized. Different labs often use different antibodies and detection methods, all of which can yield different results. This lab-to-lab variability makes it challenging for oncologists to make treatment decisions with certainty.

"Collected via a simple blood draw, nucleic acid strands floating in the bloodstream carry the same genetic information as the tumor."

Proactively treating cancer

Oncologists have adopted many approaches that enable them to routinely monitor patients' responsiveness to cancer treatments. Imaging techniques, such as computerized tomography (CT) and magnetic resonance imaging (MRI), allow doctors to monitor a tumor's size and location. These techniques can serve as rough proxies for whether a treatment is working, but don't provide any direct information about treatment resistance and are prone to false positives. For example, a working immunotherapy may appear to be failing because the treatment causes inflammation that makes tumors temporarily appear larger.

For decades, oncologists have employed blood tests to detect protein markers associated with cancer – like carcinoembryonic antigen (CEA) – as a means to track cancer progression. Although these tests can be administered on a serial basis, they do not directly measure genetic changes. Consequently, they are not as specific as genetic tests and are prone to false positive and false negative results.

To solve that problem, clinicians can employ molecular diagnostic tests like next-generation sequencing (NGS) to monitor the genetic profiles of tumors based on variants found in ctDNA. Collected via a simple blood draw, nucleic acid strands floating in the bloodstream carry the same genetic information as the tumor. Clinicians can use this information to create targeted therapies based on the tumor's genetic profile. NGS is comprehensive, highly sensitive, and invaluable for identifying novel mutations that impact the trajectory of a tumor and its responsiveness to treatment. But although NGS yields a comprehensive list of genetic variants in a tumor, it has significant drawbacks: the technique is costly, labor-intensive, and complex, and results often take weeks to receive. Results are also subject to significant variability among different panels and laboratories.

