MARCH 2019 # 52

# the Pathologist™

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**In My View**
Market-driven pathology residency
12 – 14

**NextGen**
Saliva: the new diagnostic fluid
36 – 39

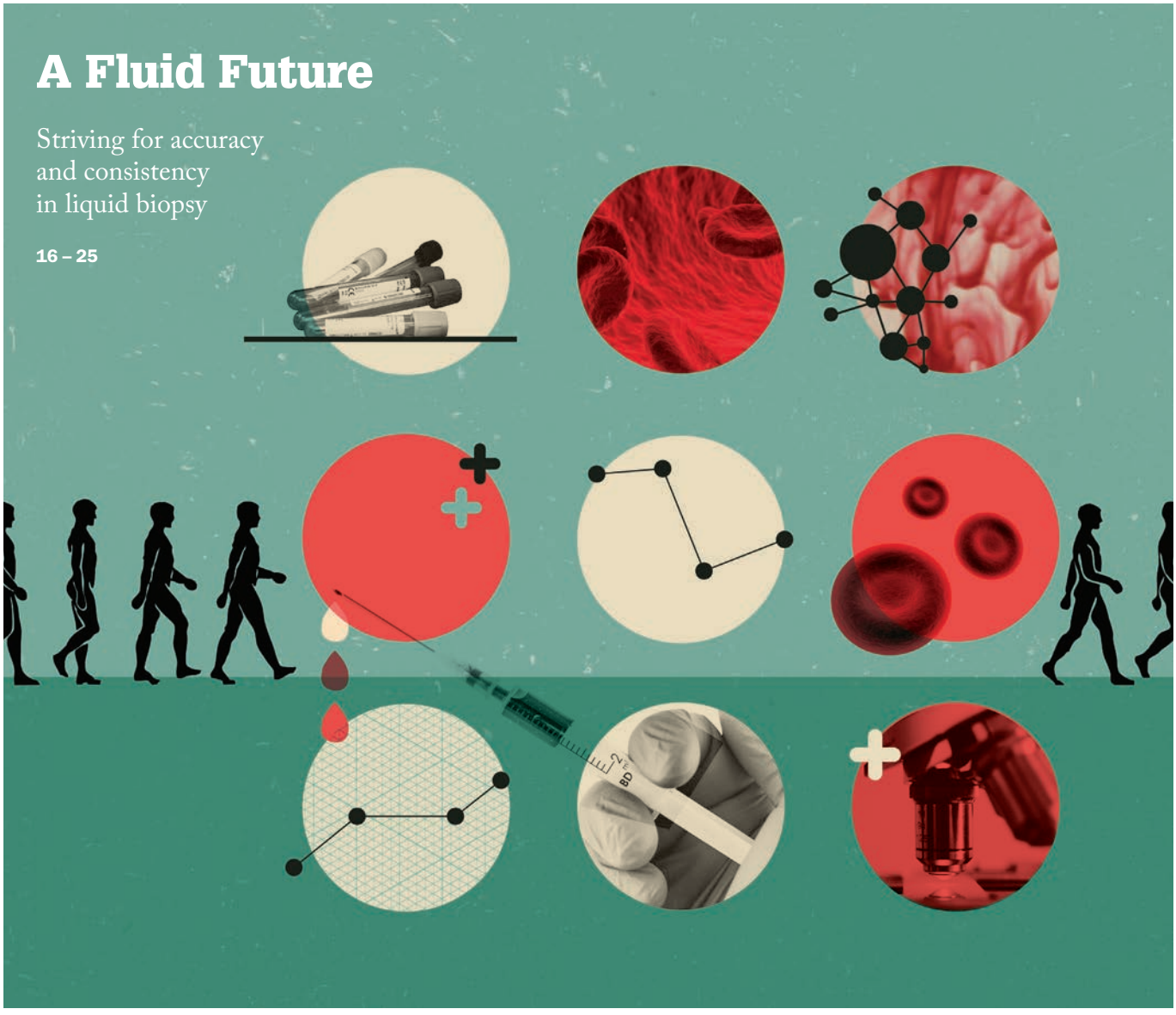
**Profession**
Career advice via social media
46 – 47

**Sitting Down With**
Chief Science Officer Dame Sue Hill
50 – 51

## A Fluid Future

Striving for accuracy and consistency in liquid biopsy

16 – 25



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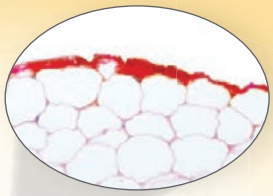

Superior

Colors show brightly on slides

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Medial

Lateral

Inks adhere throughout processing

Inferior

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USCAP 108th Annual Meeting | National Harbor, MD | March 16-21
SSO Annual Cancer Symposium | San Diego, CA | March 27-30

 References: (1) Dooley, W.C. and Parker, J. "Understanding the Mechanisms Creating False Positive Lumpectomy Margins." *American Journal of Surgery* 190 (2005): 606-608. (2) Britton, P.D.; Sonoda, L.I.; Yamamoto, A.K.; Koo, B.; Soh, E.; and Goud, A. "Breast Surgical Specimen Radiographs: How Reliable Are They?" *European Journal of Radiology* 79 (2011): 245-249. (3) Molina, M.A.; Snell, S.; Franceschi, D.; Jorda, M.; Gomez, C.; Moffat, F.L.; Powell, J.; and Avisar, E. "Breast Specimen Orientation." *Annals of Surgical Oncology* 16 (2009): 285-288. (4) McCahill, L.E.; Single, R.M.; Aiello Bowles, E.J.; Feigelson, H.S.; James, T.A.; Barney, T.; Engel, J.M.; and Onitilo, A.A. "Variability in Reexcision Following Breast Conservation Surgery." *Journal of the American Medical Association* 307.5 (2012): 467-475. (5) Singh, M.; Singh, G.; Hogan, K.T.; Atkins, K.A.; and Schroen, A.T. "The Effect of Intraoperative Specimen Inking on Lumpectomy Re-excision Rates." *World Journal of Surgical Oncology* 8.4 (2010). (6) Landercasper, J.; Attai, D.; Atisha, D.; Beitsch, P.; Bosserman, L.; Boughey, J.; Carter, J.; Edge, S.; Feldman, S.; Froman, J.; Greenberg, C.; Kaufman, C.; Morrow, M.; Pockaj, B.; Silverstein, M.; Solin, L.; Staley, A.; Vicini, F.; Wilke, L.; Yang, W.; & Cody III, H. "Toolbox to Reduce Lumpectomy Reoperations and Improve Cosmetic Outcome in Breast Cancer Patients: The American Society of Breast Surgeons Consensus Conference". *Annals of Surgical Oncology* 22.10 (2015): 3174-3183. (7) Lovrics, P.J.; Cornacchi, S. D.; Farrokhyar, F.; Garnett, A.; Chen, V.; Franic, S.; and Simunovic, M. "The Relationship Between Surgical Factors and Margin Status After Breast-Conservation Surgery for Early Stage Breast Cancer." *The American Journal of Surgery* 197.6 (2009): 740-746. (8) Gibson, G. R.; Lesnikoski, B.A.; Yoo, J.; Mott, L.A.; Cady, B.; Barth, R.J. Jr. "A Comparison of Ink-Directed and Traditional Whole-Cavity Re-Excision for Breast Lumpectomy Specimens with Positive Margins." *Annals of Surgical Oncology* 8.9 (2001): 693-704. (9) Gage, I.; Schnitt, S.J.; Nixon, A.J.; Silver, B.; Recht, A.; Troyan, S.L.; Eberlein, T.; Love, S.M.; Gelman, R.; Harris, J.R.; and Connolly, J.L. "Pathologic Margin Involvement and the Risk of Recurrence in Patients Treated with Breast-Conserving Therapy." *Cancer* 78.9 (1996): 1921-1928. (10) Menes, T.S.; Tartter, P.I.; Bleiweiss, I.; Godbold, J.H.; Estabrook, A.; and Smith, S.R. "The Consequence of Multiple Re-excisions to Obtain Clear Lumpectomy Margins in Breast Cancer Patients." *Annals of Surgical Oncology* 12.11 (2005): 881-885. | MMTHEPATH MAR 2019

# Case of the Month

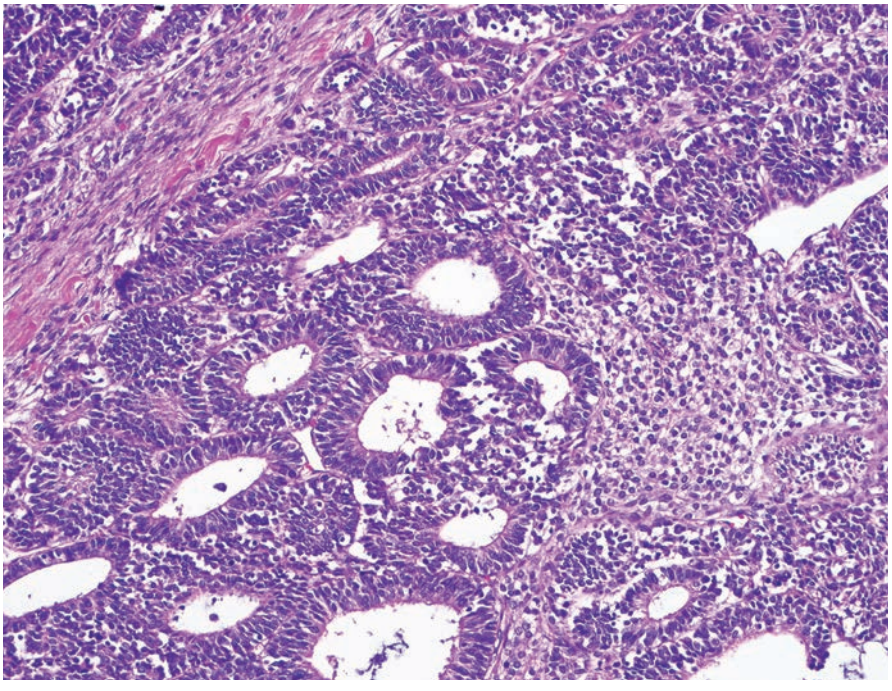
This renal tumor removed from a four-year-old child may be found in which hereditary syndrome?

a. *WAGR*

b. *Lynch syndrome*

c. *Multiple endocrine neoplasia type I*

d. *Li-Fraumeni syndrome*



## Answer to last issue's Case of the Month…

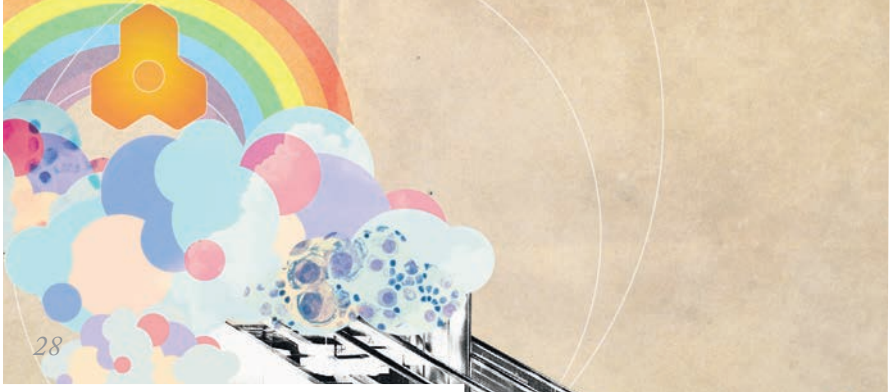
*C. Sarcomatous change*

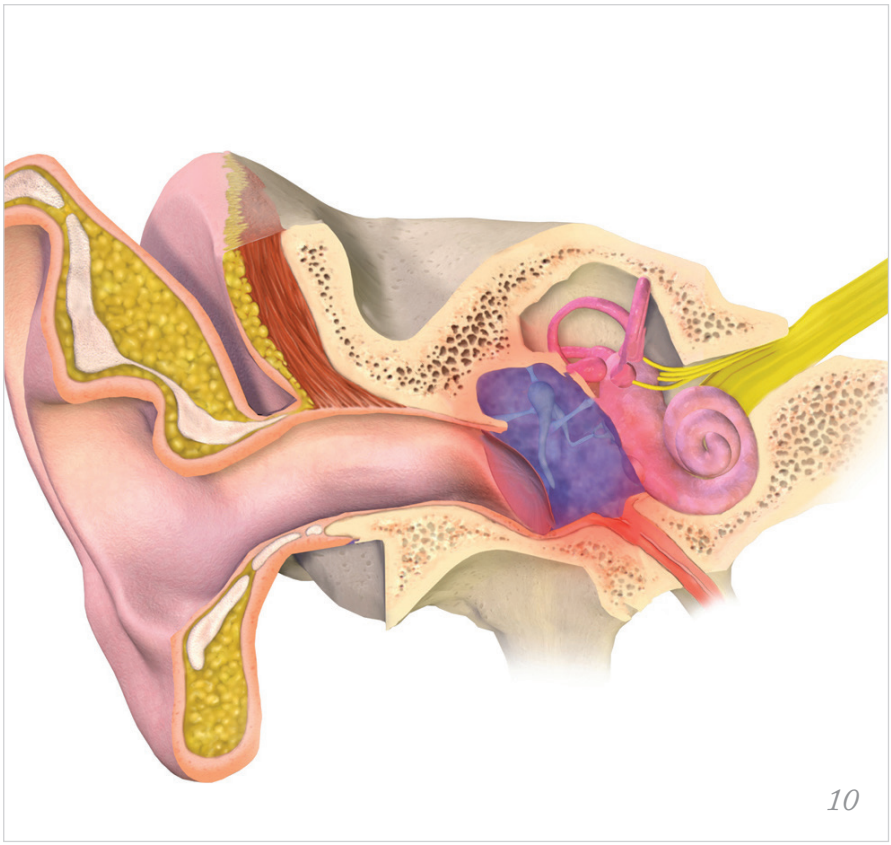
The images indicate a diagnosis of spermatocytic tumor, previously known as spermatocytic seminoma. Three morphologically distinct cell types are noted on microscopy: small cells with hyperchromatic nuclei and scant cytoplasm resembling lymphocytes; medium (intermediate)-sized cells with round nuclei, granular chromatin, and dense eosinophilic cytoplasm; and large, mono-multinucleated cells with round, indented nuclei and lacy chromatin. Sarcomatous change within a spermatocytic tumor is characterized by the appearance of undifferentiated and/or heterologous sarcomatous elements. Such tumors tend to metastasize and have a worse prognosis than usual spermatocytic tumors.

*Courtesy of PathologyOutlines.com. Case by Debra Zynger, Associate Professor and Director of the Division of Genitourinary Pathology, The Ohio State University Wexner Medical Center, Columbus, USA; discussion by Belinda Lategan, Associate Professor and Pathologist at St. Boniface Hospital, Winnipeg, Canada.*

**To register your guess, please go to http://tp.txp.to/0319/case-of-the-month**
**We will reveal the answer in next month's issue!**

# Contents






05 **Case of the Month**

07 **Editorial**
The Family and Friends Test
by Michael Schubert

## Upfront

08 Treat or Surveil

09 Scrutinizing Breast Cancer

10 Putting a Bug in Your Ear

11 Jagged Little Pill (For Cancer)

## In My View

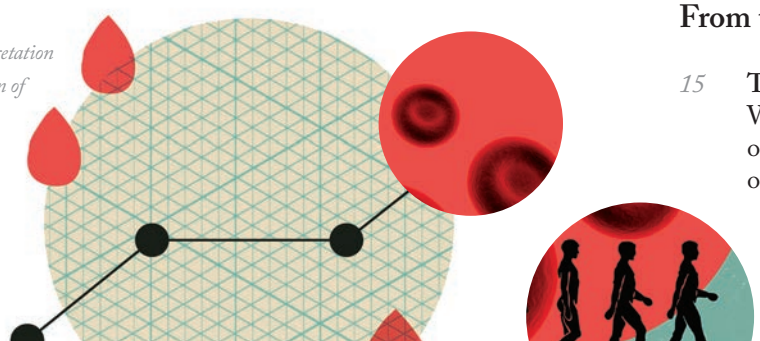
12 We hear from Michael B. Prystowsky, Jacob Steinberg, Adam Cole, and Tiffany M. Hébert about the value and relevance of market-driven pathology residency.

## From the ASCP

15 **The Importance of Giving**
We strengthen ourselves and our community when we invest our time and resources.


## On The Cover


*Artist's interpretation of the evolution of liquid biopsy*




## Feature

*16* **A Fluid Future**
Experts in the field of liquid biopsy gather to discuss the technique's strengths, its room for improvement, and its future in diagnostics.

## InPractice

*28* **Positive Steps to Tackle Triple-Negative Breast Cancer**
Breast cancer is a multifaceted disease entity, particularly in triple-negative disease. Jane Armes shares the importance of molecular subtyping for targeted treatment.

## InPractice

*36* **A Diagnostic to Drool Over?**
Blood is not our only option as a diagnostic fluid. With the average person producing one liter of saliva each day, investigating its potential seems an obvious choice.

## Profession

*42* **Peer-to-Peer with Jeffrey Myers**
One pathologist asks another to share stories of his career and the sage advice he has to offer to colleagues now and in the future.

*46* **Career Advice – In a Nutshell**
What's the most helpful career advice you have ever received? We asked, and now we share your answers!

## Sitting Down With

*50* **Dame Sue Hill,** Chief Scientific Officer for England, and Senior Responsible Officer for Genomics in the National Health Service, London, UK.



the **Pathologist**

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# The Family and Friends Test

*How do the life circumstances of laboratory medical professionals affect their perception of patients?*



I recently read a reflection by a Polish pathologist on Instagram. Roughly translated, she asked, "Does being a doctor make me a different kind of mother to my children… or am I a different kind of doctor now that I am a mother?" She noted that being a doctor made her more aware of the diseases her children might encounter – but that being a mother made her more aware of the people behind each diagnosis. "Now, looking at each patient's details, I wonder if there is a daughter or granddaughter," she wrote. "Or maybe it is a lonely father of two, or a newlywed husband."

