the Pathologist

SPECIAL SERIES

Oncology

UPFRONT

Single Test Boosts Childhood Cancer Care

A new approach sees improved diagnosis and treatment selection for children with cancer

Mutation analysis in oncology is a fast-growing field – but some mutations are trickier than others. Gene fusions present a challenge due to the rarity of individual fusions, the potential for atypical breakpoints, and promiscuous fusion partners. Nonetheless, it's believed that gene fusions play a role in up to 20 percent of all human cancers (1). In fact, several childhood cancers, including leukemias, lymphomas, and sarcomas, are distinguished by their potential for gene fusions. Accurate detection of these mutations is vital for effective patient care – and that's why a research team in the Netherlands has employed a method of RNA sequencing using the whole transcriptome in a bid to improve diagnostic accuracy.

The results stem from the Princess Máxima Center for Pediatric Oncology, which has increased its identification of relevant tumor characteristics by almost 40 percent since adopting whole transcriptome RNA sequencing for all of their patients (2). Using the new approach, the researchers were able to identify 83 fusions within 244 patients, 24 of which were missed by traditional methods and seven of which modified the original diagnosis or treatment.

And, although standard diagnostics identified many of the same fusion genes, the identification was often just one half of the two-sided fusion, yielding an incomplete picture.
"RNA sequencing was already used before, but only in children who were very ill, and for whom standard treatment had stopped working," said study co-author Bastiaan Tops (3). "In our research hospital setting at the Princess Máxima Center, we have implemented RNA sequencing into standard diagnostics. Our new study shows that this approach is paying off."

Whole transcriptome RNA sequencing is not without its disadvantages – most significantly, that the ideal sample size is 300 ng of RNA (with a minimum of at least 50 ng), whereas traditional methods such as RT-PCR require as little as 10 ng. Nonetheless, the study authors encountered insufficient sample volume or quality in only 3 percent of cases, making this a minor issue relative to the clinical benefits offered by whole transcriptome RNA sequencing.

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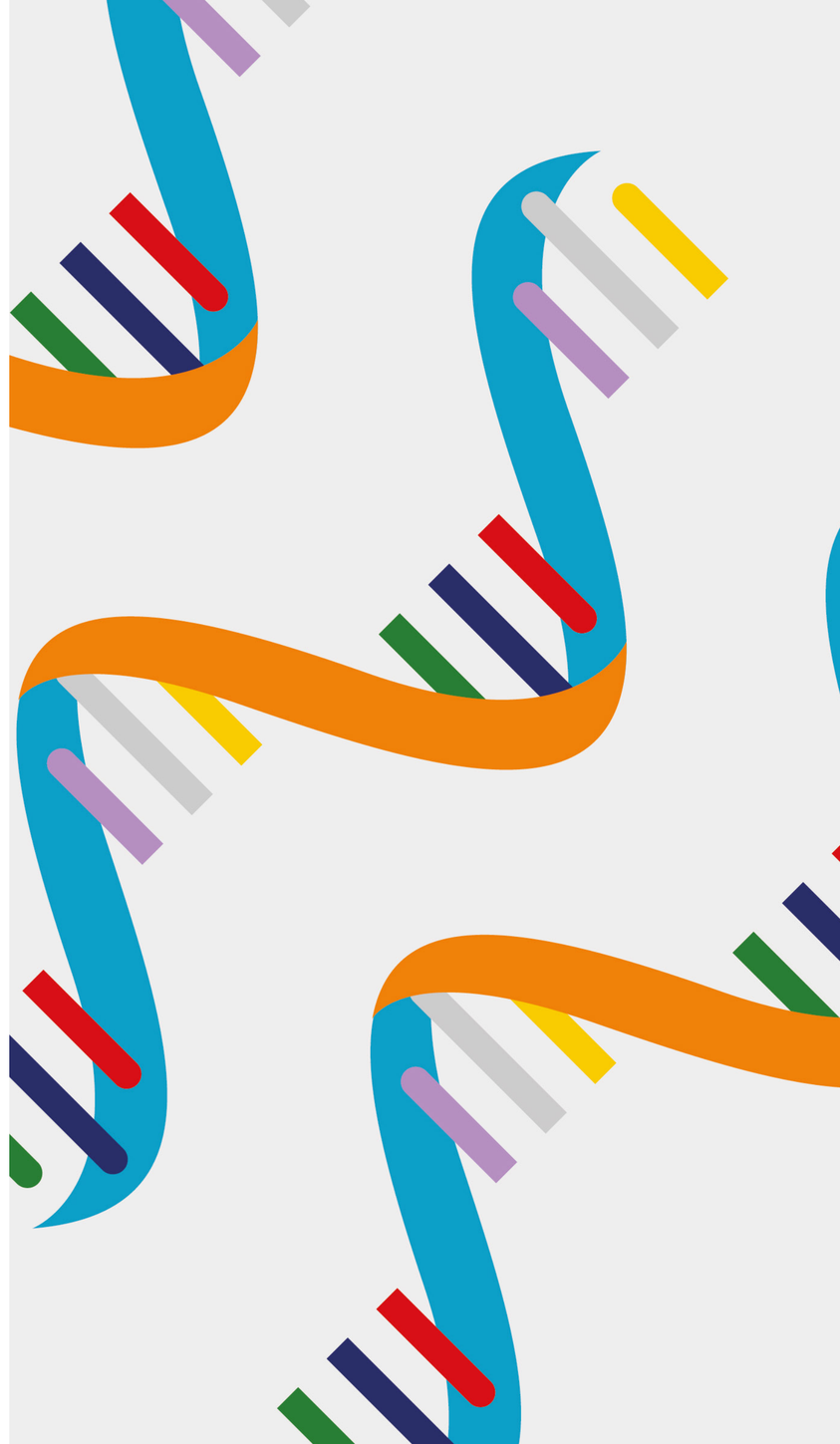