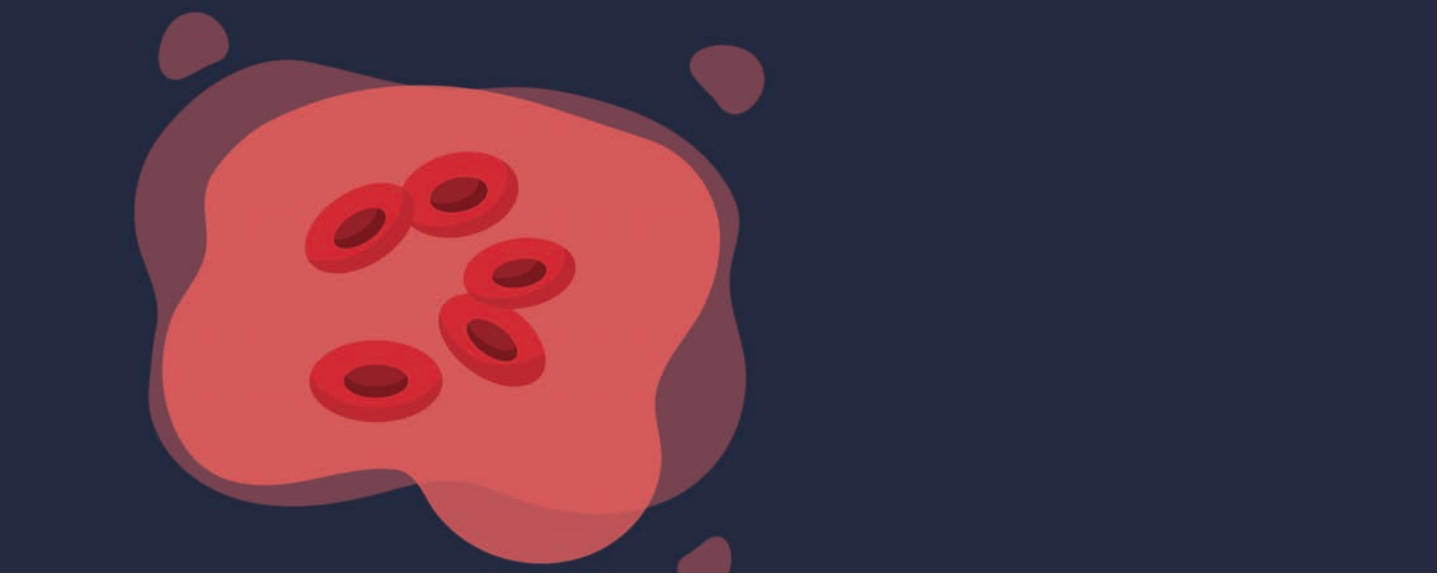
An alternative approach

To monitor for tumor progression more quickly and efficiently, clinicians can add droplet digital PCR (ddPCR) technology to their toolkit. This liquid biopsy technique can complement NGS by providing highly sensitive and reproducible biomarker information about a tumor. Many laboratories already use ddPCR to validate NGS results due to the methodologies' complementary benefits and the lack of need for library construction prior to performing ddPCR analysis. Research from several recent phase III clinical trials shows that ddPCR can provide oncologists with a rapid, reliable and cost-effective method to both predict and track the effectiveness of therapy.

Data from one such trial shows that quantifying ctDNAs in a liquid biopsy using ddPCR technology is a more accurate method for tracking treatment resistance than quantifying DNA derived from tissue biopsies (3). Sara Tolaney and colleagues at the Dana Farber Cancer Institute looked at two endocrine therapy resistance genes, *PIK3CA* and *ESR1*, in patients with HR+ metastatic breast cancer.

To compare the performance of tissue and liquid biopsies, the researchers assayed 334 plasma samples and 434 formalin-fixed, paraffin-embedded (FFPE) samples, respectively, from a total of 669 patients treated with fulvestrant or fulvestrant and abermaciclib in combination. They found that the concentration of *PIK3CA* and *ESR1* among ctDNAs in plasma correlated with resistance to abermaciclib, but did not find this correlation among FFPE samples (3). These data suggest that ctDNA from liquid biopsy may provide more reliable detection of resistance to endocrine therapy in HR+ metastatic breast cancer.

In a different phase III clinical trial for endocrine therapy in patients with ER+ breast cancer, Francois-Clement Bidard used ddPCR technology to track the onset of *ESR1* mutations to inform the design of the trial (4). Using liquid



biopsy tests prior to treatment, after one month after treatment, and every two months thereafter, Bidard observed the emergence of *ESR1* mutations as patients showed signs of treatment resistance. Based on his real-time monitoring, he was able to randomize these resistant patients to a new group for whom alternative treatment may provide better patient outcomes. This interventional study addresses one of the most pressing questions concerning the clinical utility of blood monitoring: does it benefit the patient to act on molecular changes before clinical symptoms are evident?

Liquid biopsies using ddPCR technology can also assist in predicting whether or not patients with malignant pleural mesothelioma will benefit from cancer surgery. This cancer is especially common in the elderly; according to the American Cancer Society, two-thirds of patients are 65 or older (5). Consequently, surgery carries heightened risks associated with age – such as arrhythmias, pneumonia, and loss of lung function – that can diminish or cancel out surgical benefits (6). Knowing whether or not surgery might provide a significant benefit can help patients avoid pointless and risky operations.

One indicator for this is plasma ctDNA concentration as measured using ddPCR technology. To test this, Luke Martinson and colleagues at the University of Leicester, in a proof-of-principle study, designed a ddPCR liquid biopsy for mesothelioma patients based on mutations found in tumor tissue using whole exome sequencing (7). Using pre-surgical blood, the investigators examined patients' ctDNA concentrations to predict the outcome of their surgeries. They discovered that patients survived for a significantly shorter duration following surgery if they had detectable ctDNA in their blood beforehand. This study suggested that ddPCR technology may be used to assess the risk-benefit ratio of subjecting a patient to cancer surgery.

ddPCR's potential clinical role

Liquid biopsy has already found its place in the clinic, employing tools such as DNA sequencing to provide a noninvasive view of the genetic and phenotypic nature of a patient's cancer. These tools provide additional context to tissue biopsy and imaging, which are standard of care in cancer detection and monitoring.

ddPCR technology complements these techniques by enabling a physician to capture genotypic and phenotypic cancer information through DNA (or RNA) biomarkers found in blood. After identifying actionable mutations using NGS, an oncologist can reliably track a patient's ctDNA levels using droplet digital PCR technology to monitor disease status and response to treatment. These data can help oncologists adjust their patients' treatments over time, increasing the patient's survival and quality of life.