Although not every practitioner is a parent, there are few who have no ties to family and friends at all – which is why so many pathologists and laboratory medicine professionals emphasize the need to remember that behind each sample is a person with a life and loved ones. For this mother, having children prompted her to think more deeply about each sample that came under her microscope. For others, facing a health scare with a loved one may trigger a more profound connection. Still others strive to forge a stronger link by offering contact details, conducting ward visits, or even inviting patients into the laboratory to learn more about their diagnosis.

In the past, we've had pathologists speak about the benefits of patient-centered care (1). We've also had patients write to us about their desire to interact with their pathologists (2). And laboratory medical professionals like the Instagrammer above take those ideas and run with them – because, after all, wouldn't we want our friends and family to receive that same level of concern from the laboratorians involved in their care?

How do you and your colleagues approach patient-centered care? And do you feel that your own life circumstances have affected the way you view – and treat – your patients?

**Michael Schubert**
*Editor*

*References*

1. *"Hello, My Name Is…", The Pathologist, 37, 18–29 (2017). Available at: https://bit.ly/2Sx2ViP.*
2. *S Schubert, "A Blip on the Radar", The Pathologist, 39, 15–16 (2018). Available at: https://bit.ly/2ECkR8c.*

# 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*

## Treat or Surveil

**George Vasmatzis and John Cheville explain how to harness the genetic alterations associated with prostate cancer progression**

Prostate cancer patients whose tumor is composed entirely of Gleason pattern 3 are considered to be low-risk and are often faced with a treatment conundrum. They can either select active surveillance of the tumor or proceed with surgery or radiation therapy. Results from needle biopsy specimens often don't make this decision any easier; because they only sample a small portion of the tumor, they don't reveal the full picture. To improve this diagnostic accuracy, researchers at the Center for Individualized Medicine's Biomarker Discovery Program used a next-generation sequencing (NGS) approach to identify molecular markers associated with higher disease progression risk. These insights will shed new light for patients who have to make the important decision: treatment or surveillance?

The introduction of serum prostate-specific antigen screening resulted in the detection of many cancers that were treated but were clinically insignificant. This overtreatment is one issue that we hope to address with the new molecular markers. Low-risk patients without the genetic alterations have a lower chance of disease progression and may wish to choose active surveillance instead of treatment. On the other hand, those possessing the genetic alterations may harbor a higher-grade cancer with a greater risk of disease progression, so treatment might be their best option. This knowledge won't only be beneficial for patients, though – the cost of treatment is high and a better understanding of those patients that will benefit from active surveillance rather than treatment will save billions of dollars every year.

One of the biggest challenges we faced when identifying the biomarkers was the lack of frozen tissue samples from patients with low-risk cancer. Obtaining these samples was crucial because we needed to sequence tumor cells from patients in both risk categories. Because we carry out close to 1,000 surgeries each year, we were able to locate around 50 frozen tissue sections from low-risk patients, to which we applied a specialized protocol to amplify the DNA and RNA from just a few cancer cells. We carried out mate-pair sequencing on these frozen sections and found five genes that are more frequently altered in Gleason patterns 3 from Gleason score 7 than in Gleason patterns 3 from Gleason score 6.

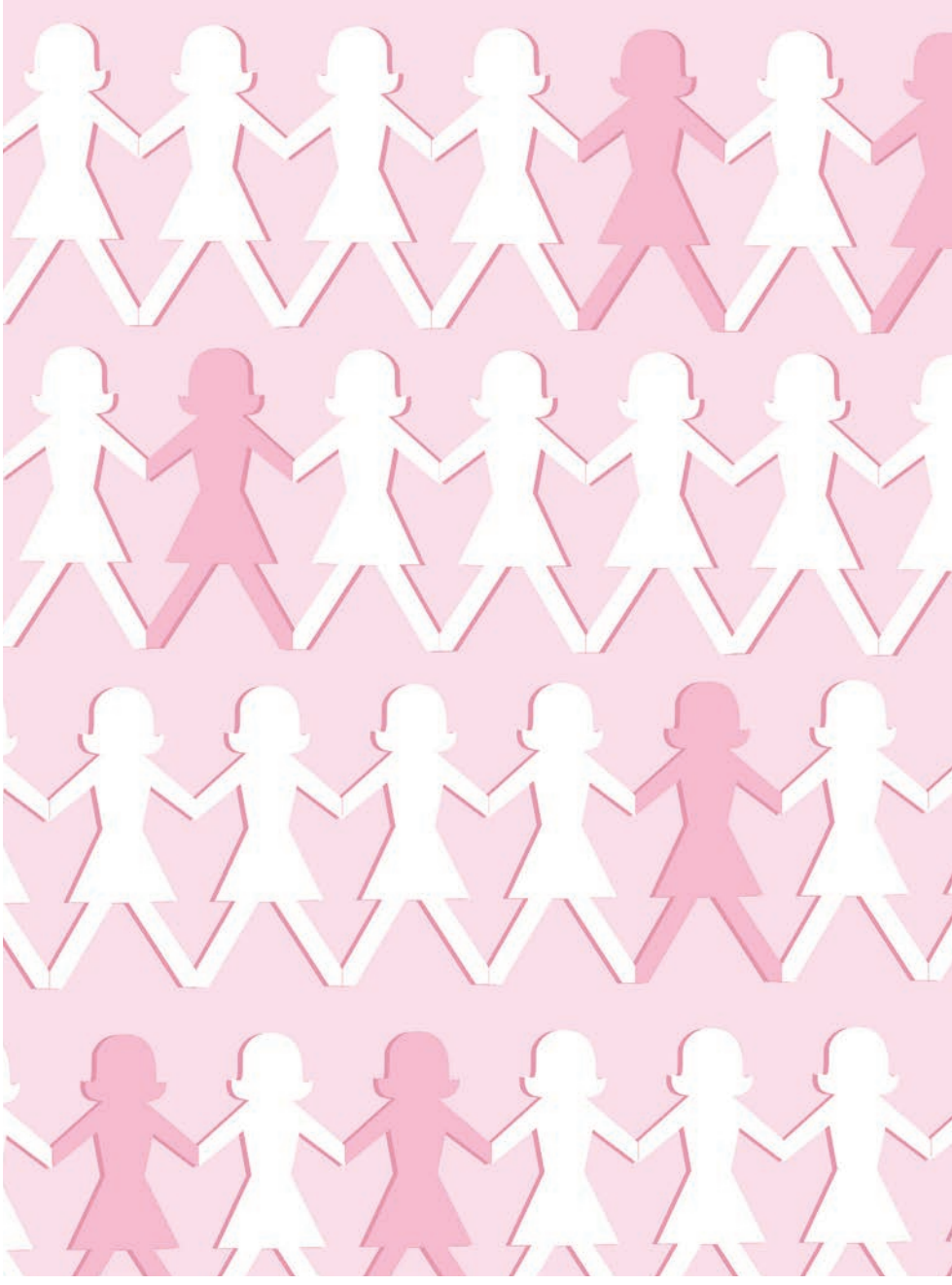
We have now converted this genetic information into a fluorescence in situ hybridization test available to patients at Mayo. This test is applied to patients that have only Gleason pattern 3 on their biopsy, and it predicts the likelihood that their prostate gland contains a higher-grade tumor that may need treatment. We are currently working to validate further biomarkers that will enhance the test's predictive power. Our ultimate goal is to provide as much information to the patient as possible, so that they can make an informed decision about the management of their cancer.

*Reference*

1. *G Vasmatzis et al., "Large chromosomal rearrangements yield biomarkers to distinguish low-risk from intermediate- and high-risk prostate cancer", Mayo Clin Proc, 94, 27–36 (2019). PMID: 30611450.*

# Scrutinizing Breast Cancer

**FLEX aims to enroll 10,000 patients and follow each one for 10 years**



If you wanted to know everything about breast cancer, where would you begin? As with many cancers, there is too much complexity to select a single aspect, such as genomics, tissue profiling, clinical history… A truly comprehensive study must capture every facet of the disease – and that's the aim of the FLEX breast cancer registry. The survey is a prospective, observational study that combines full-genome profiling with clinical data. It seeks to enroll 10,000 patients with stage I, II, or III breast cancer and follow them over the course of a decade. There are no limits on who may join the study: men and women of any age and from any ethnic group are welcome. Bastiaan van der Baan, Chief Clinical and Business Development Officer at precision oncology company Agendia, explains FLEX in more detail.

How long will the project take?

The FLEX breast cancer registry survey is set up to be a standing trial – so, as long as there are questions outstanding in relation to improving breast cancer care, it will continue to recruit patients. It collects universal clinical data and full genome data to help us understand the course of the disease and its response to therapy to find the right treatment for the right patients at the right time.

Often, involvement in clinical trials can involve extra process steps; however, FLEX has been set up in a way that makes the burden for the clinic relatively small, so non-academic sites can participate with minimal additional work. And because these sites generally see the majority of breast cancer patients, they will generate a significant amount of real-world evidence.

What kinds of gene associations do you hope to find?

The possibilities for data analysis and exploration are limited only by the population of patients entered into the FLEX registry. Because these are primarily early-stage patients with clinical details and follow-up, we can explore genomic diversity within subtypes of breast cancer. We can explore the gene signatures associated with outcomes and with sensitivity and resistance to specific therapy regimens. We can even look at the differences in breast cancer gene expression patterns between women of different genetic backgrounds, as well as a variety of clinical subsets in which there is an urgent need for in-depth genomic information.

FLEX is a US-only trial at present, but we have a very similar registry study – PRECiSE – ongoing in the Netherlands. I hope that these projects will accelerate the implementation of new insight. It still takes a lot of time to implement new diagnostic, prognostic, and predictive data – but we hope these platform trials will change that.

# Putting a Bug in Your Ear

## Gene variants may increase susceptibility to middle ear infections

Middle ear infections are one of the most common ailments of childhood. But not everyone is equally susceptible – why? A research group at the University of Colorado's Anschutz Medical Campus has discovered a way to identify those who may be more at risk. Describing for the first time the expression of the *FUT2* gene in the middle ear (1), the researchers found that levels spike within 24 hours of bacterial infection, and also that certain variants of the gene alter the overall microbiome of the middle ear.

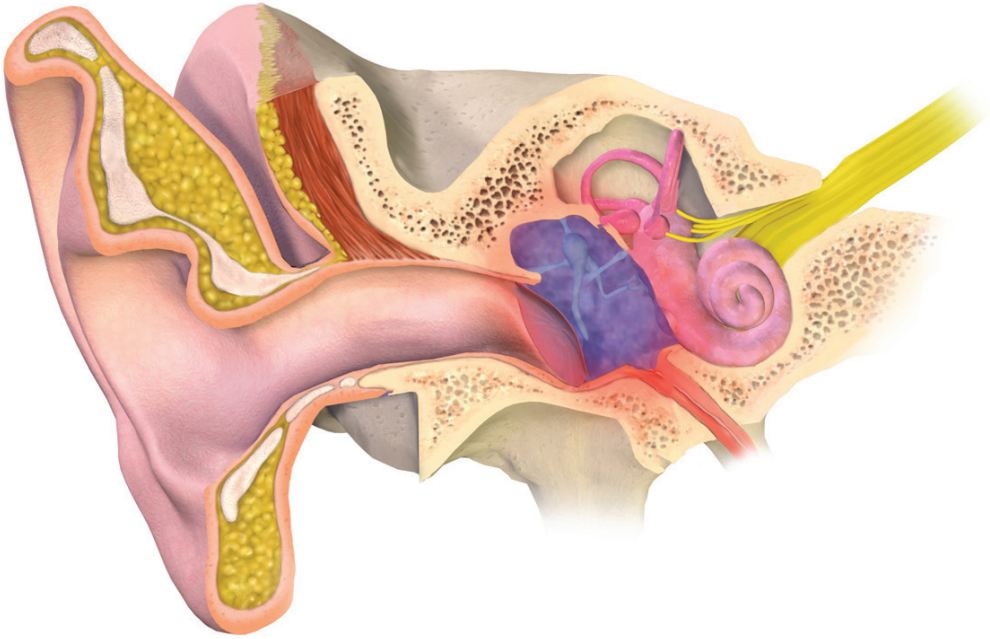
"*FUT2* was identified as a protective locus against otitis media susceptibility in a genome-wide association study (2)," says first author Regie Santos-Cortez. "However, when we looked at the sequence data from our multi-ethnic families, we saw that the *FUT2* variants conferred risk for otitis media."

How does the gene interact with the microbiome to elevate the risk of disease? "Middle ear swabs in *FUT2* variant carriers had greater bacterial diversity, meaning that the types of bacterial groups in variant carriers are different – and relatively higher in number – than in non-carriers," Santos-Cortez explains. "This suggests that the bacterial load in the middle ear, including potential pathogens, is increased in those with genetic susceptibility to disease." By decreasing the presentation of the A antigen bacteria use to enter the middle ear lining, the variants cause a shift in the microbiome, decreasing some bacterial populations and increasing others – including some known to promote chronic or recurrent infections.

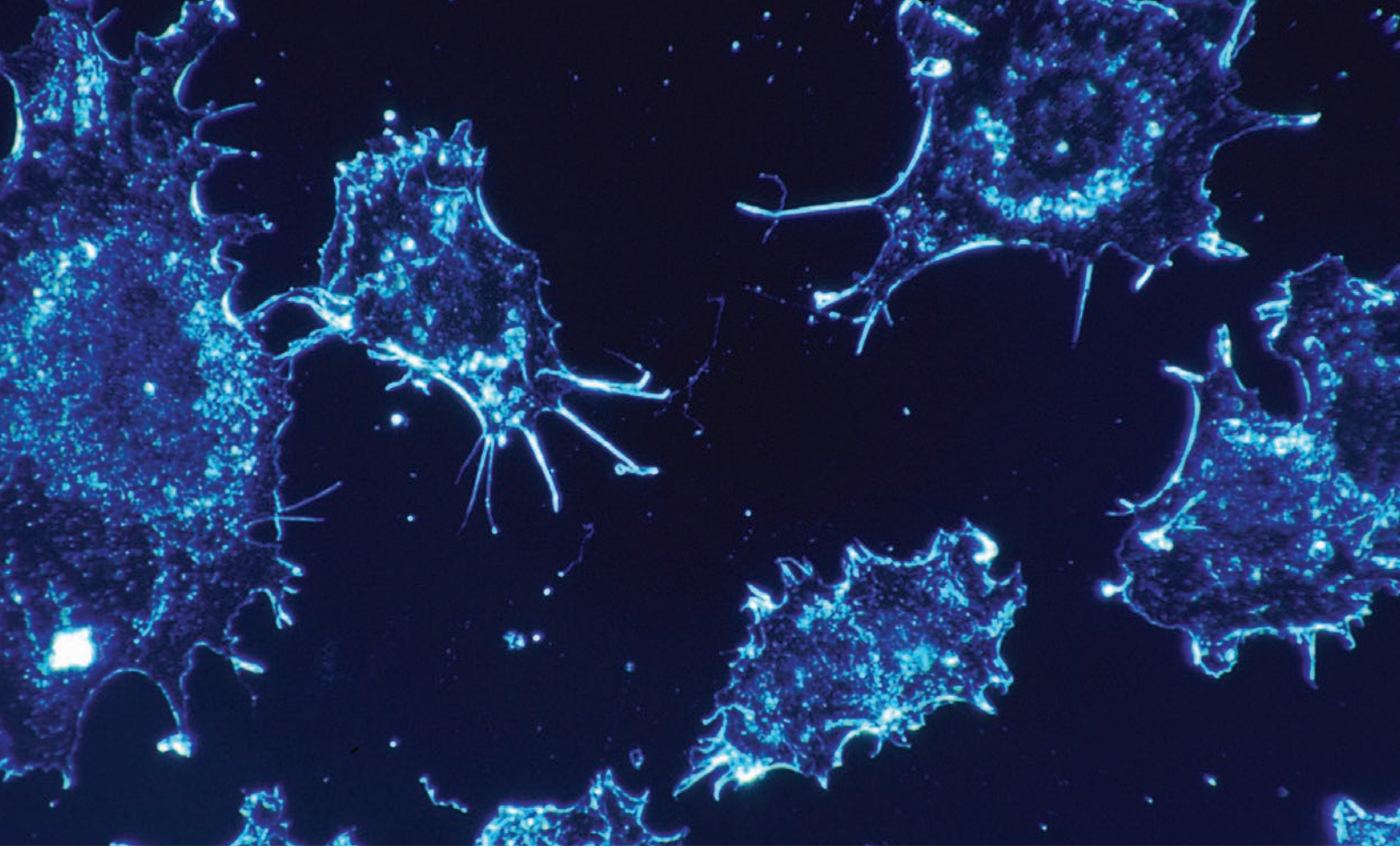
What's next? Santos-Cortez suggests that microbiota transplants might help restore the normal middle ear microbiome in variant carriers. "What we need to know at this point are the good commensal bacteria that we would want to restore to healthy levels to outcompete the pathogenic bacteria," she says. "We would also want to have more directed therapies, such as antibiotic treatments, that target specific pathogenic bacteria without affecting the healthy commensals." Meanwhile, she and her colleagues intend to continue finding genetic variants in relation to the middle ear microbiome to improve otitis media management in every population.