UPFRONT

Secrets of the Cervical Cells

New indices predict poor prognosis in patients with breast and ovarian cancer

"Cancer development is complex, with both germline genetic and non-genetic influences playing an essential role," says Martin Widschwendter, explaining the inspiration behind two new papers investigating the DNA methylome of cervical cells as a predictor of breast and ovarian cancer prognosis (1, 2). "The underlying and principal drive to our work is to develop novel, easy-to-apply primary and secondary cancer preventive measures," he continues. "To achieve this, we need to understand who is at high risk of developing cancer."

The team chose to study epithelial cells in both studies – why? Widschwendter says there were multiple reasons behind the decision. "The matrix that reflects influencing factors is the epigenome – and, metaphorically speaking, these risk factors leave an epigenetic footprint. The tissue from which this epigenetic footprint comes needs to satisfy three main requirements: i) the cells that act as a surrogate for the cells of origin must be easily accessible, because we cannot perform biopsies or surgical procedures to obtain at-risk tissue when the main purpose is to identify at-risk individuals; ii) the cancers of interest originate from epithelial cells and, because the epigenome in epithelial cells differs drastically to that in blood cells, the surrogate tissue must be an epithelial tissue; and iii) surrogate tissue must be hormone-sensitive, because several risk factors for breast and ovarian cancer are hormonal. The only tissue that meets all three of these requirements is that obtained from a cervical smear sample: easy to access, hormone-sensitive epithelial cells."

In the studies, the researchers developed two indices for predicting risk in breast and ovarian cancer patients. "The Women's Risk Identification for Breast Cancer (WID-BC) and Ovarian Cancer (WID-OC) indices are epigenetic (DNA methylation) signatures in cervical smear samples," explains Widschwendter. "They are based on the combination of DNA methylation levels at several CpG sites. WID indices were derived by comparing DNA methylation in samples from women with and without cancer."

What makes the studies unique is that they selectively included women whose cancers had characteristics already known to be associated with poorer outcomes. "By including women with these types of cancer, we ensured that the WID indices were designed to predict cancers with the worst prognoses," says Widschwendter. "We know that breast cancer is a heterogeneous disease and that some cancers are overdiagnosed – leading to harms associated with overtreatment. By designing our test to identify cancers with the poorest prognoses, we aim to avoid this type of overdiagnosis."

The WID indices have great potential for supporting pathologists and laboratory medicine professionals working in cancer prognostics, which could positively impact patient outcomes. Widschwendter says, "We are hopeful that, in the not-too-distant future, a WID test result derived from a cervical smear sample will afford women the opportunity to understand not only their risk for cervical cancer, but also their risk for endometrial, ovarian, and breast cancer. This will lead to tailored advice regarding primary and secondary preventive measures, and we will continue to work with pathologists and laboratory medicine professionals to achieve these goals. There is also great promise in the WID test approach to be utilized and delivered in a self-sampling setting."