Lisa Jensen-Long is Vice President of Marketing, Digital Biology Group, Bio-Rad Laboratories, Pleasanton, USA.

References

1. *SL Wong et al., "Variation in hospital mortality rates with inpatient cancer surgery," Ann Surg, 261, 532 (2015). PMID: 24743604.*
2. *ML Ashdown et al., "Chemotherapy for late-stage cancer patients: meta-analysis of complete response rates," F1000Res, 4, 232 (2015). PMID: 26834979.*
3. *SM Tolaney et al., "Clinical significance of PIK3CA and ESR1 mutations in ctDNA and FFPE samples from the MONARCH 2 study of abemaciclib plus fulvestrant". Poster presented at the AACR Annual Meeting 2019; March 29–April 3, 2019, Atlanta, USA. Abstract #4458.*
4. *F-C Bidard, "Clinical utility trials for CTC and ctDNA in ER+ advanced breast cancer". Presented at the AACR Annual Meeting 2019; March 29–April 3, 2019, Atlanta, USA. Abstract #SY31-02.*
5. *ACS Staff, "Risk Factors for Malignant Mesothelioma" (2018). Available at: https://bit.ly/2NcC6mG. Accessed June 24, 2019.*
6. *ACS Staff, "Surgery for Malignant Mesothelioma" (2018). Available at: https://bit.ly/2YcjLYb. Accessed June 24, 2019.*
7. *LJ Martinson, et al., "Personalized circulating tumor DNA profiling in malignant pleural mesothelioma". Poster presented at the AACR Annual Meeting 2019; March 29–April 3, 2019, Atlanta, USA. Abstract #1349.*

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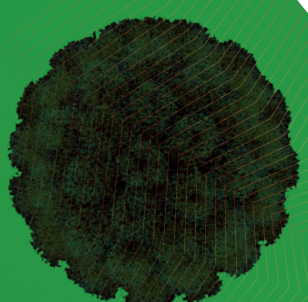

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Lessons Learned, with Jeanie Martin

From completing kidney transplants throughout the night during the Northern Ireland conflict to receiving an MBE for her work in biomedical science, Martin describes her journey and reflects on the evolution of tissue typing

By Jeanie Martin

My career in the lab began in 1964. I worked as a scientific assistant in veterinary research, which exposed me to a broad range of biomedical science disciplines. After eventually deciding to specialize in hematology and blood transfusion, I joined the Northern Ireland Blood Transfusion Service in 1966 where I became an associate of the Institute of Biomedical Science in Hematology and Blood Transfusion. In 1976 – after a short break to have my second son – I began working in Histocompatibility and Immunogenetics (H&I), also known as tissue typing. For the next 25 years I participated in the "wet work" aspect of the 24/7 on-call service, while also serving as lab manager from 1989 until my official retirement in 2011. I returned to work part time and, due to the fact that the Belfast Trust H&I lab was without a Head of Department, I took on the role of Interim Clinical Lead from 2015 to 2018.