*References*

1. *RLP Santos-Cortez et al., "FUT2 variants confer susceptibility to familial otitis media", Am J Hum Genet, 103, 679–690 (2018). PMID: 30401457.*
2. *JK Pickrell et al., "Detection and interpretation of shared genetic influences on 42 human traits", Nat Genet, 48, 709–717 (2016). PMID: 27182965.*



*Credit: BruceBlaus*



# Jagged Little Pill (for Cancer)

**How can we use JAG1 – a small protein involved in cancer metastasis – to assess the aggressiveness of tumors and prevent clustered cell migration?**

In our quest to discover the elusive "magic bullet" for cancer treatment, tumor cell heterogeneity represents a major stumbling block. Now, though, a team that spans Rice and Duke Universities has gained further insights into JAG1 – a small protein that drives metastasis by allowing cancer cells to migrate in multicellular clusters. Lead author Federico Bocci of Rice University is hopeful that the protein, which sheds new light on tumor aggressiveness, could be an important target for therapeutic intervention.

In the context of cancer, the epithelial-to-mesenchymal transition (EMT) is considered the main mechanism by which cancer cells migrate. Epithelial cells in tumors lose their cell-cell adhesion and gain motility, which plunges them into the bloodstream to metastasize. "It was recently discovered that cancer cells can migrate not only as single cells, but also as multicellular clusters that carry a higher metastatic potential," says Bocci. "JAG1 plays a significant role in this process by enabling a hybrid epithelial/mesenchymal cell phenotype characterized by both cell-cell adhesion and motility."

JAG1 belongs to a class of ligands called Jagged, which, along with Delta ligands, bind to a receptor known as Notch in a signaling pathway that regulates tissue morphogenesis. "Factors in the tumor microenvironment that flip the balance of power to make Notch-Jagged the prevalent signaling mode can lead to more aggressive tumors," says Bocci. The study also found that several inflammatory molecules released in the tumor microenvironment, such as interleukin 6, can increase Jagged levels in cancer cells and make the tumor more aggressive.

Targeting JAG1 significantly reduces tumor organoid growth for triple negative breast cancer in vitro (1); therefore, JAG1 is an attractive target for both diagnostic and therapeutic use. Despite the promise, the researchers note that Jagged ligands are important to the body's inflammatory system, so any potential therapy would need to focus on controlling the level of Jagged, rather than removing it completely. Meanwhile, Bocci is focusing on the immediate future: "Experimental investigation must validate the role of JAG1 in vivo before efficient therapeutic strategies can be developed."

*Reference*

1. *F Bocci et al., "Toward understanding cancer stem cell heterogeneity in the tumor microenvironment", PNAS, 116, 148–157 (2019). PMID: 30587589.*

# In My View

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

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

*Contact the editor at edit@thepathologist.com*

## Answering the Call

### Adapting pathology training to meet the needs of 21st-century medicine

*By Michael B. Prystowsky, Professor and University Chair in the Department of Pathology at the Albert Einstein College of Medicine and Chairman of Pathology at the Montefiore Medical Center; Jacob Steinberg, Professor of Pathology at the Albert Einstein College of Medicine and Director of Pathology Residency Training at the Montefiore Medical Center; Adam Cole, Assistant Professor of Pathology at the Albert Einstein College of Medicine and Associate Director of Pathology Residency Training at the Montefiore Medical Center; and Tiffany M. Hébert, Associate Professor of Pathology at the Albert Einstein College of Medicine and Associate Director of Pathology Residency Training at the Montefiore Medical Center, New York, USA*

Professional opportunities to practice pathology vary worldwide. In Europe and the Middle East, most positions are for either an anatomic pathologist or a laboratorian directing clinical laboratories. Residency training reflects these opportunities. Although these career paths exist in the United States, there is also substantial opportunity to practice pathology in a community hospital – a job that requires expertise in both anatomic pathology and laboratory medicine. As a result, approximately 85 percent of pathology residents seek board certification in both disciplines.

A recent workforce analysis of American pathologists forecasts a shortage of pathologists in the next five to 10 years with current practice modes (1). The shortage results from a growing population of elderly patients with chronic diseases, a "retirement cliff" of pathologists, and no growth in training programs. The average pathology trainee takes five to six years of training, including four years of residency and one or two fellowships. Such training most often yields a subspecialist pathologist who naturally desires to practice their subspecialty – but that generally doesn't appeal to a small (10 or fewer pathologists) community practice that needs an adaptable generalist with subspecialty expertise (2,3).

The question of how to train pathologists to practice healthcare based on 21st-century medicine has been a frequent topic of discussion. A group of us at a recent pathology retreat took the initiative to develop a conceptual plan that redefines pathology training in line with the requirements of the Accreditation Council for Graduate Medical Education and the American Board of Pathology (4). Parts of this conceptual plan have long been in place at Montefiore Medical Center; the rest has evolved over the past three years.

Numerous surveys of pathologists (in practice and in training) reflect what respondents think was most useful in their training, which is usually determined by the skills they use in practice (5). But although practice is an important parameter, it does not fully reflect the value of training in uncommon practice areas (such as autopsy) or the communication skills required to be an integral member of a healthcare team (6). With this concept in mind, we posed two key questions:

What should be the practice capability of our graduating resident? And how do we train our residents to be competent, without fellowship training, to practice in any given setting?

To address both questions, we developed a unique training plan. It begins one month prior to the new resident's arrival with an online onboarding exercise that includes a refresher in histology, basic principles of laboratory testing, fundamentals in quality management, and acculturation to Montefiore and New York City. The preparation frees up the first few months at Montefiore for anatomic pathology processes rather than on revisiting medical school.

Let's digress for a moment and consider a specific example: the value proposition of the autopsy as a training exercise for pathology residents. Although practicing pathologists may do few or no autopsies post-training, autopsy pathology lays the groundwork for comprehensive diagnostic capability. A successful post-mortem examination requires the resident to process the clinical history (including laboratory- and imaging-based diagnostic tests), to conduct gross and histopathologic analyses, to synthesize the findings, and to communicate them to the healthcare team. This process then segues into surgical pathology training. We expect the resident to review the clinical history before receiving the specimen, perform the diagnostic process, and render a diagnostic opinion that they report in writing and communicate verbally to the healthcare team, both directly and in conferences.

But how can we expect new trainees to perform competently if we immediately give them a full workload? They can do more – at least initially – if we treat them like technologists or pathology assistants. The problem? When we do that, we risk simply deploying them to make up for staff shortages. At our institution, we're more interested in helping our residents become useful colleagues in the later years of training, so we have developed a teaching service in surgical pathology that works toward that goal from day one.

*"We're interested in helping our residents become useful colleagues in the later years of training."*

Each resident works as an apprentice with an experienced pathologist on a subspecialty service. They are given light caseloads until they master the process of working up a case, at which point the caseload is gradually increased until the trainee can perform a full day's work. The residents get to that point much more quickly than in traditional training because they are in a less stressful guided learning environment. Likewise, they become comfortable with different specimen types more quickly when they have focused training in that subspecialty, as opposed to encountering specimens as they're dispersed through a generalist surgical pathology service. By having just one pathologist teach using a single tissue type, we eliminate stylistic differences and the nuances of different tissues, enabling residents to focus on learning the basic process of making a diagnosis. As a result, the time to competency and the need for remediation are greatly reduced.

The first two years of our program focus on process and incorporating fundamental practices (7) – including basic principles of laboratory medicine (taught in the third month via a one-month chemistry rotation), communication skills (honed through filmed presentation exercises), and the ability to perform a data-driven quality management study using a unique decision support tool. The third year of residency begins to transition the resident into the role of a pathology consultant. The rotations integrate anatomic pathology and laboratory medicine, and some even place the resident on a clinical team (such as the thyroid clinic or infectious disease rounds). The entire fourth year is elective.

In our effort to make our residents market-ready, we're also enhancing our mentoring program, which is still a work in progress. In year one it functions more as a coaching program, ensuring that each resident is on track with their learning goals. It's our view that residents are forced to make career choices much too early in their training; we believe in the need to mentor and advise our trainees when it comes to choosing a fellowship. It's our hope that helping residents design individualized fourth-year programs tailored to particular job opportunities will increase their fellowship opportunities and future job eligibility. For now, the fourth-year program augments the training experience for those with a particular fellowship in mind. For example, if a resident knows she'll be doing a gastrointestinal pathology fellowship, but also knows that she wants a community practice job, she may spend more time in other surgical pathology subspecialties and specific laboratory medicine rotations during her fourth year.

There are several key factors that we considered during the development

*"We're finding that our current crop of trainees are more satisfied than their predecessors and are performing at a higher level in a shorter period of time."*

of our program, which we feel are significant to the future of pathology training in general:

1. Initiating an open discussion with faculty based on the frank acceptance that we could do a better job at training. Incorporating frequent and timely feedback into our daily routines improves both resident progress and our ability to identify areas where we can intervene to help them. Likewise, providing residents with more one-on-one time with faculty allows us to better tailor learning experiences to an individual resident's performance level.
2. Defining the essential skills and capabilities required for our graduating residents. Using the pathology residency training competencies, milestones, and entrustable professional activities as a guideline, we focus on imparting the foundational practice habits and processes that we feel are necessary to succeed in any practice milieu. Basic practices – such as knowing how to evaluate a patient's clinical history and correlate it with clinical and laboratory values, how to construct a cogent and succinct pathology report, and how to communicate with colleagues on the healthcare team – are the foundation of our training program. Such a strong foundation also gives trainees the skills needed to adapt to changes in practice and content that they may encounter later in their career.
3. Drawing on the collective faculty and resident experience to design a trainee-centric program with agreed-upon, desirable outcomes in mind, rather than a program aimed at the short-term goal of filling staff shortages. Investing in our pathology assistant staff is key to reaching this goal in surgical pathology. As such, we continue to increase our complement of assistants with an eye toward hiring those who are interested and invested in resident education. As residents move beyond the introductory months to their third- and fourth-year rotations, we give them more opportunities for graduated responsibilities. These include clinical-laboratory liaison in clinical rounds, frozen section hot seat, junior attending rotations in surgical pathology, and expanded duties, such as test utilization approval and transfusion medicine call responsibilities.

We're finding that our current crop of trainees are more satisfied than their predecessors and are performing at a higher level in a shorter period of time. Even those who have not personally benefited from our curriculum can see the advantages; nearly two-thirds of residents surveyed who went through our old curriculum stated that they would choose to go through on the new curriculum if they were to start residency again. Residents view the fourth year as an opportunity to complete one or more "mini-fellowships" with the added bonus of graduated responsibilities throughout the year. We're hopeful that this focused training will produce more confident, market-ready residents with the requisite adaptability to work within any practice setting – and we call upon other institutions to take up the same challenge.

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# The Importance of Giving

## When we give of ourselves, we and our professional community grow stronger

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

Burnout is a well-known pitfall within our profession. We work nights, weekends, and holidays; we're on call for emergencies; we cover extra shifts for sick colleagues. We're integral to the overall healthcare system, but often receive little public recognition. In some professions, that's a recipe for disillusionment and apathy. But in pathology and laboratory medicine, our personal wellbeing is an integral component of providing optimal patient care, so how can we combat burnout?

Perhaps paradoxically, the answer is actually quite simple – by giving. You may say, "I give so much of myself already! How can giving more help keep me from experiencing burnout?" Here's a case in point: if, as the Dalai Lama suggests, the purpose of our lives is to be happy, and that happiness comes from our own actions, then we are responsible for our own happiness. If we overlay the Dalai Lama's words onto a mission statement for ourselves and our profession, it would be: when we give of ourselves, then "we" – the team – become stronger.

*"By giving our time, resources, and money, we help those in need while also helping our profession soar."*

What might investing in our profession look like?

- Dedicating yourself to education; for example, continuing education for yourself or passing your knowledge on to your colleagues, residents, and medical laboratory science students. You can write chapters or even textbooks in your area of expertise. You can author journal papers, magazine articles, and contribute to medical laboratory blogs to increase awareness and knowledge of the field.
- Becoming a mentor. It might be as simple as introducing yourself to a new colleague and offering to have coffee once a month to discuss career goals and concerns. It can be as involved as partnering with a professional society or medical school to create a mentoring program for new professionals. Or perhaps you'd feel more comfortable joining a mentoring program that already exists. Mentoring benefits both parties; the mentee gains professional advice, while the mentor is reminded why they chose this profession.
- Volunteering. Whether it's planning Lab Week activities for your colleagues or applying for opportunities within your professional society (you can find ways to become involved with ASCP at tp.txp.to/ASCP/volunteer), volunteering is a great way to meet people and generate enthusiasm for the profession.
- Financial giving. You don't have to donate a million dollars to have an impact. Whether it's donating textbooks and reference books to your laboratory, sponsoring a journal subscription for the library, or giving money to organizations such as the ASCP Foundation (tp.txp.to/ASCP/foundation), every little bit pays it forward.

By giving our time, resources, and money, we help those in need while also helping our profession soar. At the same time, we fight burnout within ourselves and our community, which makes for a better workplace, ensures a solvent culture, and generously invests in the future of our profession. It's yet another way we're stronger together.

# A FLUID FUTURE

Why are there inconsistencies between different liquid biopsy tests – and how can the field evolve to maximize accuracy and impact?

Advances in disease profiling over recent years have opened new doors in the quest for early diagnosis and personalized treatment, with metastatic prostate cancer being a case in point. As the most widespread malignancy affecting men in the USA – 174,650 new cases and 31,620 deaths are predicted for 2019 – prostate cancer research is crucial (1). One such breakthrough is the use of liquid biopsy tests to detect circulating tumor DNA (ctDNA) in patients' blood, offering a minimally invasive method of disease profiling.

The field is still young, but already there is a wide range of tests that all claim high performance. And that's no surprise; the global liquid biopsy market is predicted to grow from US$310 million in 2016 to a staggering $1.2 billion by 2023 (2). With respect to prostate cancer, fierce competition for an early foothold in the market has led to a flurry of commercially available liquid biopsy tests in the US, all with the same ambition: to improve diagnosis and management.

Metastatic prostate cancer is a highly heterogeneous malignancy associated with a wide range of potentially actionable mutations (3). The application of next-generation sequencing (NGS) techniques to primary tumors has begun to identify potential biomarkers for prostate cancer detection and characterization. These targets include circulating tumor cells (CTCs) and ctDNA, which reveal unique and complementary information about the tumor.

Liquid biopsy can be used in a variety of ways with solid malignancies – to detect actionable mutations, to inform treatment decisions, to monitor treatment response or measure efficacy, to detect disease recurrence, and to identify resistance mechanisms (4).

Although ctDNA appears to hold great potential in monitoring and profiling the evolution of tumors, a study by Gonzalo Torga and Kenneth Pienta compared two commercially available liquid biopsy tests – Guardant360 (Guardant Health) and PlasmaSELECT (Personal Genome Diagnostics) – and reported high levels of incongruence (5). We spoke to four experts to unpick the inconsistencies, explore areas for improvement, and ask where liquid biopsy is headed.


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## WORK IN PROGRESS

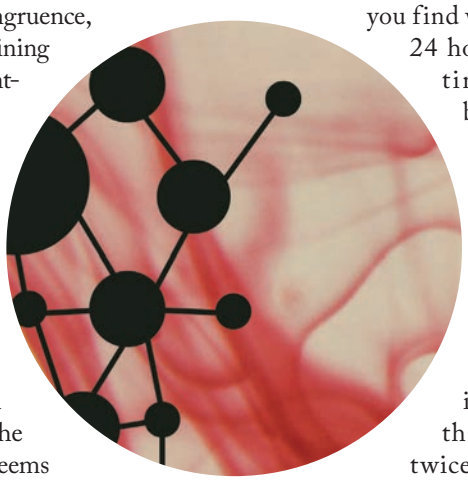
Gonzalo Torga is a postdoctoral fellow at John Hopkins Medicine who conducts translational research while developing new therapies for prostate cancer.

### Why did you compare the two tests?

Actually, that wasn't our original plan; it stemmed from wanting to know which of the two tests worked best. We were considering how to best serve patients in the clinic when my boss told me about two new, commercially available platforms that used targeted NGS of ctDNA to detect actionable mutations. I simply asked, "Which is the most accurate test for our patients?"

### What did you find?

First, we only considered genetic alterations that both platforms claimed to cover, which amounted to 42 different genes. Of the 40 patients in our study, 12 showed complete congruence between the two tests, six had partial congruence, and 16 had no congruence at all. The remaining six patients were not evaluable for patient-level congruence (5).



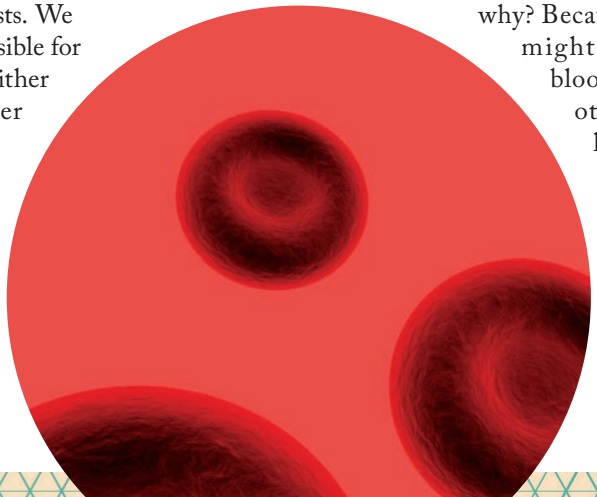
One of the problems we encountered was the high number of reported mutations in the samples. The results showed approximately a 40–50 percent mutant allele fraction, so I contacted the companies and asked them to check whether this was due to germline DNA or from the tumor itself. Although germline DNA mutations can be used to make treatment decisions, the issue is that the PlasmaSELECT test seems to report germline mutations as somatic mutations, giving rise to the divergence between the two tests. We believed that germline contamination was responsible for the relatively high allele fraction; however, neither company was able to confirm for us whether germline DNA was influencing the results.