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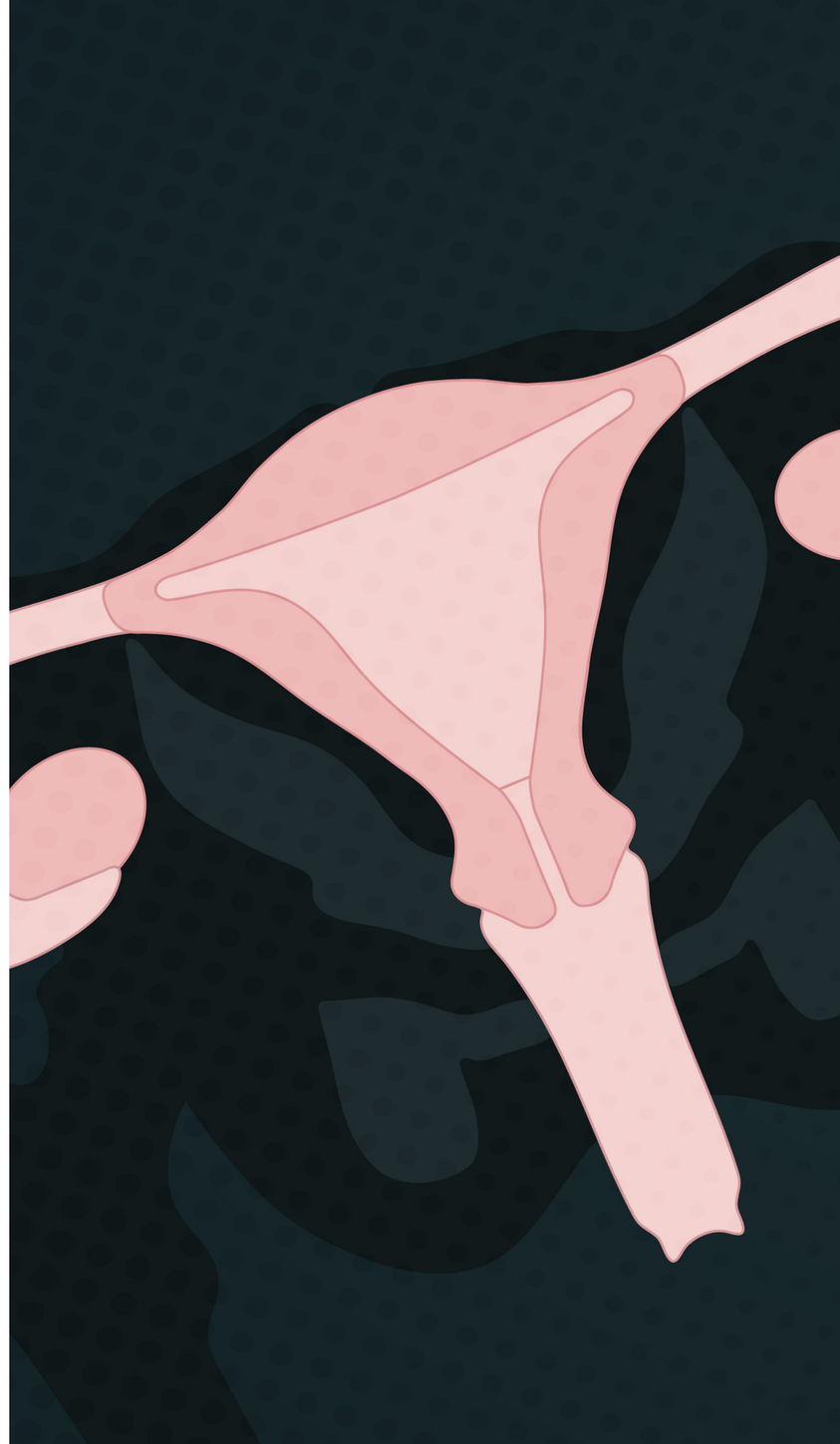

UPFRONT

Blood-Based Biopsy for Cancer-Predisposed Patients

A new liquid biopsy approach makes cancer diagnosis in high-risk patients easier and more accurate

In patients with inherited conditions such as neurofibromatosis type 1 (NF1), tumors are a frequent occurrence. Although many of these tumors are benign, some can turn malignant – but there's no easy way to tell which tumors do this and when. To address this gap, Aadel Chaudhuri and colleagues at the National Cancer Institute and Washington University School of Medicine have developed a new blood test that could free NF1 and other cancer-predisposed patients from painful biopsies and extensive imaging procedures (1).