One of the most important aspects of this job – and of my career – is working on kidney transplant matching, which I've seen evolve considerably. After starting out by using complement dependent cytotoxicity crossmatching and then moving toward flow cytometry, we now try to maximize the use of virtual crossmatching technology for patients awaiting deceased donor transplantation. Our ambition has always been to reduce the amount of time it takes for the kidney to reach the recipient once a donor has been found. Whenever a patient in intensive care is identified as a potential donor and permission is given, the first step is to conduct human leukocyte antigen (HLA) typing on the donor. That data is then sent to a central location in the UK, where it is used to select the best match for that kidney. Once the donor dies, the retrieval and transportation process can take several hours, and by the time a crossmatch has been completed with the

At a Glance

- *It is vital to minimize the time taken to conduct a kidney transplant once a donor has been found*
- *Technological advancements such as virtual crossmatching have moved the goalposts for patients who can receive a transplant*
- *Developing a close relationship with transplant clinicians enables laboratorians to develop a personalized approach to patients*
- *Laboratories have gone overboard with the amount of documentation required, which can detract from the important work*

recipient – which can take six hours in itself – there can be a 15–25 hour wait between donor selection and transplantation.

A virtual crossmatch made in heaven

Our solution to this, like that in other centers around the UK, has been to conduct extensive work on all our patients before a donor is even identified. With virtual crossmatching, we are able to use solid phase and bead-based HLA antibody identification assays to predict the outcome of a physical crossmatch, so that we can decide whether or not to accept a kidney donor based on the predicted risk of transplantation. Rather than wait six hours for a physical crossmatch to be completed on the day of transplantation, we can move forward with the transplant as soon as the kidney arrives in our center. Over the past few years, we have consistently had the lowest cold ischemia times (the period between the deprivation and restoration of blood supply to the organ) for donation after circulatory death in the UK, which is due to successful optimization of the technique in our unit in Belfast. Other units are catching up with us now, because H&I is such a specialized field that all of the laboratories in the UK collaborate effectively; if one lab develops something, everyone else quickly takes it on board.

The impact of virtual crossmatching for patients is huge because they can receive transplants sooner than they previously would have. The shorter cold ischemia times mean that donated kidneys work much more quickly – and the transplants are more successful. We now have extremely low failure rates in the people who have virtual crossmatches, and patients without additional health issues do particularly well. It has rapidly taken off across the UK; almost 90 percent of Northern Ireland patients were transplanted with a virtual crossmatch in 2018.

For the deceased donor transplant program there is a lot of pre-work involved, which is all about trying to predict what is going to happen on the day of the transplant. Some patients will never be suitable for virtual crossmatching, but we can list the various HLA antigens that aren't acceptable for the patient due to the presence of HLA antibodies, as well as considering those that will be suitable. When I started working in tissue typing 43 years ago, we were using very basic serological techniques; now, we are able to carry out high-resolution HLA typing to characterize HLA alleles within the patient and define detrimental antibodies to a similar level. It's amazing how far the field has come.

"When I started in the 1970s, we would never have transplanted patients older than 50 – but today, we regularly transplant patients who are well into their 70s."

The transformation of tissue typing

There have been several major milestones in the evolution of tissue typing, the biggest of which is the transition from serological HLA typing in the 1970s to the molecular methods we see today. Using serological techniques to determine a patient's HLA antibodies and to crossmatch potential donors was extremely labor-intensive. The introduction of flow cytometry for crossmatching gave us a much more sensitive technique; however, we still use both approaches because in some instances it is possible to transplant with a crossmatch that is serology-negative, but flow-positive. It's flow cytometry that most accurately predicts long-term survival for the more difficult-to-transplant patients. A positive cytotoxic crossmatch with a sample taken from the patient on the day of transplant is still a contraindication for transplant.

For me, the most important change is that the combination of these technologies has allowed us to move the goalposts for the patients we can transplant. For example, when I started in the 1970s, we would never have transplanted patients older than 50 – but today, we regularly transplant patients who are well into their 70s. It's very rare for us to come across a person who isn't transplantable (provided, of course, that they are physically capable of coping with the surgery and immunosuppressant treatment).

Although the birth of HLA typing took place in the 1950s, we only started transplanting in Belfast in 1968. Today, half a century down the line, there is a lot of talk about applying sequencing to HLA typing. Unfortunately, sequencing doesn't lend itself well to solid organ transplantation because it can't currently be performed within the ischemic time allotted for the survival of a transplanted kidney. For an intervention like stem cell transplantation, on the other hand, next generation sequencing is more suitable, so we can expect it to feature much more heavily in the future. From our point of view in Belfast, one focal point for research is to analyze masses of data that go as far back as the 1970s. We want to look at the people who have shown long-term survival after kidney transplantation in the days

when we didn't have this current level of technology at our disposal. That will allow us to investigate whether the new technology has made us oversensitive – we may, in fact, not be transplanting patients whom we would have before the advent of molecular typing and sensitive antibody screening. It will also be interesting to study the patients who survived when we didn't have such sophisticated immunosuppression available.