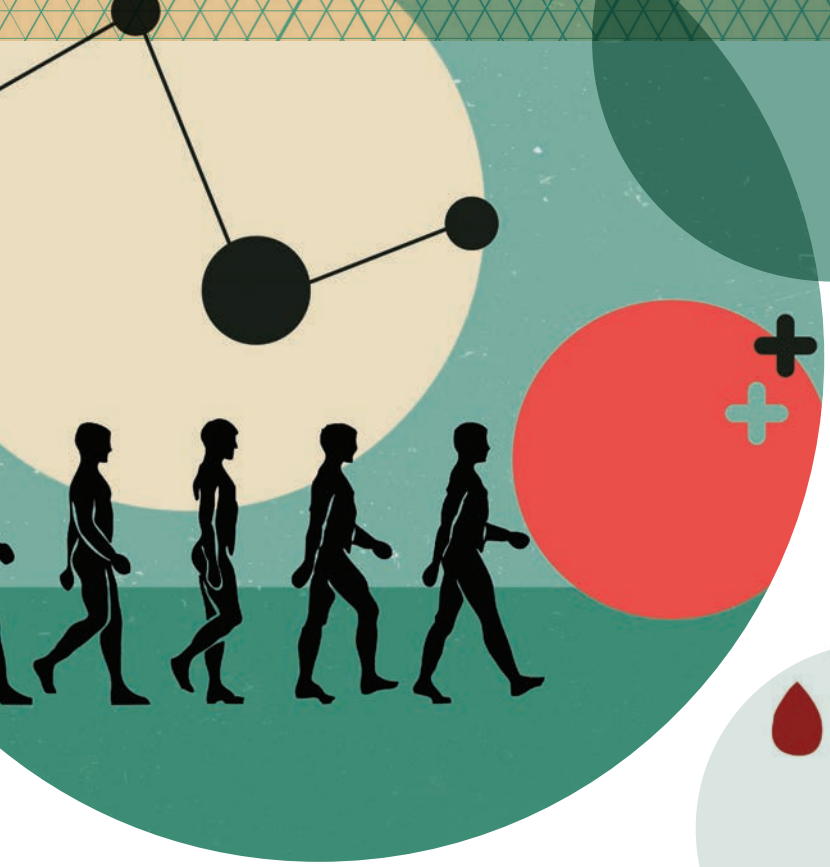
The concerning part is that we won't be able to base any clinical decisions on these tests until they can either rule out germline contamination or report germline mutations as such. Only then will we be able to trust the consistency of the results.

### How could this inconsistency affect patients?

At the time of our study, we were trying to select patients for clinical trials. However, one of the big problems was that the tests have different turnaround times – one takes two-to-three weeks, whereas the other takes four weeks. You can imagine the difficulties this caused. For example, when we were about to take a patient forward for a clinical trial based on the results of one test, the results from the other test would come back with notability different results from the same blood sample. This was obviously controversial and prompted us to put these results into a paper, because you simply can't make any reliable clinical decisions with this level of uncertainty.

Other problems currently impeding the development of the tests are processing times and methods. Different blood processing methods have a strong influence on the levels of cell-free DNA (cfDNA) and ctDNA and this should be considered when evaluating ctDNA in peripheral circulation. This could mean that if you analyze DNA after it has been processed for 12 hours, the mutations that you find will be different to those that appear after 24 hours. Consequently, the long processing times currently associated with liquid biopsy tests result in inconsistencies when reporting mutations. This disparity could represent an important roadblock for the standardization of these tests (6). I also found that, although these companies are confident in the tests' ability to detect mutations, the letters that they submitted in response to our paper indirectly acknowledge poor inter-assay performance. This means that, if you submit the same blood sample twice on the same day to the same company, you might still get different results – why? Because a single mutation might be present in one blood sample but not the other. The main issue here is that, if a rare mutation is present in an earlier sample but then isn't detected again at a later date, you might wrongly think that





*"A major concern with liquid biopsy at the moment is that there's no way to distinguish between benign clonal expansions and cancer by looking at ctDNA from the plasma."*

it has disappeared and conclude that a certain treatment is working. In fact, it may only mean that the second sample does not contain every mutation present in the first.

Alongside germline contamination, we believe there could have been issues with clonal expansions. These appear in the blood – especially in older people – and resemble tumor cells in terms of the mutations they carry, but they are not associated with cancer. A major concern with liquid biopsy at the moment is that there's no way to distinguish between benign clonal expansions and cancer by looking at ctDNA from the plasma. I think that one way to fix these problems would be to analyze DNA from white blood cells instead; this would reveal the patient's germline status and whether mutations have originated from clonal hematopoietic expansions. It would also mean that no further sampling is required, because white blood cells will be present in the original blood sample.

## How can the issues be resolved?

Ultimately, these tests need to be standardized. For example, when we started testing for prostate cancer, we were advised to use the same lab every time because test location affected the results. We would find differences in prostate-specific antigen (PSA) values that were purely due to technological differences between labs. Over time, those tests became more standardized, and now it doesn't matter where they are carried out. We need to reach a point where liquid biopsy tests will report the same mutations and the same mutant allele fractions every time, and I think that regulatory agencies need to play their part in achieving this standardization. At the moment, the tests are only approved by the Clinical Laboratory Improvement Amendments (CLIA); however, the ultimate ambition is obtaining approval from the US Food and Drug Administration (FDA), which can only happen if the tests demonstrate clinical utility. The American Association of Pathologists and the American Society of Clinical Oncology have recently published a joint review of these tests, contraindicating their use for monitoring or making treatment decisions at any stage of cancer (7).

## What are your hopes for the future?

The technology is exciting for the oncologists and pathologists who will carry out these tests in the future. The prospect of monitoring the evolution of a patient's treatment frequently and noninvasively is enticing – it means treatments can be changed before something goes wrong.

Alongside the development of plasma-based assays, tests are also being developed that include proteins in their analysis. The cancerSEEK test is one such liquid biopsy intended to detect early-stage cancers with high specificity to minimize false negative results. The test has shown early promise in detecting multiple tumor types and localizing cancers to their anatomic sites of origin (8).

Liquid biopsy is definitely the future; these tests will play a vital role for cancer patients. But it is clear that much more work needs to be done before they are ready for routine clinical use.

# SEEKING NEW STANDARDS

John Simmons is Vice President of Translational Medicine at Personal Genome Diagnostics.

### Why was there incongruence between the PlasmaSELECT and Guardant360 tests?

When we look at the study carried out by Torga and Pienta, several things come to mind. One of the most important issues is standardization; much of the incongruence is related to where reporting thresholds fall. The problem is that ctDNA may be present in the blood at very low levels that, for currently available tests, are near the limit of detection. Alterations at such low levels comprise very few mutated molecules and might not even be detected by the same assay after repeat testing due to sampling probability (the chance that any given sample may not contain a particular mutation). The PlasmaSELECT test therefore categorizes these mutations as indeterminate, because they are below the threshold of consistent detection. The study that found high levels of incongruence didn't distinguish between these types of mutations, meaning around half of those labeled discordant fell below PlasmaSELECT thresholds.

There is a need for standardization in this space. We're working to resolve issues with reporting thresholds by going to the FDA and other regulatory bodies. Another important factor is the clinical evidence; both of these tests are accredited by CLIA, but have yet to go through the FDA. For a diagnostic test to be approved by the FDA, you have to be able to demonstrate clinical validity. Therefore, much of the information about how and when to use the assays would be part and parcel of an FDA application – something on which we are currently working. At the moment, ctDNA tests are relatively new to the field; although we're experiencing some inevitable growing pains, I think access to this cutting-edge technology can only be a good thing for oncologists and pathologists. Our next step will be to take the test through the regulatory authorities and to hone in on clinical validity and standardization.

### How will you work toward standardization?

Standardization has many different interpretations. In our case, there are two parts to what we're trying to achieve. First, a degree of standardization comes from taking a test through the FDA's clearly defined intended use requirements; you need transparency in the analytical performance and clear definitions as to what you are reporting. Another layer of standardization that would greatly help the interpretation and comparability of ctDNA tests as a field concerns filtering approaches for germline variants and mutations associated with clonal hematopoiesis of indeterminate potential (CHIP). At the moment, there is no gold standard for applying germline and CHIP filters, and as such, you see a variety of different approaches that can ultimately lead to incongruence in reporting.

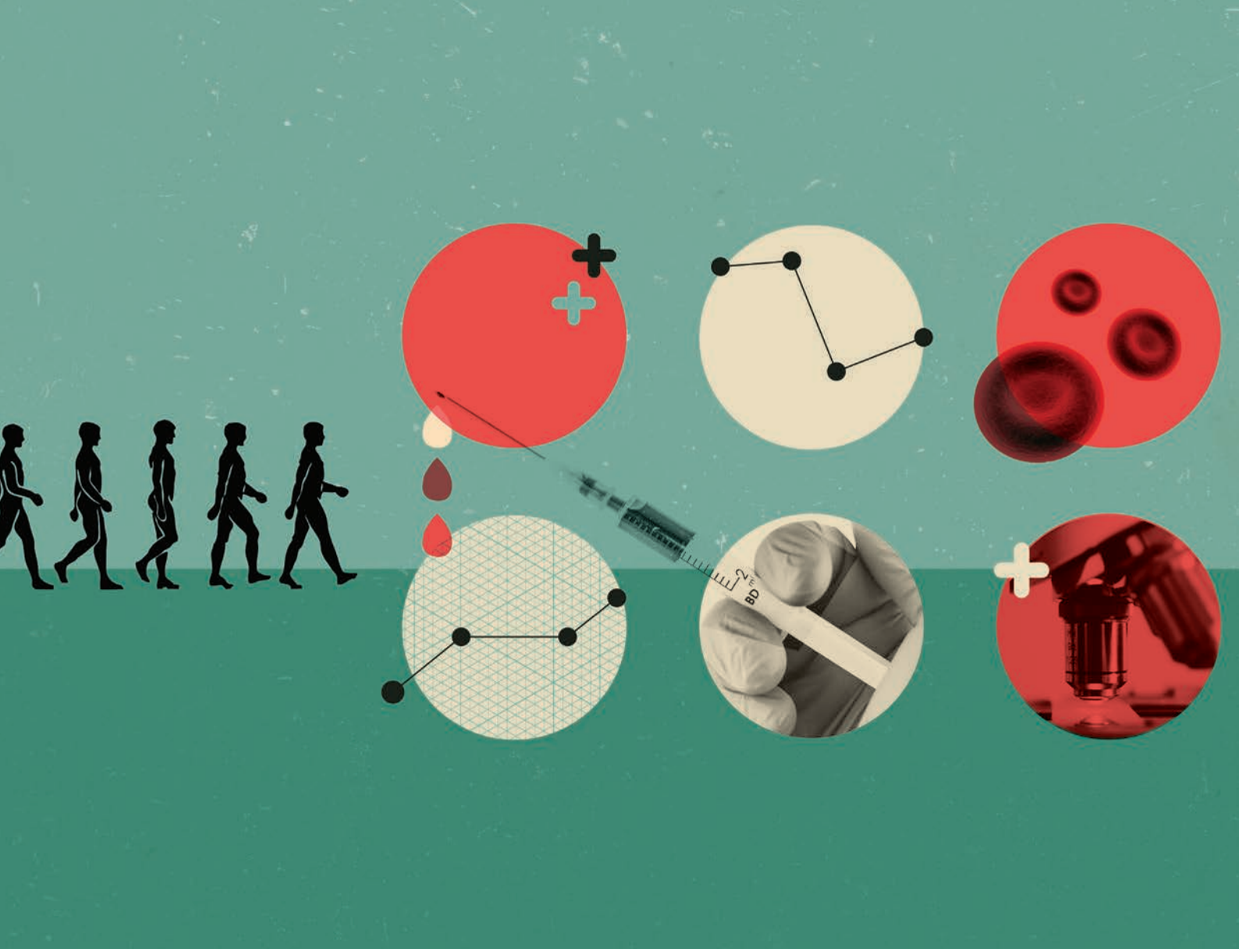
### Why do germline variants pose such a problem?

These tests use total cfDNA from patient plasma. This means that DNA from both tumor cells and non-tumor tissue is being sequenced, but as the tests are only intended to identify somatic mutations, germline single nucleotide polymorphisms (SNPs) must be removed bioinformatically, which is known as filtering. Currently, as a field there is no standardization of filtering approaches, resulting in variation from test to test. Most filtering approaches rely, at least in part, on information from publicly available databases that catalogue germline polymorphisms along with information on frequency in different populations. Even when using the same databases, different tests apply distinct filtering thresholds that can lead to incongruent reporting of rare germline variants.

There are also algorithmic approaches that can be applied beyond the use of databases, but again, these approaches vary by test and can also be confounded in cases with high levels of ctDNA in the blood. To add to the complexity, there are some drug treatments where efficacy is related to alterations in certain genes regardless of whether the alteration was germline or somatic. There are definitely technological limitations in this space, but it's also crucial for us as a community to devise some standards for germline filtering in both plasma and tissue-based tests.

### Where are you directing future research efforts?

We are using our in vitro diagnostic assays in clinical trials to demonstrate the clinical validity and utility of ctDNA-based tests in a variety of tumor types. This requires collaboration between academic researchers and pharma partners to amass the evidence we need to take the test through regulatory agencies.



I think there is a growing amount of retrospective evidence from a number of solid tumor types that indicates we can use ctDNA to identify treatment response and disease progression more quickly than with imaging alone. The next important step for us is to demonstrate prospectively the clinical utility of changing treatment based on changes in ctDNA levels or detecting minimal residual disease by ctDNA. Many of these studies will be focused on earlier stages of disease than those where we use ctDNA assays today.

## What does the future hold for liquid biopsy?

I see liquid biopsy tests entering a more defined role in treatment selection for late-stage patients for whom tissue testing is not appropriate or tissue is not available. I would say that is the lowest-hanging fruit for these assays and this is where we are directing many of our clinical studies. Beyond that, I see additional applications of ctDNA-based assays in treatment response monitoring and detection of minimal residual disease.

NGS tests – both tissue- and ctDNA-based – have reached the marketplace at an incredible speed. Now, we are starting to see strong indications that these tests are developing clinical maturity, meaning that they're likely to be used in routine settings, rather than just as a last resort. But to realize the great potential of liquid biopsy, I think that we as diagnostic manufacturers need to provide education and outreach to oncologists and pathologists in routine practice. We also need to think about how to incorporate the interpretation of tissue and plasma NGS assays into medical education and residency, so that people coming into the field are comfortable with these tests. That way, oncologists and pathologists will be well-positioned to use the results in their routine clinical practice once the tests are approved by the regulatory authorities.

# THE QUESTION OF INTENDED USE

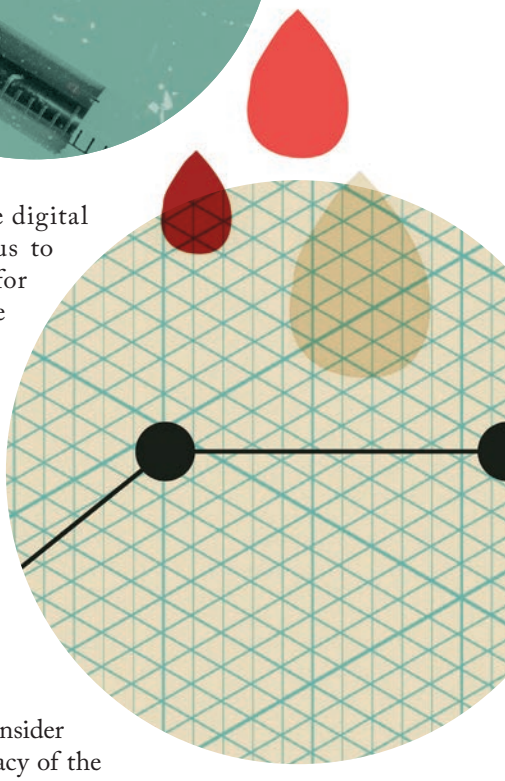
Ryan Dittamore is the Chief of Medical Innovation and Head of Translational Research Partnerships at Epic Sciences.

## What is the AR-V7 liquid biopsy test?

When a patient with metastatic prostate cancer has failed a first androgen receptor (AR)-directed therapy (either abiraterone or enzalutamide) the oncologist is faced with a difficult question. The patient can either receive the other hormone therapy or start chemotherapy. As you can imagine, patients are often reluctant to go on chemotherapy because of its high toxicity, whereas the AR-directed therapy – an oral pill with low toxicity – is a more attractive prospect. The stakes surrounding this decision are high; if the patient doesn't react well to the second line of treatment, the disease can progress rapidly. Another factor to consider is the high cost of AR-directed therapies. These drugs bring in over $4 billion globally, so they are extremely expensive for healthcare systems to administer, and there is no way to predict how a patient will react to them.

To address this issue, we tested almost 20 different biomarkers to identify one that could help determine whether AR-directed therapy will work. The outcome of this search was the androgen-receptor splice variant 7 messenger RNA (AR-V7), which encodes a functional protein detectable in clinical specimens. We found that the presence of AR-V7 is associated with resistance to abiraterone and enzalutamide. This is because the protein that AR-V7 encodes has no ligand-binding domain, which is the target of androgens – it is blocked directly by enzalutamide and indirectly by abiraterone. Therefore, neither of these therapies will work if you have an AR-V7 protein that is essentially constitutively signaling the cell to grow.