How will the new test affect diagnostic professionals? "In the future, we see our research improving clinicians' ability to detect and track cancer in high-risk patients predisposed to the disease, such as NF1 patients at risk for malignant peripheral nerve sheath tumors (MPNSTs). One could envision our test being run routinely in pathology labs to track high-risk individuals at each clinic visit, with pathology results integrated with the clinical picture and radiology results to inform decision-making and tumor board discussions."

But the development process was not entirely smooth sailing; Chaudhuri and his colleagues encountered several challenges. When they first realized that a standard targeted hybrid-capture approach wouldn't work well for NF1 MPNST due to the relatively low burden of single-nucleotide variant hotspots, they shifted to a low-pass whole genome sequencing approach to detect and track copy number aberrations such as aneuploidy – but even that approach was not sensitive enough.

"We then observed that MPNST patients have shorter cell-free DNA fragment sizes than their plexiform neurofibroma precursor counterparts," Chaudhuri says. "Applying cell-free DNA selection for short fragment sizes and then performing genome-wide copy number analysis yielded the sensitive, specific approach we showcased in our paper for distinguishing MPNST patients from those harboring only the benign precursor lesion. We also showed that our test enables precise tracking of MPNST tumor burden, including the detection of minimal residual disease in plasma prior to radiographic recurrence." Taken together, the results suggest that plasma cell-free DNA analysis in NF1 patients has the potential to facilitate early detection of MPNST, which would enable earlier intervention and improve patient survival.

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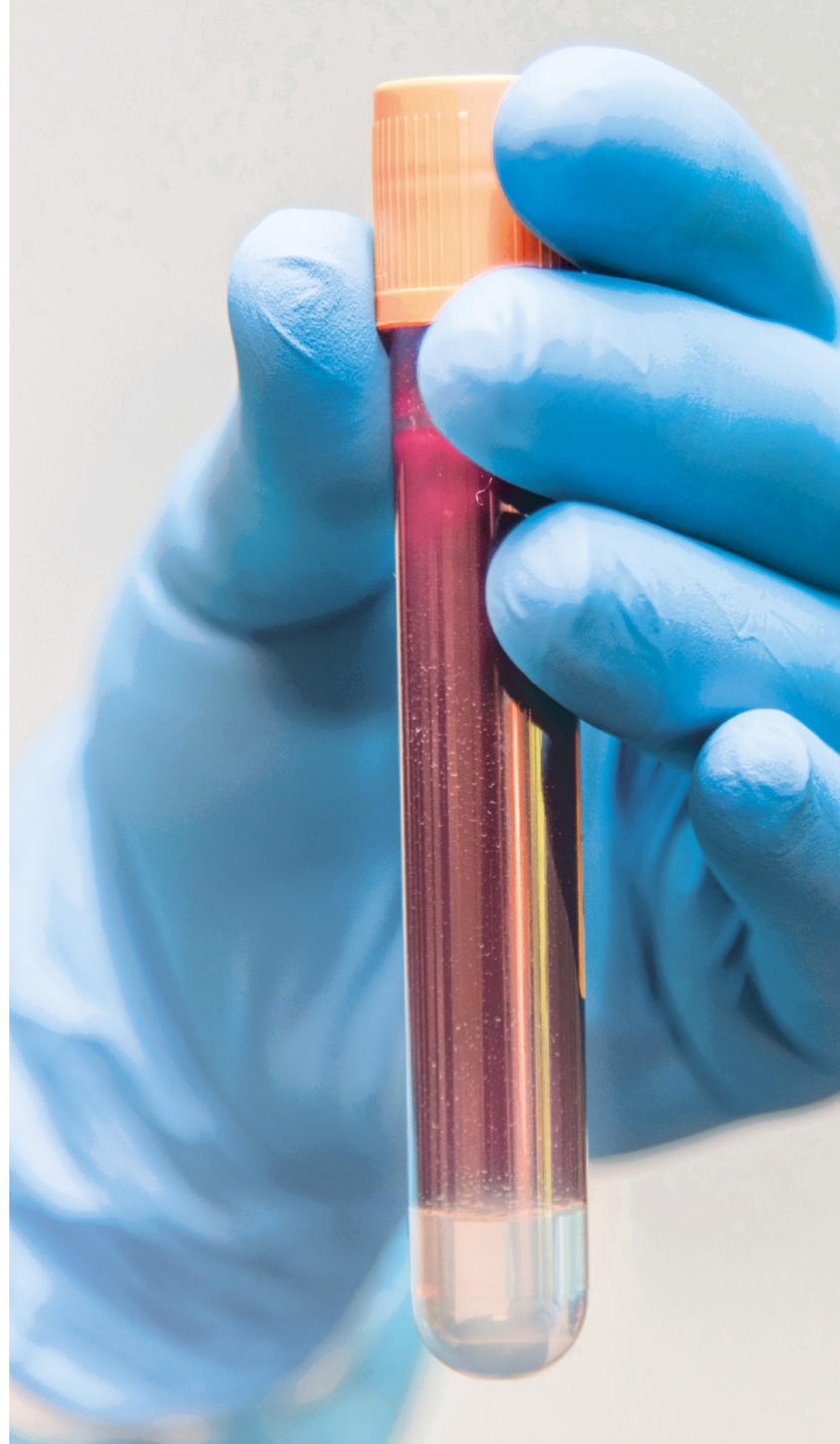