Clinical collaboration

We have a brilliant relationship with the clinicians in Belfast, and I think that is really important. One of my greatest achievements was developing this connection with Aisling Courtney, the consultant nephrologist, who came to the laboratory to speak to me about a particular patient. We discussed the possibility of having regular meetings to resolve issues with patients who are difficult to transplant, and that's how our personalized medicine approach for transplantation came into being. We sit down every three weeks to discuss the patients on the transplant list and talk about the tests we need to complete before a kidney becomes available for them. She has been instrumental in what I have been able to achieve; our collaboration has facilitated the implementation of personalized medicine for our patients because each one is considered on an individual basis.

This collaboration has also assisted Courtney in her quest to advance living donor transplantation in Belfast. When she was appointed in 2009, approximately eight such transplants were being performed annually. She developed a program bringing together clinicians, nurses, and laboratory staff, which has enabled the Belfast Trust to perform more living donor transplants per million head of population than anywhere else worldwide. This has led to several awards and numerous invitations to speak at conferences about Belfast's success.

I believe the importance of multidisciplinary teams should not be underestimated. These collaborations help the laboratory staff to become familiar with the clinicians and develop a better ethos throughout the lab. Although we know the names, antibody profiles, and HLA types of our patients, we would never know if we passed them in the street. By learning about their personal and social circumstances from the clinician, our work becomes much more meaningful – those little details confirm for us just how important the work is.

The main thing I've learned in my role as lab manager is that you can't achieve anything without the rest of the team

behind you. I always try to bring out the support and enthusiasm of those around me. Of course, I don't push them into doing things; rather, I encourage them to bring their own ideas to the table. For example, I always bring different people to the multidisciplinary meetings and set teams so that everyone can come to hear what is going on. I make sure that everybody is involved in that process because it's crucial to hear the opinions and views of the entire lab team. We all learn things from each other.

Troubles and triumphs

One of the toughest parts of the job is having to come to work during the night. If you get the call to say that a donor has become available – and, in years gone by, this always seemed to happen during the night – then you would have to be in the lab for seven or eight hours. That aspect is certainly difficult and, during the early days, there were very few people who worked the "on-call" rota, so I would often find myself in the lab at an unsociable hour. Now, we have 10 people on our rota and virtual crossmatching in place, so we're not called in as often to perform "wet work" crossmatches.

Most of my time on call was spent during the Northern Ireland conflict, which made my journeys through the night very difficult. I was never sure I would be able to travel without being stopped by the police or the army. Also, many of our donors came from the intensive care unit in the Royal Victoria Hospital, which was right in the thick of the troubled area. Those days were particularly difficult because we had to pick up the samples ourselves – but everyone in Northern Ireland was suffering hardships at that time, not just those of us who worked in the lab.

Despite these difficult times, there are many positive aspects of the role and I get a great deal of satisfaction from it. Being able to contribute to a patient's journey, all the way from initially understanding their individual issues and needs to getting them transplanted, is so rewarding; you feel a real connection with the patient. There's one particular young boy who sticks in my mind. He remains the longest patient we've ever had on dialysis in Belfast and four years ago, we managed to finally get him a transplant. The amazing thing is that we actually met him at an event last year; it was a great moment and we found out that he is doing remarkably well. All of the lab staff even had a photograph with him (see opposite)! It's hard to pick out particular moments because they're all special, but it's always a highlight when we achieve a successful transplant in a child.