Because the AR-V7 protein is most specific in the nucleus, we adopted a "no cell left behind" approach to search for it in the nuclei of CTCs. Instead of trying to sort tumor cells from leukocytes and potentially losing CTCs, this platform places every single nucleated cell from the blood on a series of glass slides. From there, we stain those slides – each containing three million nucleated cells – and use digital pathology to identify CTCs from a multiparametric feature. This is essentially the same process that pathologists go through when diagnosing cancer using tissue morphology, architecture, and protein chemistry, but the digital imaging aspect enables us to analyze six million cells for each patient sample. Once we have sorted the cells and identified the abnormal CTCs, the test looks at the AR-V7 protein in the nucleus and analyzes its chemistry to determine whether or not AR-directed therapy will work.



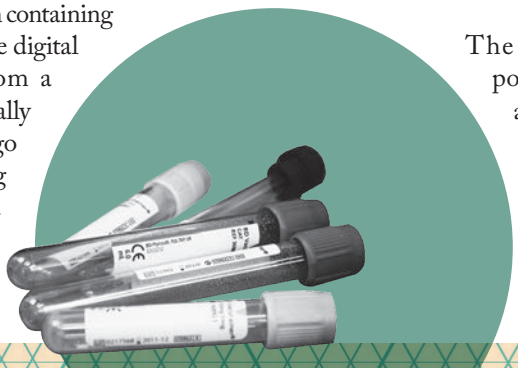
## How accurate is the test?

There are two aspects to consider when it comes to the accuracy of the test. In terms of detecting single tumor cells, the limit of the test is essentially one cell per milliliter of blood. From a protein perspective, there's no way to definitively say how much AR-V7 is present in a patient, because AR-V7 itself is a resistance mechanism that occurs only after a patient has been treated with hormone therapy. It is also only found in a subset of cells, so the only way to measure it would be to take a biopsy from every tumor lesion in a patient. The problem is that this can't be done – tumors usually spread to inaccessible bones.

Therefore, when talking about accuracy, we have to look at the clinical certainty of the test. We focused on what a positive result actually means for the patient, which is that they experience a primary PSA resistance and immediately resist AR signaling inhibitors. Essentially, they don't respond to any further AR-directed therapy and their overall survival is very short. For this reason we can't afford any margin for error in the test results, so it has to be incredibly specific. It's also predictive – the data suggest that patients who test positive could as much as double their life expectancy if they revert to chemotherapy over AR-directed therapy.

## What are the differences between this approach and the PlasmaSELECT and Guardant360 tests?

The main difference is the intended use population. The AR-V7 test is directed at a very specific subgroup of patients with metastatic, castrate-resistant prostate cancer who are considering a second AR signaling inhibitor. The other two tests are



*"I think that, with liquid biopsies, we need to think very carefully about the intended use population along with validating the tests to clinical endpoints."*

aimed at making treatment decisions for many stage III or stage IV metastatic cancer patients, which includes millions of people. The problem is that, because the tests return a long list of mutations, it's not always clear what action to take.

For a test to have clinical utility it must be fit for purpose, which is achieved by designing it for a specific clinical indication that therapy decisions can be based upon. In addition, the test must impact clinical outcomes, such as patient survival, when its use is compared with non-use. I think a great example of this is the programmed death ligand 1 (PD-L1) question, which pathologists deal with extensively at the moment. PD-L1 expression is a mechanism of immune evasion that various malignancies exploit; it's usually associated with poorer prognosis. There are a variety of PD-L1 tests that focus on different cell types and different potential treatments – PD-L1 is not the same across different cancer types and decisions points. Just because you find a biomarker and develop a test, that doesn't necessarily mean it has the same clinical interpretation or clinical value as you switch from one disease to another.

If, for a single clinical question, you have two different tests with contrasting ways to report genomic information that return differing results, how is the physician meant to make a treatment decision? The problem occurs when we don't know what these biomarkers mean in the context of the clinical question that the physician is trying to solve.

I think that, with liquid biopsies, we need to think very carefully about the intended use population along with validating the tests to clinical endpoints. Understanding – and using – the PlasmaSELECT and Guardant360 tests would have been much easier if the manufacturers had provided some form of clinical interpretation. "If you see this biomarker in biochemically recurrent prostate cancer, we know that the patient will or will not respond to the drug that we're administering." But those studies haven't been done, so the result is a lot of confusion. If we want to avoid this and accelerate precision liquid biopsy tests, we have to be very careful about answering a clinical question.

### What are your next steps?

In terms of the AR-V7 test, we are continuing to commercialize it in the US with Genomic Health. We are also evaluating opportunities to globalize the test outside of the US; for instance, in Europe, Canada, Asia, and South America. In addition, we have a deep pipeline of tests in development – those are focused on therapy selection, treatment monitoring, recurrence monitoring, and identifying resistance to therapies. Going forward, I expect a number of tests to come to fruition that have a very focused clinical question. The key, though, is that they must be supported by clinical data and studies that demonstrate their value, so that physicians aren't left guessing with the results.

# A BRIGHT FUTURE

Jacqui Shaw is Professor of Translational Cancer Genetics at the Leicester Cancer Research Center and leads the cfDNA Advisory Group for Genomics England.

## What are liquid biopsy's main advantages and limitations?

A liquid biopsy test that is being used to detect a particular mutation – or other tumor specific change – needs to have specified limits for sensitivity and specificity. The current limitations to liquid biopsy have already been explored in detail but, put simply, you can't accurately report back on a test that hasn't been validated. The most sensitive tests at the moment are those that will detect few molecules of tumor DNA with high sensitivity and specificity; either using high-depth sequencing with a molecular barcoding or target enrichment strategy, or droplet digital PCR. Each of these approaches will have a reported minimum detection threshold; for example, in 20 nanograms of DNA, the minimum might be five molecules of tumor DNA. In this case, if you detect two mutant molecules, then the test result should be reported negative because it's below the minimum accepted threshold for the test. The advantage of liquid biopsy, though, is that you can easily carry out repeated sampling – so if you repeat the test two months down the line and there are six tumor molecules, then the result becomes positive. With a tissue biopsy, there is always the possibility that results are affected by sampling bias or intratumoral heterogeneity, alongside the fact that biopsy might not be possible anyway. Despite the current caveats associated with different liquid biopsy technologies each with slightly different sensitivity and specificity readouts, the offer of real-time, repeated sampling and monitoring potentially gives them a big advantage.

## Do you think the genomic complexity of the tests hinders the interpretation of results?

I think you could make a case for both sides of this argument. Some people think there is too much information involved with genomic tests and that a lot of the data may not be relevant to the clinical question. But I think the point of a genomic test is to produce a baseline of information to store and use as a reference point over time. The pathologist or oncologist can, of course, choose to filter these data, allowing them to see only particular genes of interest or actionable mutations. One of the big breakthroughs with liquid biopsy is that we are now looking at a genome-wide analysis. Although there are challenges associated with the large amount of data that needs to be stored, mined, and analyzed, it's crucial to have this information to build cancer genome profiles so we can better understand how cancers change over time.

## How does the commercial side of liquid biopsy affect the field's development?

We're living in a world where there's an increasing amount of engagement between industry, research institutions, and healthcare providers – and I don't think that's going to change any time soon. In my opinion, having a number of competitors developing various technologies may lead to the delivery of high-quality tests in a shorter timescale. Generally, in the world of science, it's great to have collaborators and competitors alike; they drive the field forward, and I don't think that's any different in liquid biopsy.

There are a number of large, ongoing clinical studies and trials in the field that will inform the future role of liquid biopsy in clinical decision-making. If they perform well, then I believe that liquid biopsy tests will lead to more patient-tailored interventions or changes in treatment at earlier stages. Obviously, the ultimate success is improving outcomes – which is why one of the most enticing possibilities of the technology is profiling cancers in patients where it's not possible to physically access the tumor. The future is bright because there are so many potential opportunities for liquid biopsy!

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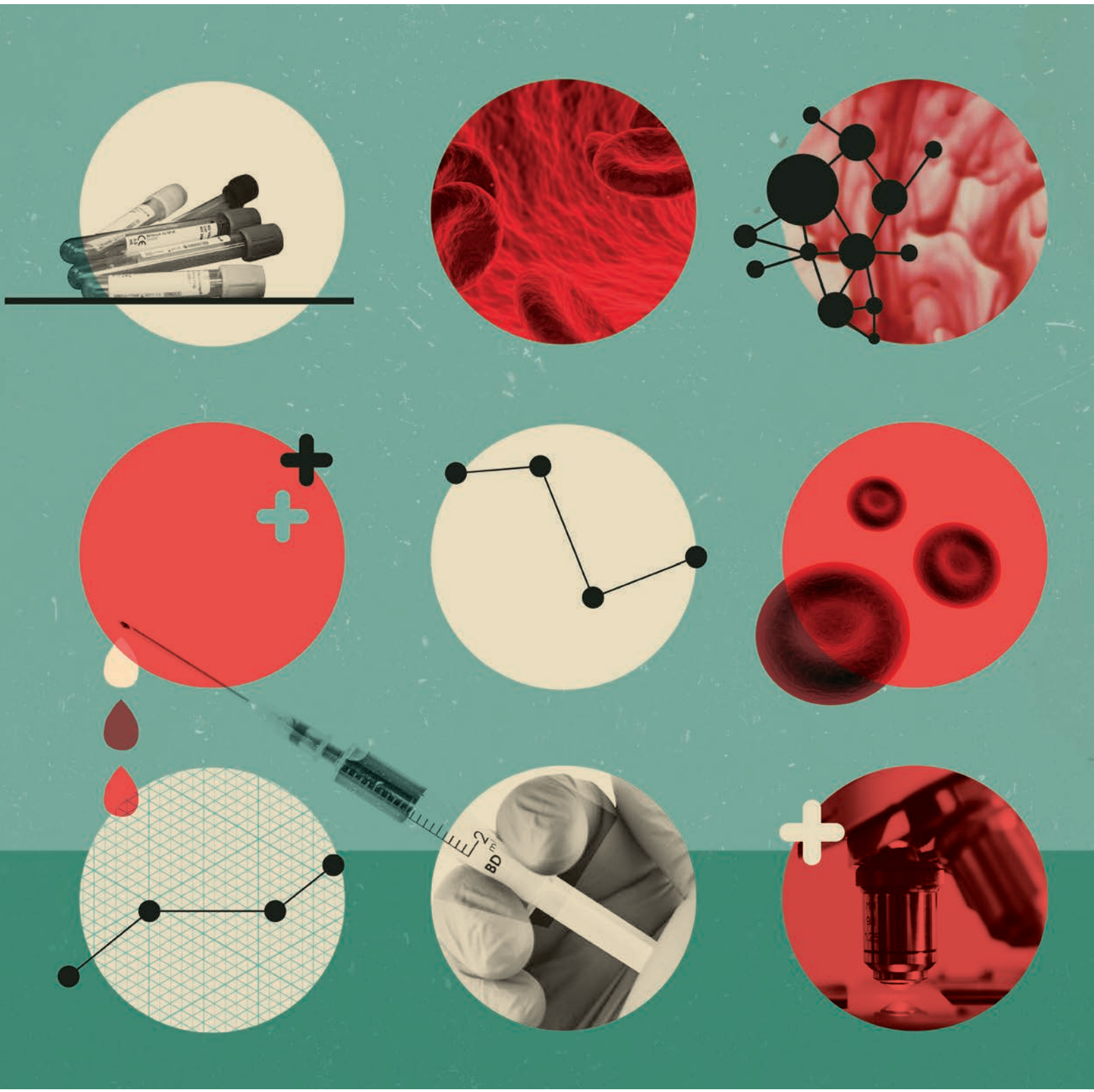
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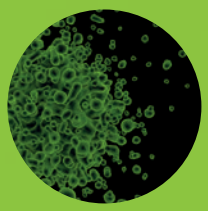

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*28-33*
Positive Steps to Tackle Triple-Negative Breast Cancer
This disease features multiple molecular subtypes, each of which differs in prognosis and treatment approach – so identifying a patient's subtype is vital.

# Positive Steps to Tackle Triple-Negative Breast Cancer

## How to distinguish between different molecular subtypes of TNBC – and why it's so important

*By Jane Armes*

No two breast cancers are the same – and the more we learn about each type, the more we uncover the truth of that statement. Once classified only by location, then later by hormone receptor status, we are now discovering new molecular subtypes of each breast tumor. This includes the triple-negative breast cancers (TNBCs), noteworthy for their lack of expression of nuclear hormone receptors and HER2. These tumors typically have poor outcomes and cannot be treated with hormone therapies like their positive counterparts; however, the discovery of multiple distinct molecular subtypes of TNBC means that detailed analysis could lead to better targeted treatment and, ultimately, improved outcomes for patients.

*At a Glance*

- *Triple-negative breast cancer (TNBC) is not a single entity, but rather multiple distinct molecular subtypes*
- *Each TNBC subtype shows different alterations in gene expression and has the potential to be targeted by different types of therapy*
- *To develop better TNBC therapies, we need to establish exactly what constitutes "triple-negative" and find a cost-effective way of identifying such tumors*
- *Digital image capture, artificial intelligence, and deep learning may help us spot patients who would benefit from TNBC targeted treatment*

### Current testing in breast cancer

At the moment, we diagnose breast cancer by pathological assessment of the diseased tissue. A histopathologist assesses the microscopic morphology of the tumor to confirm the clinical diagnosis of breast cancer and to assess features – such as type, grade, and lymphatic invasion – that are prognostic of cancer outcome. The pathologist then applies a set of predictive analyses to the tissue, using immunohistochemistry (IHC) to determine specific protein expression or in situ hybridization (ISH) to detect gene amplification. These techniques are all microscopy-based, meaning that a pathologist is needed to identify the correct cells for analysis. The standard predictive tests include IHC detection of expression of the nuclear hormone receptors – estrogen receptor (ER; see Figure 1) and progesterone receptor (PR), which are predictive of response to hormone therapies – and either IHC or ISH detection of HER2 status (Figure 2) to determine which patients may benefit from HER2 targeted therapies. Expression of these three proteins is also prognostic of overall outcome. In addition, many laboratories perform IHC

detection of the Ki-67 protein, a marker of cell proliferation that can be used both to determine the utility of chemotherapy and for an indication of overall prognosis.

The disadvantages of this current method of diagnosis is that the tests are semi-subjective. Why? Because they depend on the pathologist's interpretation of the tissue. Of course, because pathologists undergo rigorous training, the subjectivity is kept to a minimum – but it still exists. Also, data quantification is difficult via histopathology techniques, which are essentially visual observations at a microscopic level.

Over the last decade, we have made great progress in understanding the molecular drivers of breast cancer – and, as a result, we understand much more about the complexities of how these cancers develop. Such analyses have also emphasized the different molecular types of breast cancer, underpinning the morphological diversity histopathologists have noted for many years. A major molecular dichotomy in breast cancer typing is whether or not ER is transcribed. There is then further stratification, at a gene transcription level, of ER-expressing and non-expressing cancers. To some extent, the lessons we have learned from the molecular analysis of breast cancers have led to a more formalized assessment of IHC prognostic and predictive analyses, with increased emphasis on PR expression as a surrogate for a functioning ER transcriptional activation pathway. (Why? Because a functioning ER pathway causes upregulation of several genes, including PR.) We also emphasize analysis of the proliferative activity of the tumor, beyond a mitotic count, using Ki-67 IHC.

Based on the data built up over the last decade via molecular profiling of breast cancer, there are now commercially available platforms for transcriptional analysis of diagnostic breast cancer samples, which can be used in addition to standard histopathology-based tests. At the moment, though, these panels are only discriminatory in the prognosis of ER-positive, HER2-negative, early-stage breast cancers. These tests are based on expression profiling of between 12 and 70 genes (depending on the platform) that aim to predict the long-term outcome of such a cancer and therefore to assess whether there is a need to use chemotherapy, as well as hormone therapy, in the management of a patient.

## The nature of TNBC

Because it is simply a matter of detecting absence of expression of ER, PR, and HER2 in breast cancer cells, TNBC is not difficult to diagnose. However, as with any diagnosis based on absent expression, it is important to prevent false negatives. We can achieve this relatively easily with ER and PR, as long as we examine a slide that contains both breast cancer and normal tissue (because normal breast epithelium expresses ER and PR, which can be used as an internal positive control; see Figure 1b). Normal tissue can also be used as an internal control of HER2 expression – but HER2 overexpression should not be detected in the normal breast epithelium, so HER2-negative cancers will have similarly minimal expression, whereas a HER2-positive breast cancer will show strong overexpression (Figure 2a). Additionally, because HER2 protein overexpression in breast cancer is almost always due to gene amplification, HER2 amplification status can be assessed by ISH. In the case of TNBC, there is neither HER2 gene amplification nor protein overexpression.

Because of the absence of ER/PR expression or HER2 gene amplification/protein over-expression, and because of TNBC's general poor outcome, it is generically treated with chemotherapy. However, molecular data – largely based on transcriptional +/- gene copy number analyses – have further defined TNBC into several subgroups, which has led to the discovery of potentially clinically actionable therapeutic targets. Right now, there are many ongoing clinical trials of targeted therapies and immunotherapies for TNBC. It is likely that patients' responses to these therapies will be determined by their specific TNBC subtype, which means that the pathology laboratory will need to perform TNBC subtyping. Unfortunately, molecular-based subtyping, which includes transcriptional and/or gene copy number analysis, is relatively difficult to access and expensive to perform.