UPFRONT

The Shapeshifting Cancer

Understanding the heterogeneity and plasticity of glioblastoma

Glioblastoma is an aggressive cancer that can form in the brain or spinal cord, with an average survival outlook of 12 to 18 months – even with the wide range of treatments available. Where does the disconnect lie between relentless treatment and survival? It's near impossible to prevent glioblastoma recurrence, meaning that patients face therapy-resistant relapses with a bleak outlook.

Researchers from the NORLUX Neuro-Oncology Laboratory at the Luxembourg Institute of Health (LIH) decided that we can do better. By reviewing recent literature on glioblastoma plasticity and its role in creating heterogeneous cells, they uncovered several reasons these cancers are so good at coming back (1). Some glioblastoma cells carry stem cell properties, allowing the establishment of heterogeneity that can be difficult to overcome. To make matters worse, glioblastoma cells can also change the way they look and function in response to their environment. This plasticity, combined with the recurring nature of the tumors, makes glioblastoma an even deadlier enemy.

"Cellular states interact dynamically with each other and with the surrounding brain to shape a flexible tumor ecosystem, which enables swift adaptation to external pressure, including treatment," explained lead author Yahaya Yabo (2). Therefore, the aim of the literature review was to identify insights into potential new treatments that could target the cancer's plasticity.

The review highlighted the need for a shift in how glioblastoma and other aggressive tumors are treated, but left the door open for exactly which therapies could be effective. "[Glioblastoma] eradication will require targeting the dynamic states rather than single entities," said Simone Niclou, director of the Department of Cancer Research at LIH (2). "Further studies are needed to reveal the drivers of plasticity and treatment escape. These should address which of the changes are fast and reversible, and which are retained in tumors long after treatment."