For those considering a career in laboratory science, I would say that it's very difficult to find any other specialty that gives you as much job satisfaction as working in H&I. The fact that you work with so many different patients, while continually checking for antibodies and completing crossmatches, gives you a sense that you are truly helping someone's life along. I can't see myself doing anything else in biomedical science that would give me the same level of involvement.

Life beyond the lab

I have now worked in the lab for 54 years and, later in 2019, I will finally retire from my role as a biomedical scientist. To be honest, I am not sure how I am going to spend all the extra time. I will definitely miss the day-to-day lab routine! Although a lot of work needs doing in my garden, I am going to need to find something to occupy myself mentally – I'm thinking about doing something completely different. Perhaps I'll take a course in Ancient History at the Open University.

One thing that I won't miss about the lab is the sheer amount of documentation that is now required. I appreciate that health and safety is an important part of our work, but I think we have gone overboard and may be focusing too much attention on whether or not a particular document is correct. It can take a lot of time, and I believe that it sometimes distracts us from the really important work we carry out. Laboratories have always been ahead of the game with safety; the people who work there are naturally focused on quality control and don't need masses of documentation to prove it – their outcomes do the talking. The amount that has to be done simply to gain accreditation is excessive, and without accreditation you can't function. Having experienced the evolution of laboratory science over the last 54 years, one thing I would change about the current system is to reduce the amount of unnecessary but required documentation.

Member of the British Empire

I was absolutely astounded and delighted when I received the award letter for my MBE; it was completely unexpected. My immediate thought was of my father – he received an MBE in the military division for his work during the war, so I have since joked with my sons that there is no pressure on them! It was a great experience to visit Buckingham Palace and meet Prince William; I feel very honored to have been recognized by my peers in this way.

I am just one cog in the wheel of a very successful team in Belfast. Although I had been the lab manager since 1989, I initially retired in 2011. But they struggled to recruit a new clinical lead for the lab, so I took over again and have been running it on a part-time basis. I think this was the main reason that I received the MBE – if I hadn't returned, then the lab would not have maintained its accredited status, thus making it difficult to function. I have worked in that lab for over 40 years now, but I just see myself as part of the overall team. If they could give an MBE to the whole team, that would be great!