## TNBC subtypes

Probably the most widely accepted subtyping of TNBC is based on transcriptomic

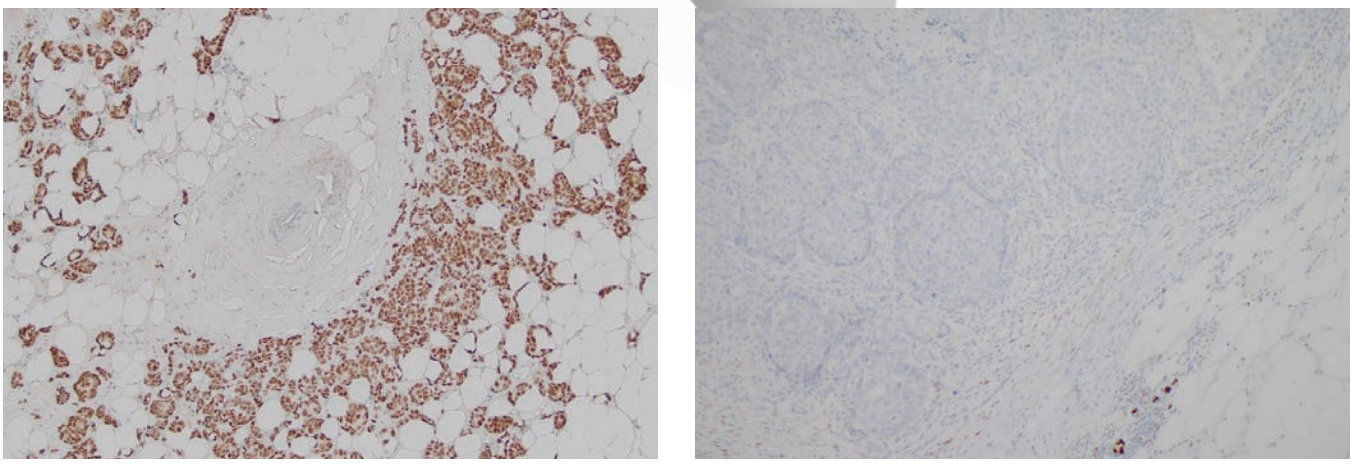

Figure 1. A) ER-positive breast cancer. IHC shows strong nuclear expression of ER in the breast cancer cells, which have infiltrated into fat. B) ER-negative breast cancer. The islands of breast cancer cells are seen across the top left. A few residual benign breast epithelial cells, which strongly express ER, are seen at bottom right. This indicates that the cancer is a true ER-negative breast cancer.

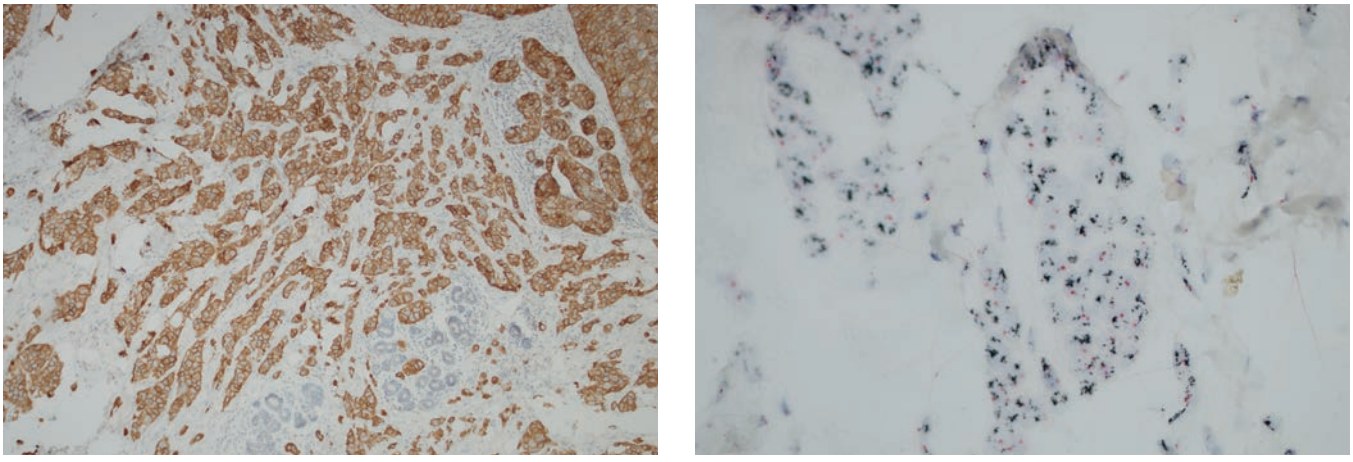

Figure 2. HER2-positive breast cancer. A) IHC shows strong overexpression (3+) of HER2 protein, a cell membrane protein. The invading cancer surrounds normal breast lobules, which do not overexpress the HER2 protein. B) ISH using a dual probe, with red dots hybridized to the centromere of chromosome 17 and black dots hybridized to the HER2 gene on chromosome 17. There are numerous black signals compared with red, indicating high-level amplification of the HER2 gene.

analysis of TNBC (1). Initially, six subtypes of TNBC were proposed: basal-like 1 and 2 (BL1 and BL2), immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like (MSL), and luminal androgen receptor (LAR). What distinguishes these subtypes?

- BL1 subtype cancers are characterized by altered transcription of genes involved in the cell cycle, in cell division, and in DNA damage response pathways.
- BL2 subtype cancers show alteration of growth factor signaling as well as glycolysis and gluconeogenesis.
- IM subtype cancers are transcriptionally enriched for immune cell signaling.
- M and MSL subtypes are enriched for genes involved in cell motility, epithelial to mesenchymal transition and cellular differentiation pathways.
- In addition, MSL show altered growth factor signaling and angiogenesis. Interestingly, these cancers are said to express low levels of cell proliferation genes and also to have a low level of expression of claudin genes (previously described by other groups and exhibiting a particularly poor prognosis).

Although six subgroups were first described, the same researchers then compressed these six subtypes into only four (2), declaring that the IM and MSL

transcriptional profiles were probably due to the transcriptional profiles of tumor-infiltrating lymphocytes and stromal cells, respectively, rather than of the tumor cells.

Other groups have proposed TNBC subtyping based on both transcription and gene copy number data (3). RNA and DNA genomic profiling have also been used to identify and confirm four distinct TNBC subtypes (4): LAR, M, basal-like immunosuppressed (BLIS), and basal-like immune activated (BLIA). BLIA was shown to have the best and BLIS the worst prognosis of these four groups. Ultimately, Ahn and colleagues (5) summarized TNBC subtypes that had been described independently by different groups and determined that the combined data identified four molecularly distinct TNBC subtypes, each amenable to different targeted therapies:

- basal-like, amenable to platinum based therapies and PARP inhibitors
- mesenchymal, amenable to MET, FGFR, and mTOR inhibitors
- immune, amenable to immune checkpoint inhibitors; and
- luminal androgen, amenable to androgen blockade and PIK3CA inhibitors.

## The future of TNBC

We are on the cusp of offering targeted therapies to TNBCs. However, current diagnostic categorization of breast cancers is based on ER/PR and HER2 expression, which is overly simplistic – and will be even more so when targeted therapies for TNBC subtypes are available. We will need a robust mechanism of identifying the different TNBC subtypes.

A corollary of developing targeted therapies for TNBC subtypes is to redefine our interpretation of what exactly is "triple-negative." Prior to any targeted therapies, if even a low level of

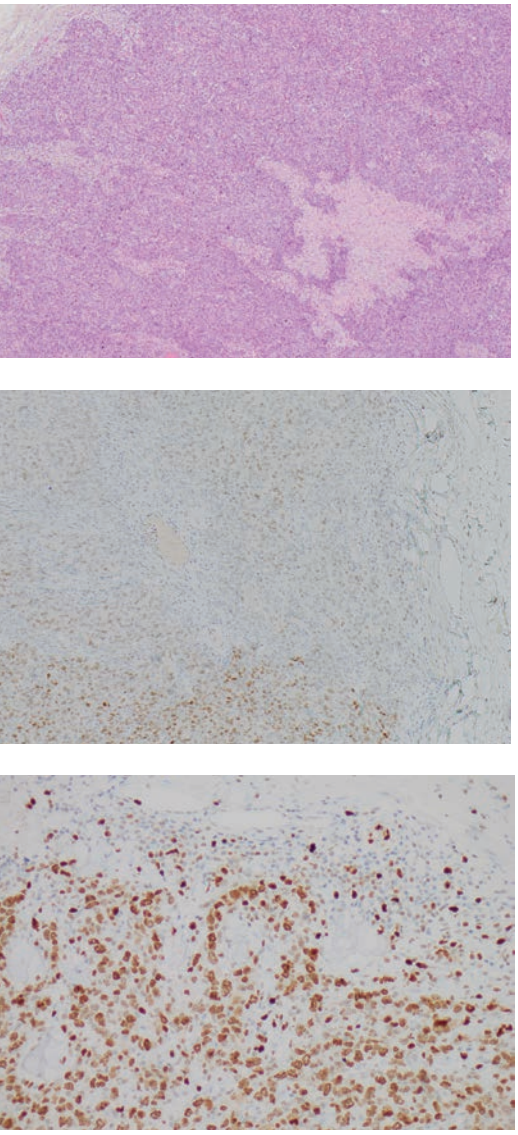

Figure 3. This breast cancer has the classical morphology of basal-like breast cancer (i.e., TNBC), but would be classified as ER-positive (non-TNBC) due to weak-to-moderate focal ER expression detected by IHC. A) This high-grade breast cancer has morphological features of a basal-like breast cancer (H&E stain). B) The same cancer shows focal ER expression and is therefore classified as an ER-positive breast cancer. C) The cancer has a very high Ki-67 expression index (>90 percent of tumor cells positive), indicating a high proliferation rate – another feature of basal-like breast cancers.

ER expression was detected in a breast cancer, it would be treated as an ER-positive breast cancer and the patient offered hormone therapy. However, ample data suggest that some low-ER-expressing breast cancers behave, in both outcome and response to hormone therapies, much more like TNBC (Figure 3). Now and in the future, though, categorizing such cancers as ER-positive – rather than as one of the TNBC subtypes –would likely mean denying that patient much more effective TNBC-targeted therapy. Therefore, not only will we have to diagnose different TNBC subtypes, but we will also have to positively identify cancers that would be amenable to TNBC targeted therapies, despite some (usually weak and focal) expression of ER/PR.

My personal belief is that even small-panel genomic analysis of breast cancer is too expensive to be used in a diagnostic sense to identify TNBC subtypes. I also suspect that there is probably insufficient skilled manpower to interpret genomic

data for such a common cancer – and, even then, any technique that does not identify and analyze tumor cells within a population of non-tumor cells in a tissue sample may lead to an erroneous result. Equally, given the advent of immune therapies, we are now interested in the interplay between the genomics of a tumor and the patient's response to that tumor – in effect, a precision medicine approach to therapeutics. The interplay between the tumor and the patient's individual reaction is presented and captured in the microscopic morphology of that tumor. In fact, it is possible for a breast pathologist to diagnose basal-type and luminal androgen receptor-type TNBC on morphology supported by selective IHC. This morphology can be recognized even if the tumor also weakly expresses ER/PR. It will also disclose the extent of any immune response to the tumor that may be amenable to immune checkpoint inhibitors. Therefore, I believe that we should take a renewed interest in the morphological classification of breast cancers – and possible expansion of the morphological classification to identify new determinants of tumor outcome – by analyzing the patient's individual response to the tumor, perhaps by accurate assessment of tumor infiltrating lymphocytes or stromal-tumor interface factors.

It is likely that histopathologists' standard morphological analysis of breast cancer can be greatly enhanced by applying digital image capture, artificial intelligence (AI), and deep learning techniques to tumor morphology. Such an approach may consolidate the morphological factors identified within a tumor section that relate to that tumor's molecular constitution, and hence its TNBC subtype. It may also be able to recognize new morphological factors, such as tumor-stromal interactions, that are important for tumor outcome. We also know that digital image analysis of tumor sections is more accurate than pathologists' interpretations of important quantitative data, such as the quantification of tumor infiltrating lymphocytes. And that's why I think it is important to revisit tumor morphology in the post-molecular era using these new techniques, which are capable of capturing and analyzing large sets of data on a similar scale to genomic data – except that the inputs are morphological. The ultimate outcome may be that digital imaging, AI, and deep learning techniques will help us spot previously unrecognized prognostic and predictive factors, and that digital image interpretation may be well-suited to assess quantitative data within a microscopic section. We can apply the integration of these techniques, and the enhanced morphological analysis they will yield, to accurately interpret different types of TNBC.

My colleagues and I have developed an international collaboration to investigate TNBCs via digital image capture and AI on the background of known patient outcome, standard morphology assessment, and genomic data. We are performing this research to clarify how morphology can be used to subtype TNBC.

## A part of the future

My own career as a histopathologist has spanned almost 30 years and definitely started in the pre-genomic era. I have seen the giant leap we have made in patient outcomes, partly by integrating molecular data into patient management. I am also interested in seeing just how far immune therapeutics can be successfully applied to cancer management. But throughout all these developments, I have held on to the belief that the histopathologist, with their detailed knowledge of disease processes and their visual knowledge of the complexity of disease, should be at the forefront of integrating new techniques into diagnosis. I think that we have to be wary of "sound bite" pathology that is easy for non-pathologists to understand, such as the categorization of weak ER-expressing cancers as "ER-positive" when, in fact, the complex morphological information (much more difficult for a non-morphologist to comprehend) suggests that it should be in a different category of breast cancer. Therefore, my recommendation would be for an integrated approach to breast cancer diagnostics, where morphology is understood to be the visual picture of tumor–host interaction – and, therefore, the foundation of precision medicine.

*Jane Armes is Clinical Director, Tissue Pathology and Diagnostic Oncology, ICPMR, Westmead Hospital at NSW Health Pathology and Clinical Professor of Pathology, Sydney University Medical School, Australia.*

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*36-39*
A Diagnostic to Drool Over?
Saliva is abundant and easy to access, making it a great choice for a diagnostic fluid – and now, researchers have discovered salivary circulating tumor DNA.

# A Diagnostic to Drool Over?

## Dispelling stigma and building credibility for a little-used bodily fluid by harnessing its potential in cancer screening

*By Luke Turner*

New diagnostic methods continually arise – something that is driven by our determination to detect disease as early as possible, and thus give patients the best chance of successful treatment. Although liquid biopsy testing has primarily focused on blood, an innovative approach seeks to use an even less invasive bodily fluid – saliva.

Originally trained as a dentist, David Wong has spent the last 12 years working on the use of saliva for health surveillance – a goal that he says is compelling and achievable. Despite humans producing around one liter of it every day, the diagnostic potential of saliva has not been evaluated with as much vigor as other less accessible fluids, such as plasma and spinal fluid. Wong believes that existing social and cultural stigmas surrounding saliva need to be uprooted to harness its potential for the early assessment of disease.

*At a Glance*

- *Liquid biopsy is a rapidly evolving field that commonly uses blood and urine as source material for diagnostic tools*
- *Despite the volume of saliva humans produce each day – 1 liter – the fluid isn't currently used for the detection of circulating tumor DNA*
- *Saliva contains genetic material and proteins, and can be collected through noninvasive techniques*
- *David Wong, who has been working on "saliva-omics" for 12 years, hopes to curb negative social and cultural associations to advance saliva as a diagnostic fluid*

### Within spitting distance of success

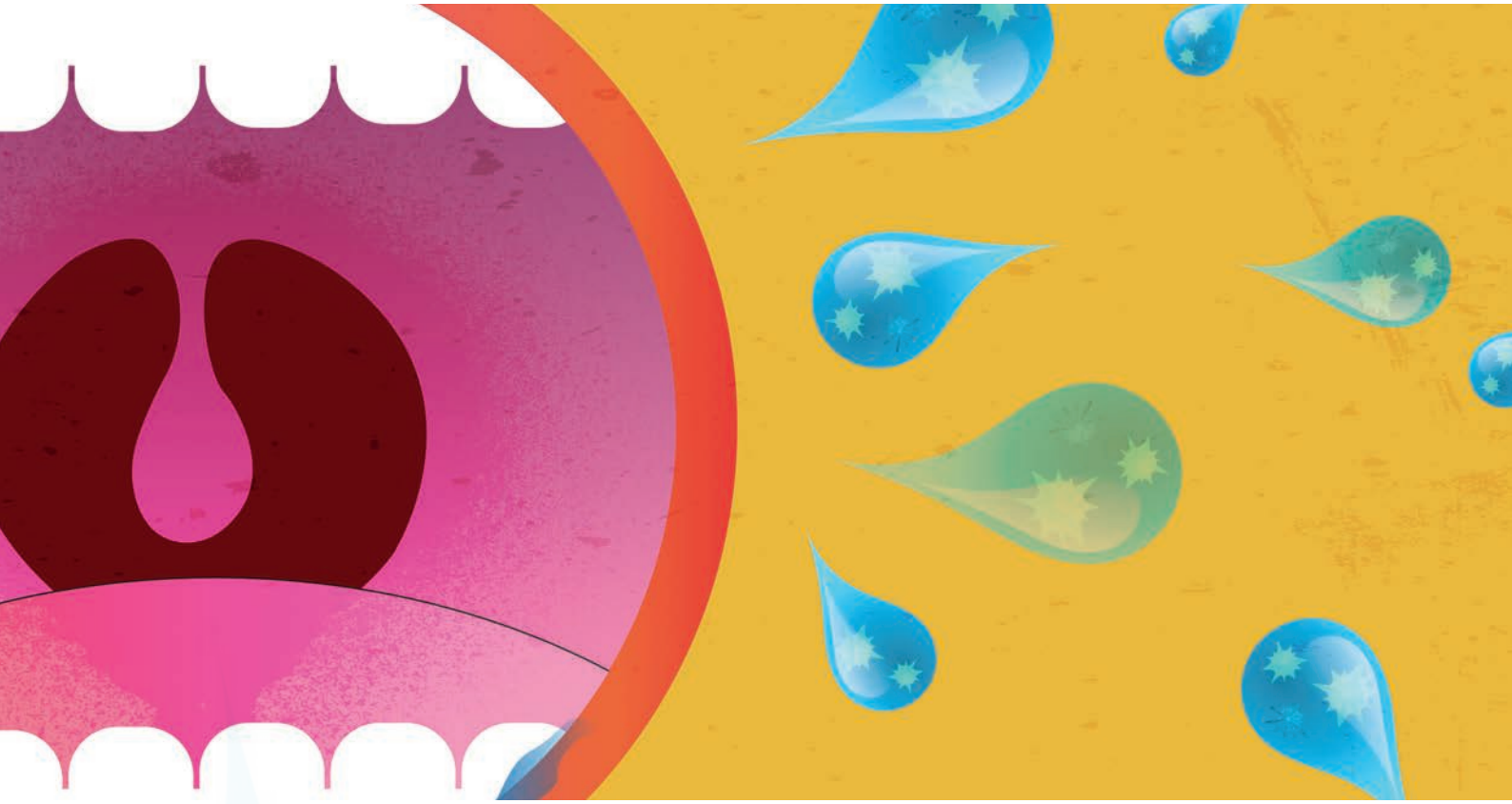
"It seems strange that, as such an abundant fluid, saliva has never been used in the same way as blood and urine in terms of liquid biopsy. There could be a whole host of reasons for this. Perhaps it is the negative social and behavioral associations with the fluid that discourage scientists from appreciating the clinical value of saliva," says Wong. "This needs to change, and we are taking baby steps to achieve these changes in perception."

Given that the ultimate goal for early disease detection is noninvasive diagnostic testing, the fact that every one of us produces enough saliva to fill three soda cans per day makes it an appealing target. Its collection is about as noninvasive and painless for the patient as any sample could be. But Wong recognizes that accessibility is just one parameter: "Obviously, the most important aspect of any screening tool or diagnostic is performance. Although noninvasive fluids are easy to obtain, that's irrelevant if their diagnostic ability is marginal or less than current practice."

*"Obviously, the most important aspect of any screening tool or diagnostic is performance. Although noninvasive fluids are easy to obtain, that's irrelevant if their diagnostic ability is marginal or less than current practice."*

"Twelve years ago, the National Institutes of Health directed investment into this landscape in an attempt to give it scientific credibility and prove that saliva has clear clinical utility. And that's how the journey first began – the effort was bolstered by the finding that mixed constituents, such as genetic material, proteins, microRNA, and microbial content, are equally as endowed in this fluid as others," says Wong.

Wong and his team are currently focusing on the use of saliva for lung cancer detection. Shed by tumor cells and released into the blood, circulating tumor DNA (ctDNA) fragments are known to be fingerprints of human tumors. "In our first application – using saliva to detect circulating tumor targets in non-small cell lung carcinoma – the performance of saliva was as good as that of blood, and in one case even better," (1,2). As the ability and accuracy of liquid biopsy testing has advanced in recent years,



*"I think the next steps for us will be to continue the scientific credentialing of salivary testing by seeking clinical context that will have a big impact."*

it has set a high bar for saliva to achieve the same early detection credibility as other methods, including the traditional tissue biopsy.

Wong believes that saliva could be used for screening and risk assessment. "In cases where a computed tomography (CT) scan of someone's chest shows that there is a mass present in the lung, the majority turn out not to be a tumor. There's no way an image can determine whether it is, in fact, a tumor, so it is routine practice for the patient to return six months down the line for another scan. If we could obtain a drop of saliva in tandem with the radiological image and evaluate whether or not a tumor is present, it would be a game-changer in assisting clinical decisions." The use of saliva also extends to measuring stress hormones, enzyme levels, and developmental disease biomarkers.

In addition, salivary testing is highly relevant for head and neck cancer, which is rising in prevalence with nearly 690,000 new cases annually (1). Future efforts to use saliva to detect this disease are boosted by the finding that about 70 percent of head and neck cancer squamous cell carcinomas show detectable circulating tumor DNA mutant fragments (3).

## Driving potential into credential

In addition to the collection of saliva being truly noninvasive, only a single drop is required, which Wong considers far more practical for

liquid biopsy than the use of blood. "Repeatedly drawing 10 mL of blood is not trivial, so having the ability to access a bodily fluid that i) has these discriminatory contents and ii) can be harnessed noninvasively is truly empowering. Notably, oncologists are also able to more easily access a diagnostic fluid in small children, because you can't simply perform repeated blood draws on infants whenever you need a new sample."

Another benefit of the technique is the speed with which it can be completed. The entire assay – from loading the sample onto a microarray detection platform to obtaining the results – can be performed within 20 minutes. Currently, saliva samples must be processed within an hour of collection from the patient, and factors such as the time of collection – due to diurnal variation – and variable consistency between patients also need to be accounted for (4). Despite these considerations, the future still looks good for salivary testing. Recent research found that an absorbent that can be inserted into the mouth, along with a filtered nozzle to remove cellular debris and salivary mucins, will facilitate easier collection, storage, and transportation of saliva (5).

"I think the next steps for us will be to continue the scientific credentialing of salivary testing by seeking clinical context that will have a big impact," says Wong. "The most important outcome, in our opinion, is advancing toward using saliva in a specific clinical context. For instance, we could use it to monitor patients at risk of lung cancer to detect malignancy, which would then facilitate regulatory approval. That's the ultimate goal."

Within the next three to five years, Wong hopes that saliva will become a widely used sample type for liquid biopsy testing. He offers one potential "killer app" in the form of codon 12 mutation testing for pancreatic cancer – a disease that, at the moment, is generally diagnosed late in its course, often only after it has metastasized. Although not diagnostic, 90 percent of pancreatic cancer patients have a mutation on codon 12; early screening through saliva samples could help physicians to detect high-risk individuals.

Looking ahead, Wong hopes that the negative social and cultural connotations surrounding saliva won't impede its development as a diagnostic tool. "Even with regulatory approval, there will inevitably be reluctance within clinics to say, 'Now I'm going to stop drawing blood from my patients and start working on spit.' This process won't happen quickly, but if there's demonstration of superior performance, clinical utility, and credible scientific data, I think people will begin to view the prospect differently. It may take another decade or two, but if saliva continues to sustain itself in terms of its performance, I think it has huge potential in years to come."

*David Wong is a Professor in the Division of Oral Biology and Medicine, and Associate Dean of Research and Director of the UCLA Center for Oral/Head and Neck Oncology Research.*

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# Profession

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*42–45*
Peer-to-Peer with Jeffrey Myers
Pulmonary and thoracic pathologist Jeffrey Myers talks about the evolution of his career, the changes he has brought about, and what he considers most important – including music!

*46–47*
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# Peer-to-Peer, Featuring Jeffrey Myers

## How to build a rewarding career by following instincts and aiming to do the right thing

*Ivan Damjanov interviews Jeffrey Myers*

Jeffrey Myers is the A. James French Professor of Diagnostic Pathology and Vice Chair for Clinical Affairs and Quality in Michigan Medicine's Department of Pathology at the University of Michigan. He also serves as Director of MLabs, the institution's reference laboratory division. A general surgical pathologist, he is increasingly focused on his subspecialty interest in thoracic pathology, but also has a longstanding commitment to quality and safety, accompanied by a more recent focus on patient- and family-centered care.

His career has given him plenty to think about, and the many people he has had the privilege of mentoring or being mentored by have taught him valuable lessons. Now, in an interview with professional colleague Ivan Damjanov, Jeffrey Myers shares what he has learned to benefit his colleagues in the pathology sphere.

*At a Glance*

- *Creativity and collaboration are vital to laboratory medicine – and physical spaces should be designed to facilitate both*
- *A good lab medicine professional should be honest, curious, and dedicated to the fair and equal treatment of all patients and colleagues*
- *Success doesn't always mean having a definite plan; you might be surprised at how far you get by simply trusting your instincts*
- *It's important to have outside interests to maintain work–life balance*

You wear many professional hats. Which duties take up most of your time, and which bring you the most pleasure?

My role as Vice Chair for Clinical Affairs and Quality consumes more of my time than anything else listed on my CV. To some extent, that reflects increased demand over the last couple of years, largely related to a move of our non-stat clinical operations to a new site about three miles away from the main medical campus. We designed and built a facility that reflects the future of our discipline: laboratories designed using Lean principles and tools, paired with non-laboratory space designed to facilitate collaboration and creativity in a digital age. The spaces are partially divided by the increasingly parochial interests peculiar to subspecialization. Although we got many things right, we have also encountered unanticipated challenges. Even those aren't all bad news, though; they offer an endless string of opportunities for continuous improvement.

It is still hard to beat signing out as the thing that gives me the most pleasure. This has only gotten better since the launch of our pathology-based Patient and Family Advisory Council (PFAC), which has given me back a sense of purpose. More than anything else I do, signing out helps connect me to the reason I went into pathology in the first place: tending to the welfare of those who look to us for answers and hope.

Is that what you always wanted?

I have never been clear enough about my future to identify anything in particular that I "always wanted." My undergraduate inspirations for wanting to do pathology in the first place, Jack Spier and Les Torgerson, modeled what it meant to be a "doctor's doctor" and touch the lives of countless patients every day. Those are the things to which I aspired when I redirected my career away from social work toward medicine.

*"I followed my passions with nothing resembling a career development plan, focusing on doing the right things for the right reasons and walking through doors when they opened."*

Anna-Luise Katzenstein – my lifelong mentor, collaborator and friend – showed me what it meant to be true to the work, curious about everything, and dedicated to fair and equitable conduct in all things. When I finished medical school, those things became my aspirations – and it's hard to think of a better place in which to practice those things than the Mayo Clinic, where I spent 16 wonderful years learning from colleagues who "walked the walk" when it comes to excellence in clinical care.

I came to the University of Michigan

to understand opportunities unique to a larger university setting, and to explore a powerful combination: a highly functioning clinical enterprise resting on a foundation of world-class research and a legacy of educational excellence. I was the beneficiary of a whole new set of mentors and colleagues in multiple departments and schools, with new opportunities to explore different approaches to quality and patient- and family-centered care.

The care of patients has always been, and always will be, my chief priority – it is my "non-negotiable." Any other priorities evolved organically and were driven by challenges and opportunities to serve the pathology community through member organizations, mentor trainees and peers, and learn through collaborative, clinically focused science. I followed my passions with nothing resembling a career development plan, focusing on doing the right things for the right reasons and walking through doors when they opened.

### What are the most important changes you have introduced in your department?

Changes that might qualify as novel and innovative over the last 13 years here in Ann Arbor include:

- a unique frozen section practice to support breast cancer patients in an ambulatory setting;
- an integrated center of excellence in forensic medicine that combines the strengths of our university-based autopsy practice and regional medical examiner (ME) offices, including Wayne County in Detroit;
- the design of the non-laboratory space into which we recently moved, focusing on collaboration and


creativity across subspecialty silos;
- creating a Division of Quality and Health Improvement to increase our capacity for value creation from a laboratory platform; and
- launching a pathology-based PFAC to nurture a culture of patient- and family-centered diagnostic medicine in our department, our health care system, and our discipline.

Unfortunately, I remain frustrated at my inability to advance standardization of practice supported by tools to motivate

*"We must learn to do better; our goal should be to optimize patient care, and that means being accountable for the choices we make."*

individual practitioners. We still have work to do when it comes to achieving essential levels of consistency; only in that way can we, as a foundational element of diagnostic medicine, become as cost-effective and efficient as we should – and could – be. We cling to increasingly anachronistic paradigms that may have been more relevant in a volume-based healthcare ecosystem. We must learn to do better; our goal should be to optimize patient care, and that means being accountable for the choices we make when it comes to resource utilization.

Which of your many papers are your personal favorites?

I loved working with Anna-Luise Katzenstein for all sorts of reasons, chief among them her very clear and logical thinking, her deep understanding of both the problems and the solutions, and her discipline when it came to translating the work into a manuscript. I was especially proud of the work we did in the 1980s using electron microscopy to advance our understanding of fibrotic lung diseases as models of abnormal wound healing. This work included a case report that defined fibroblast foci as sentinel clues to the pathogenesis of lung fibrosis (1) and a study of organizing pneumonia that identified features common to other forms of acute lung injury (2).

In a larger sense, I am not sure that I'd say I have done anything to advance either the practice or theory of pathology. If I have, it was to embrace the privilege of training others who have collectively done far more to advance both.

How did you approach the task of making an excellent pathology textbook even better?

The task of editing the new edition of Rosai and Ackerman's Surgical Pathology was intimidating; it felt like there was no way to make it better. At first, I thought that the best we could hope for was to avoid making it less than it already was! My fellow editors and I were determined to preserve the voice unique to a single-author textbook while acknowledging that no human being other than Juan Rosai himself could possibly accomplish this task in the same way that he did. We did the best we could to maintain his voice; at the same time, we updated information and images to serve the needs of an international community for whom the world has changed, impacted by a march toward subspecialization and the application of increasingly sophisticated diagnostic tools. For me as an editor and author, it meant often leaving something in place that I might have been tempted to say a little differently, or deciding to leave a photomicrograph rather than replace it with one of my own. I told myself it was foolish to imagine that I could possibly say better, or more effectively convey in images, what I could never understand in the same way that he does.

What else are you working on?

In 2011, I went to a Jeff Beck concert with my friend and colleague, Joel Greenson. It reawakened in me an interest in playing rock 'n' roll which is something I had given up nearly 40 years before. We joined forces with our colleague, Ulysses Balis, and some very talented local musicians to form a band called Lost in Processing. Organizations like the American Society of Clinical Pathology and the Texas and California Societies of Pathology have been generous in offering us opportunities to perform and we are having a blast! The problem is that there are not enough hours in the day or week to make the sort of musical progress that I would like to make, especially given an advancing tremor that makes some once-easy things harder. I would love to see our dreams come true for this band while we still can! That will mean figuring out ways to work more intensely to bridge the gap between my skills and those necessary to make the sort of music that others may want to hear – but I have faith that we can do it.

*Jeffrey Myers is A. James French Professor of Diagnostic Pathology, Pulmonary Pathology, Thoracic Pathology, and Vice Chair of Clinical Affairs and Quality, Michigan Medicine, University of Michigan, Ann Arbor, USA.*
*Ivan Damjanov is Professor of Pathology at the University of Kansas School of Medicine, Kansas City, USA.*

*References*

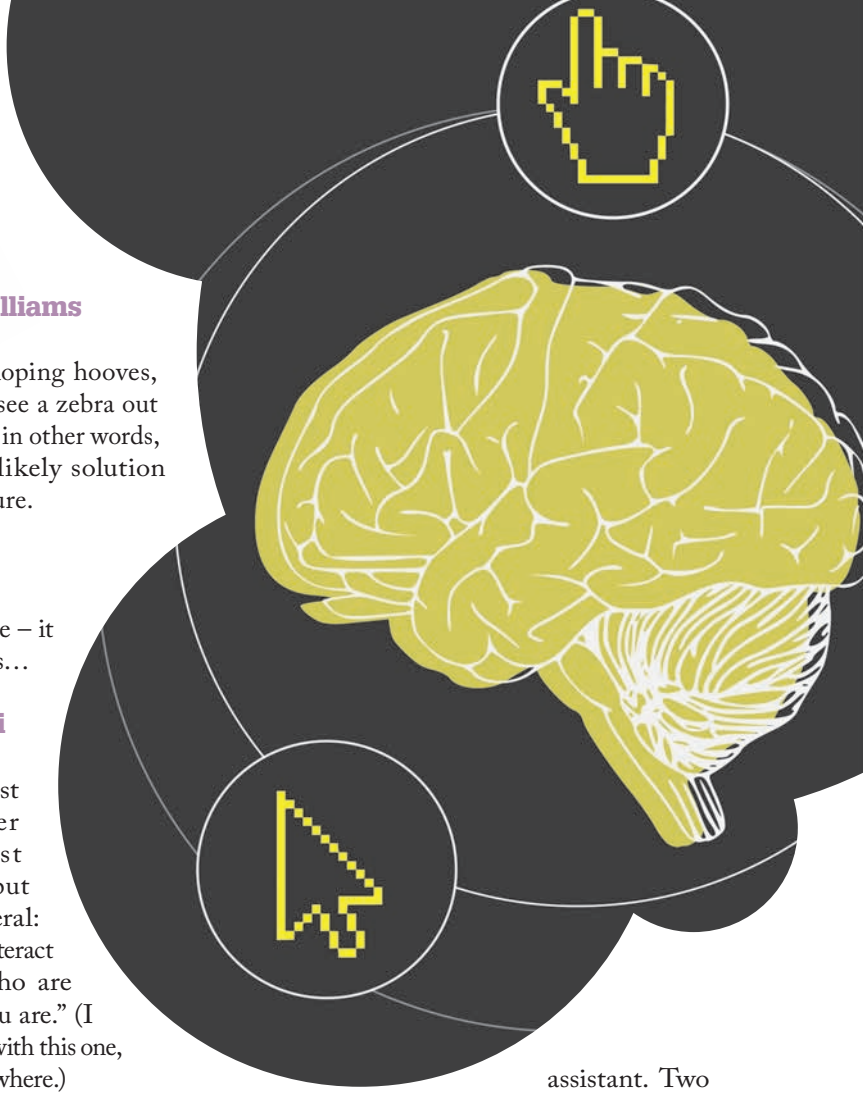
1. *JL Myers, AL Katzenstein, "Epithelial necrosis and alveolar collapse in the pathogenesis of unusual interstitial pneumonia", Chest, 94, 1309–1311 (1988). PMID: 3191777.*
2. *JL Myers, AL Katzenstein, "Ultrastructural evidence of alveolar epithelial injury in idiopathic bronchiolitis obliterans-organizing pneumonia", Am J Pathol, 132, 102–109 (1988). PMID: 3394793.*

# Career Advice – in Brief

## Bite-sized words of wisdom from pathologists and laboratory medicine professionals at every stage of their careers

What's the best career advice you've ever received? We recently asked that question of you, our readers, so that we might share those wise words with the whole community. Your responses came from every corner of the laboratory: from pathologists, clinical laboratory scientists, pathologists' assistants, medical technologists, and more.