Spotlight on... Technology

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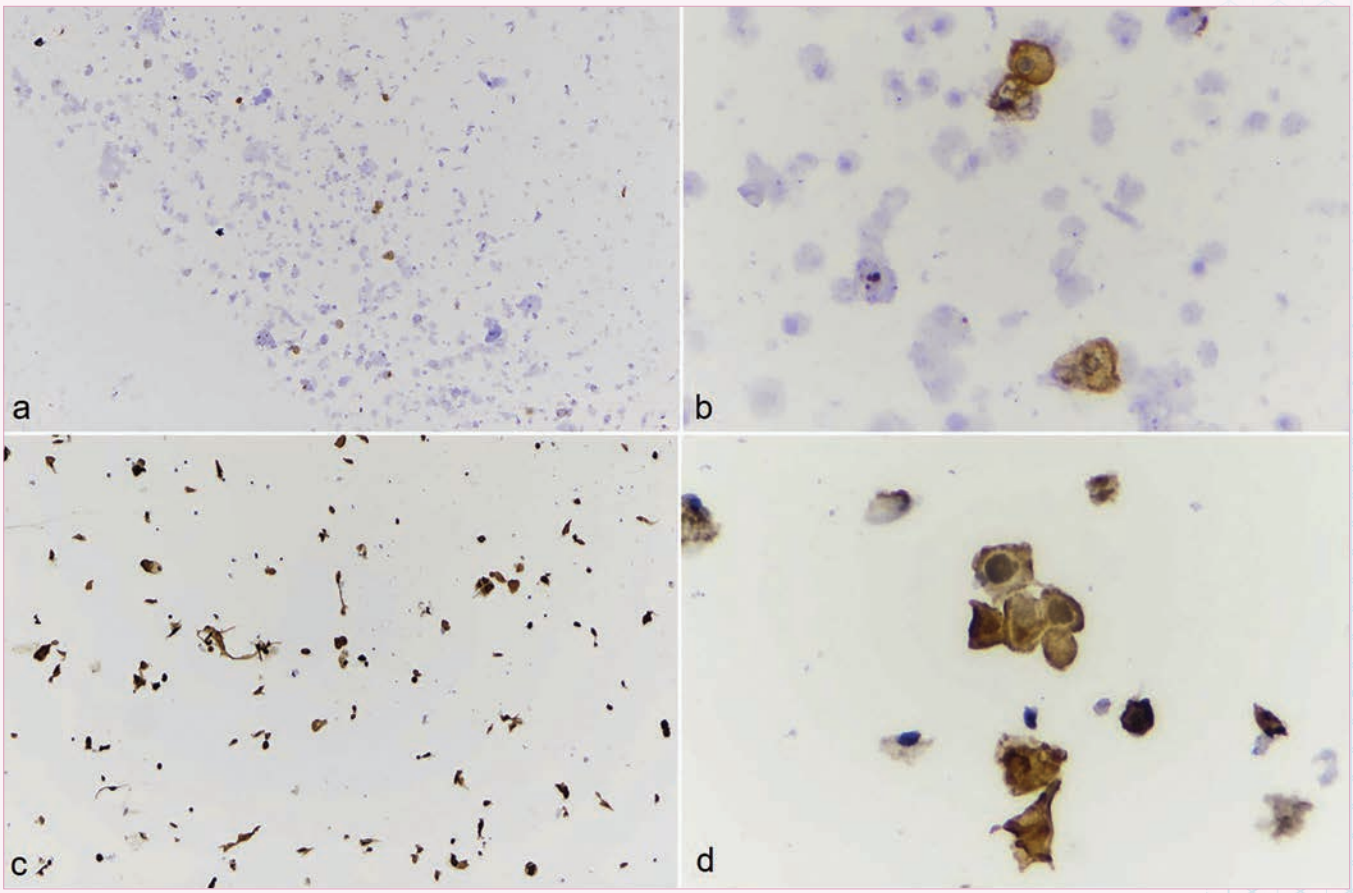

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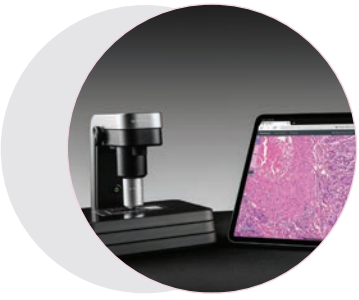

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The Truth Seeker

Sitting Down With… Bennet Omalu, President and Medical Director of Bennet Omalu Pathology, Stockton, USA

Did you always want to go into medicine?
Growing up, I never wanted to become a doctor – I wanted to be a pilot! But I grew up in Nigeria and, at the time, the societal norm was for the smartest kids to go to medical school. I didn't want to go against my parents or the tide of societal expectations, so I took the exams and began studying medicine. I actually dropped out temporarily due to some difficult personal circumstances but, once I returned, I promised myself that I would specialize in a field far removed from traditional medicine. That's how I found myself in forensic pathology!

Having said that, I love taking care of the most vulnerable in our society – the dead. My favorite part of the job is speaking to them, understanding them through the language of pathology, advocating for them, and blessing their loved ones with my findings. Through our interaction with the dead, we bless the living and enrich their lives.

You were the first to describe chronic traumatic encephalopathy and highlight its link to contact sports in 2002. Why did it take society so long to realize and react?
It's because of a phenomenon I call "conformational intelligence" (CI), whereby a person's intelligence, mentality, and perception of the environment is unknowingly controlled by the norms, traditions, and expectations of their community. I believe that CI dampens emotional intelligence and inhibits innovative or disruptive thinking. When objective evidence is presented that challenges someone's conformed mind, they engage in cognitive dissonance and reject it. Eventually, they become emotional and tribal to protect that conformity.