No stage of the journey is too early – or too late – to benefit from the wisdom of others (a sentiment with which many of you agreed). Here, with your permission, we share the most digestible career advice you're likely to find.



### UHW Cellular Pathology
*@CellularUhw*
"No matter how difficult or complex the diagnosis can be, keep it as simple as possible for the clinicians to understand." This is the most helpful advice I got! – Ioulia Evangelou, consultant histopathologist, UHW.

### Phillip Templo Jr.
*@thejourneymate*
One of my mentors said that if you don't have many opportunities, you should create your own opportunities. I think this advice is even more relevant now in the era of social media. I was able to expand my horizons through teaching, research, and overseas training.

### Gynae Path
*@GbGynae*
There is a person at the end of every biopsy.

### Doris-Ann Williams
*@DorisAnnW*
If you hear galloping hooves, don't expect to see a zebra out of the window – in other words, look for most likely solution before the obscure.

### Gino Somers
*@GSomersPath*
Be nice to people – it pays off in spades…

### Marc Ladanyi
*@MLadanyi*
My vote for best piece of career advice not just in pathology but science in general: "Seek out and interact with people who are smarter than you are." (I did not come up with this one, just read it somewhere.)

### Elizabeth Montgomery
*@LizMontgomeryMD*
Be generous with forgiveness and opportunities; be humble with successes.

### Wendy Frankel
*@WendyFrankelMD*
Look for the best in others.

### Emily Shaffer
*@DrMissWV*
As a #pathologist, you need to understand the consequences of every call you make. Will this patient require more frequent screening? More invasive testing (with increased risks)? An additional procedure/surgery due to unclear margins? What will it cost them in medical expenses and lost earnings? It matters!

### Cory Nash
*@iplaywithorgans*
Not a pathologist, but a pathologists' assistant. Two things come to mind: 1) My preceptor during clinicals would always start the day by saying, "Who's ready to save lives?" Always important to remember why we do this. 2) From the same preceptor: Never assume anything. Know.

### Joe Chaffin
*@bloodbankguy*
Do what you love to do! Don't just chase the "hot" subspecialty or what "everyone else" wants to do. Figure out what really makes you happy professionally; the thing that, when you're doing it, time just FLIES, and DO THAT! Life's too short to hate your job.

### Sarah Bean
*@DrSMBean*
Self-reflect to understand your #values. Then develop a personal #mission and #vision statement that aligns with your institution. Use these to assess

opportunities. Don't be afraid to say #no when there is not alignment. Also, remember to revisit this exercise regularly.

### Lija Joseph
*@lijjoseph*
Find a mentor you trust as early as possible in your career! Be true to your conscience, and be kind even when no one is watching! Never ever give up on someone or something you believe in!

### Kenneth Tang
*@drkennethtang*
Each case is a consult for your histopathological expertise and there is a patient (in fact, your patient) behind each case. Try to correlate with the relevant info, which is often not on the form but it will be on the EMR or one phone call away.

### Jennifer Laudadio
*@JLaudadioMD*
1. Great mentors open doors. Successful mentees walk through them. (From @jhuntpath.)
2. The three As… be available, affable, able.

### Mary Kinloch
*@saskmary*
Research is the one time in your career when you get to choose who you work with. Choose to work with your friends. – Blake Gilks

### David Grenache
*@ClinChemDoc*
Not a pathologist, but a clinical chemist for 16 years. Career advice: "Go to graduate school and earn your PhD." Practice advice: "Quality is everything."

### Sara Jiang
*@Sara_Jiang*
I've benefited from so many great mentors – one of my favorites (especially in our fast-paced get-it-done-yesterday world) is, "First get good, then get fast."

### Aadil Ahmed
*@AadilAhmedMD*
Out of sight, out of mind! Focus on what's in front of you!!

### Eiman Adel Hasby
*@eimanhasby*
"Talk to clinicians in their language."
"Your decision is a decision in another's life."

### C. Moreno Sainz
*@cmorenosainz*
Years ago, welding the mirror of a Spec 20. It was good; I tried to improve it, and it stopped working. Tip: "Sometimes, the best is the enemy of the good." When you fix a device and it works well, do not insist on better.
Our reason for being. Tip: "Do what you think is best for the patient."

### Joseph G. Keary
*@GI_Joe_K_4_CLMA*
The best advice I received early in my military career was the saying, "Bad news doesn't get better with age." It has many applications, but I use it to teach young clinical lab scientists that, if they make a mistake, be upfront with it and correct it before it gets worse.

### David Gaze
Best advice: when writing a paper, print it to proofread it; don't read on the PC screen. I now advise all my students to do the same with their own assignments.

### Khaled Lounis
Behind every slide is a human being!

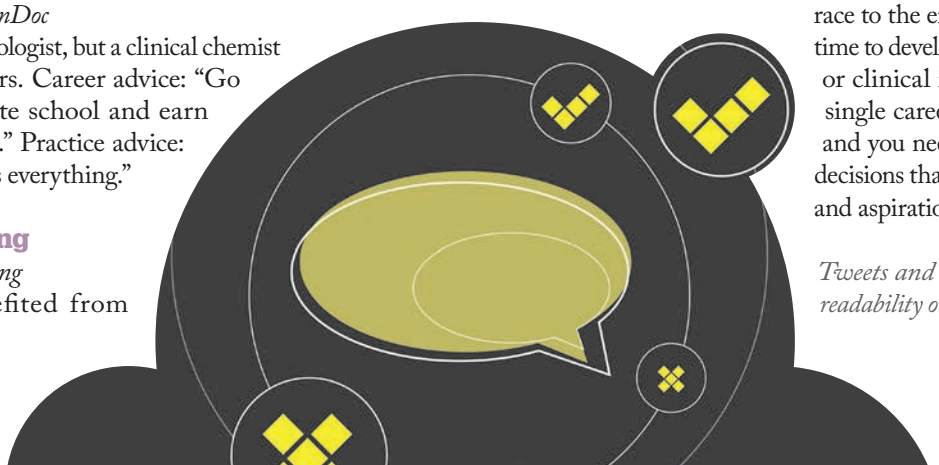
### Shazia Tabassum Hakim
"Six hours' sleep fail… five hours' sleep pass," given by my mentor, teacher, and first boss, Prof. Dr. Essa M. Abdullah!

### Pat Fournier
Forty-three years ago I started my amazing career as a medical technologist with such interest and energy for lab sciences. I still teach my students to think outside the box all the time; if you feel bored, move on to another area of the field where you experience discomfort, but never stop learning and experiencing the new areas of science and the development of new tests. Do your work with passion. One needs to learn something new every day. Your pathologist is your best friend and teacher in the lab.

### Ralph Ioder
During my internship as a clinical lab scientist, the education coordinator for the program told me to consider being a generalist for my first few years in my profession. This move not only cemented the knowledge I gained during training, but kept more options open to me in my career. I also helped train CLS interns and I passed that belief onto them.

### Bethany Williams
Put your career in perspective – it is not a race to the end. If you feel you need more time to develop skillsets or pursue academic or clinical interests, do so! There is no single career path that suits everybody, and you need to concentrate on making decisions that suit your development needs and aspirations.

*Tweets and messages have been edited for readability only.*

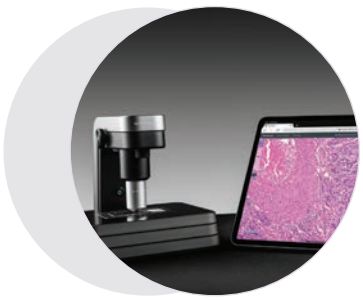


# *Spotlight on...* Technology

## Patient Safety Redefined with Signature Cassette Printer

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

*www.dtm-medical.eu*



## Grundium Ocus – Portable Microscope Scanner

A monumental leap in personal digital pathology. Ocus is a precision tool small and affordable enough to be on every medical professional's desk. It is truly portable and it can be brought anywhere. Wireless connectivity means telepathology is now possible practically anywhere on the planet. Supports all work flows.
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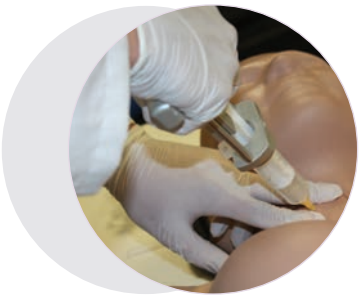

## FioNA™ Fine Needle Aspiration Simulator

Diagnostic Cytopathology recently concluded that "a realistic simulation model, in combination with a standardized training program with formal assessment methods is a valuable tool to teach FNA." Learn more about FioNA and how she improves training medical puncture and fine needle aspiration.
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## Visualize Pathology With the Hyperion™ Imaging System, powered by CyTOF®

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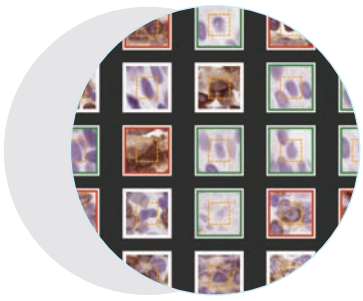
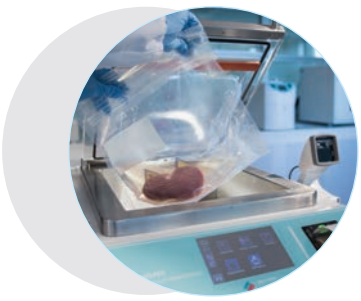


## Cell Detection Studio – a do-it-yourself tool for pathologists by DeePathology.ai

Cell detection is an important task in Pharma research and medical diagnostics. We created the most advanced cell detection platform that lets you create cell detection algorithms on your own data with extreme efficiency. Do you want to see how our Cell Detection Studio can work for you? Contact us at: *cds@deepathology.ai*
*www.deepathology.ai*



## Milestone SealSafe – Vacuum system for biospecimen management

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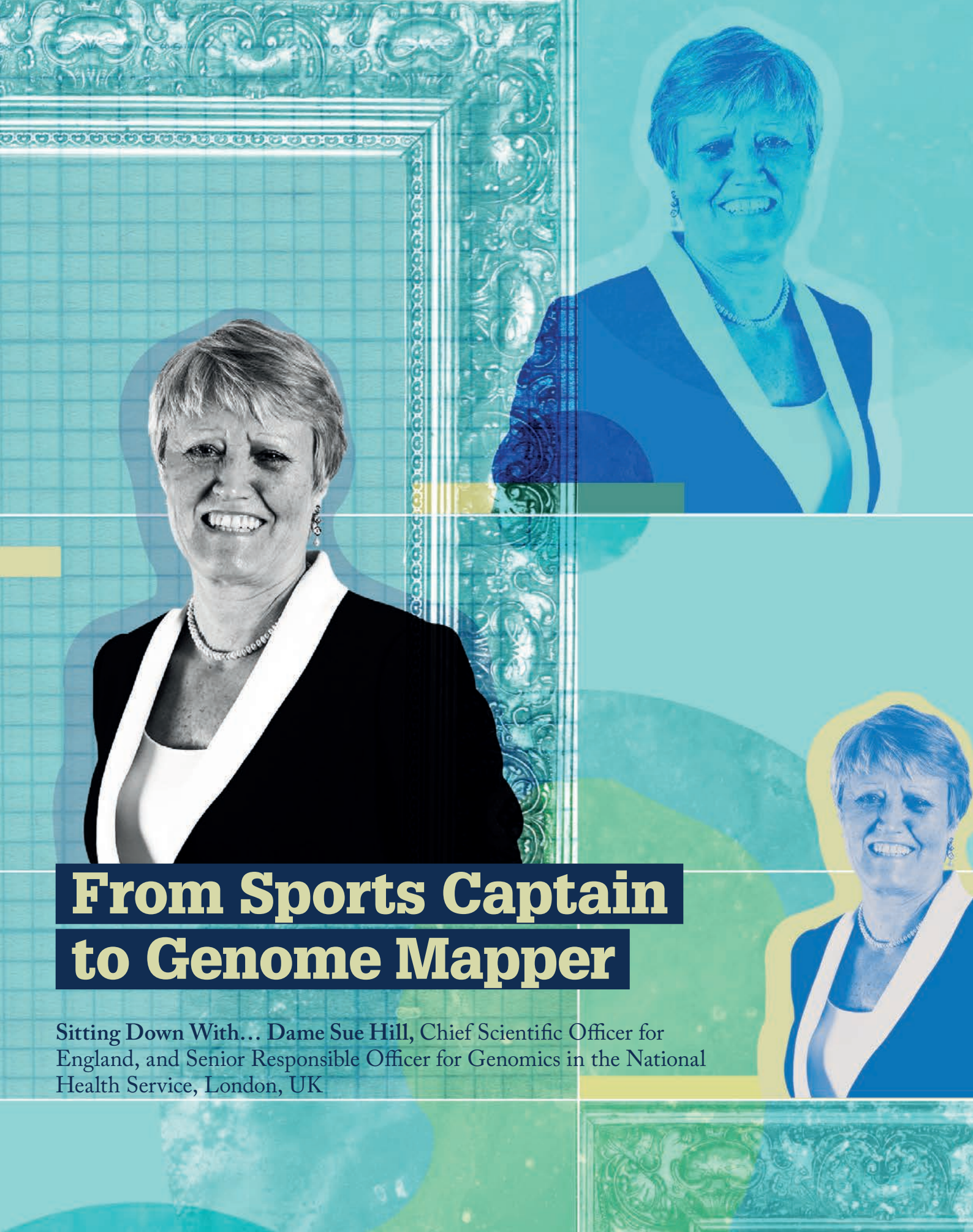

# From Sports Captain to Genome Mapper

**Sitting Down With… Dame Sue Hill,** Chief Scientific Officer for England, and Senior Responsible Officer for Genomics in the National Health Service, London, UK

What led you to a career in pulmonary pathophysiology?

My personal sporting journey drove my initial interest in human physiology. As a schoolgirl, I represented my county in hockey. I also played a variety of different sports and therefore appreciated the importance of having a highly tuned body. I became particularly interested in the development of the cardiorespiratory system, so pursued this route in my training and joined the National Health Service (NHS), where I was able to use physiological and biochemical principles to study how disease affects the respiratory system.

One turning point in my career was completing a PhD in basic science that was sponsored by, and closely linked to, industry. During my career in academia, I learned the importance of not only aligning basic research with routine care, but also working with industry on drug discovery and clinical trials. In addition, while working at University Hospital Birmingham, I led several service development and transformation projects, where I saw things from a service management and delivery perspective. When I started at the Department of Health in 2002, I brought all these elements together into the role of Chief Scientific Officer for England. There, I'm involved in everything from combining service management and planning with transformational change, right through to aligning research with routine care to drive improvement in healthcare.

What lessons have you learned about taking research to the clinic?

The first thing to consider is the basic science perspective. The crucial part here is finding the right question and ensuring that the methodology is suitable to answer that question. It's also important to be prepared to adjust the hypothesis and methodology if necessary, and to make sure everything is clearly documented. The other part is the significance of the research in terms of eventual implementation, specifically when going from basic science to the possible translational research questions that arise from initial results. Whether we're using evidence in policy to set a strategic direction or carrying out a basic science project, it's always about truly understanding the question that we're trying to answer, how we see it providing benefit, and what needs to be done to translate it. My belief is that, unless you look at it broadly from one end to the other, it's very difficult to understand where you're going to have an impact.

We've followed the above rules in genomic research in recent years. From a policy perspective, we have taken scientific evidence from research such as the 100,000 Genomes Project and applied it to clinical care so that we can drive change quickly. Our main challenge has been going from a microcosm within a single organization to implementing these changes across a very large, complex system.

What skills are required for the role of Chief Scientific Officer?

I think science is all about the spread of best knowledge and expertise. Leading scientific research projects and being involved in delivering policies to health services demands effective leadership skills – great knowledge, expertise, and the ability to influence. I think I draw a lot of my leadership skills from the lessons I learned growing up in a small village in the Cotswolds and playing sports. To get a team performing effectively in hockey, for example, you have to work with individual members of that group and build a community. I believe that, as a leader, you must have a single-mindedness. I've always been able to see and set a vision – and then take people with me on a journey based on the ultimate goal. Whether that's winning the hockey championship or leading a major transformational project in the NHS, it's the same set of principles.

Where do you see pathology and genomics going in the future?

There's no doubt that both genomic and pathologic investigations are going to be at the heart of improving disease characterization through producing an individualized set of objective measurements for patients. I think pathology and genomics are moving toward more personalization and targeted treatments, but we will only achieve the best outcomes if all of this information can be integrated. With cancer, for example, we need to integrate the histopathology findings with a breadth of other indicators; for instance, biochemical markers or diagnostic investigations, such as liver function tests.

Starting with genomics – and looking specifically at DNA – will give us a greater understanding of the underlying drivers of disease, enabling us to be more proactive and prognostic in our approach. We will continue to produce world-leading infrastructure and work toward the availability of whole genome sequencing as part of a diagnostic repertoire. I think part of the challenge is to make sure that, with genomics at the cutting edge of technology, the rest of pathology keeps up and works hand in glove to support genomics where it needs to, providing an integrated picture of an individual patient.

If you could go back to the start of your career and offer yourself advice, what would you say?

As a woman working in what, at times, has been quite a male-dominated field, one of the things I would tell myself is to be resilient. Resilience is key to being a great leader. I would also say to have fun, and always to focus on the end goal – which, for me, has always been the patient.

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