American football is accepted by society as America's beloved national game. If I had grown up in the US, there is no way I could have carried out Mike Webster's autopsy; I would never have saved his brain and examined it to discover chronic traumatic encephalopathy (CTE). It took an outsider without any conformational intelligence around football to think objectively. American society dismissed me when I first discovered CTE and, when I persisted, many people – including the National Football League (NFL) – reacted emotionally by calling me all types of names. Even fellow doctors rejected me and attempted to nullify my work, claiming that they were the ones who discovered CTE! In the 21st century, especially in this era of social media, we need to beware of CI because it has the potential to hold society back.

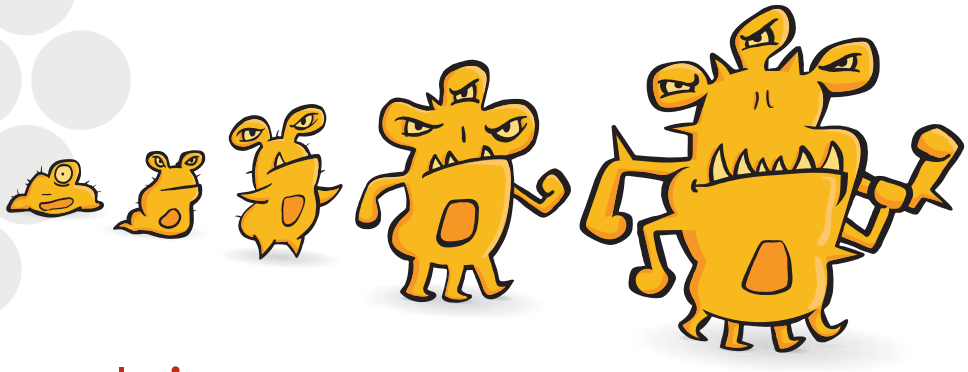
How has perception toward sports-based head injuries changed over the past decade?
I think there has been a phenomenal transformation in how people perceive high-impact sports, including football, ice hockey, and boxing – and even lower-impact sports like soccer and lacrosse. The movie "Concussion" played a crucial role in inducing this information. I call it the Will Smith effect: members of society who are better-educated and have higher socio-economic status are now removing their children from dangerous, brain-unfriendly sports. I believe that, with time, only children from lower socio-economic backgrounds will play these sports – an epidemiological trend that is already beginning to manifest itself.

Knowing what we do today, there is no reason for children to play high-impact sports. Science has shown that, if a child plays even one game of American football, there is a 100 percent risk of exposure to brain damage. The life of a child is the greatest gift to humankind and we should not degrade or undermine that life by intentionally exposing them to wholly avoidable brain damage.

How did you feel when your work on CTE was transformed into the Hollywood movie "Concussion?"
I was humbled! However, looking back now, I wish the movie had never been made, because it stole my life away from me. It's a very difficult and painful experience for people to know who you are and recognize you as you walk down the street. It can sometimes be miserable, but if the movie has impacted lives in positive ways and even saved lives, then who am I to complain? I am willing to sacrifice my life for that.

What goals do you still want to achieve?
Today, I run a successful private corporation and practice, for which I am deeply thankful. I want to continue to be myself as an African-American man despite the unique challenges we face as a cohort. Whatever tomorrow brings, I hope to enhance and restore the dignity and humanity of other human beings, one person at a time. My ultimate goal is to continue to be happy and joyful!

What advice would you give to others who wish to follow in your footsteps?
I would never advise anyone to follow in my footsteps; every person should create and follow their own unique path! Be bold, confident, and joyful in who you are and don't be afraid of the judgment of others, because you are not being yourself if everyone likes you. The greatest gift you can give society is you! Never be a conformist. The truth will always prevail – even if it takes a while – so, with everything you do, ask yourself whether you're going to be on the side of the truth. What is the truth? It's what enhances and uplifts the humanity and dignity of us all, and not just a select few or one person alone. And that's the essence of my CTE story. By using my knowledge and education to vindicate Mike Webster, I was able to uphold his dignity and humanity and that of his family. It was this mindset that allowed me to succeed. It has never been about me, but about living the truth.



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- *Listeria monocytogenes*
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  - *Streptococcus pyogenes*

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- *Candida auris*
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