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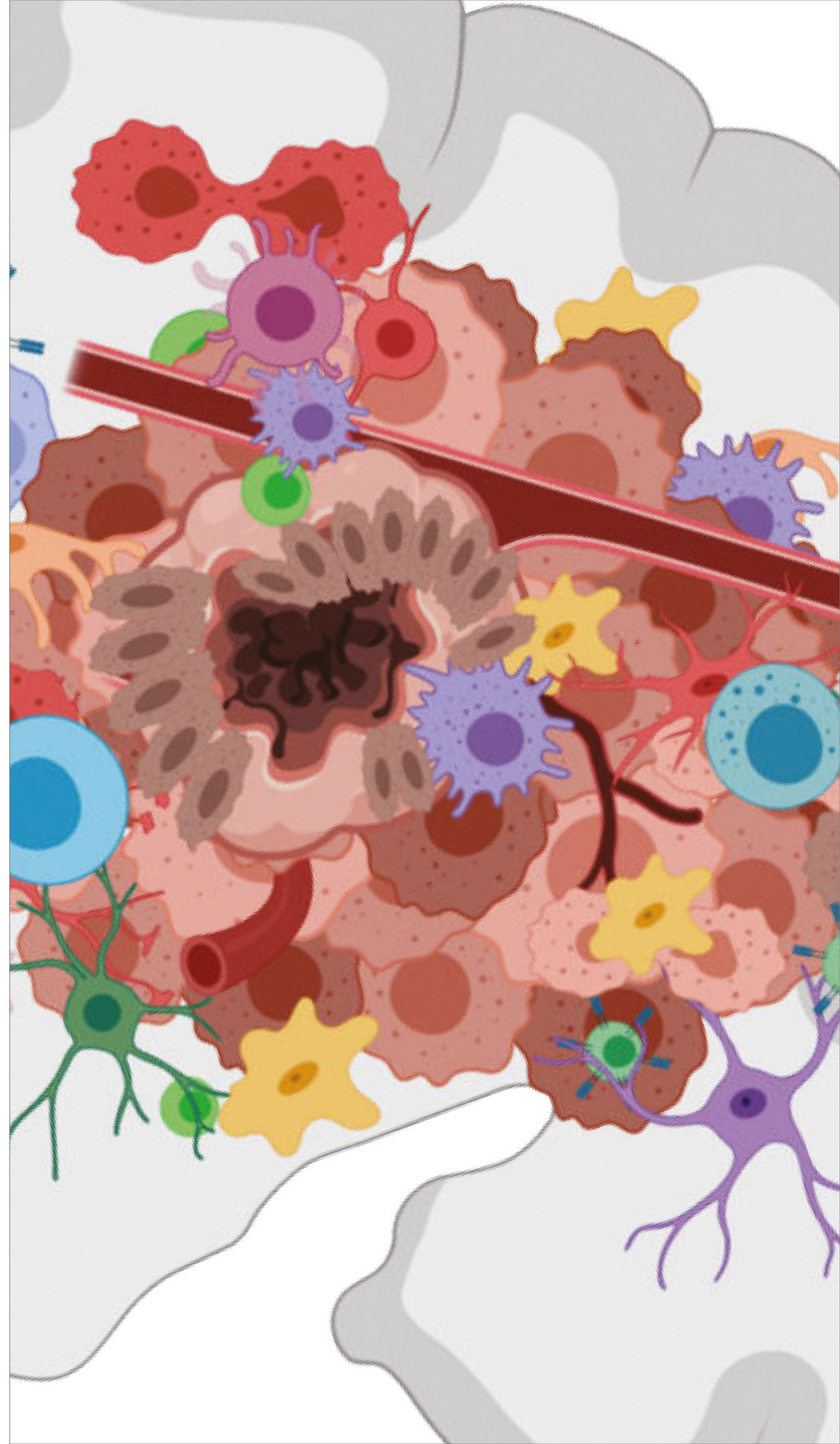

Credit: YA Yabo et al.

IN MY VIEW

Check-In Time for Cancer Screening

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

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

Beyond its direct toll on public health, the COVID-19 pandemic has also resulted in other healthcare challenges – perhaps none more noticeable than in routine cancer screening over the past year. For at-risk individuals, this could have devastating consequences. I believe that pathologists have a clear role to play in getting cancer testing back on track to reduce the chances of negative outcomes from prolonged delays.

In the last year, there has been a major drop in the number of patients seeking testing for cancer diagnosis and recurrence monitoring. A survey of more than 4,000 US adults run by the American Society of Clinical Oncology found that 24 percent of adults had delayed or canceled routine cancer screening tests due to COVID-19 (1). Separately, the Epic Health Research Network reported in May 2020 that preventive cancer screenings in the US had plummeted, with 86 percent fewer colon cancer screenings and 94 percent fewer breast and cervical cancer screenings than in prior years (2).

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

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

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

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

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IN MY VIEW

A Light in the Darkness

Spectroscopic liquid biopsy testing – a new route to brain cancer diagnostics

Matthew J. Baker is Chief Technical Officer and Co-Founder, Dxcover, Glasgow, UK

Paul Brennan is Reader and Consultant Neurosurgeon, University of Edinburgh, UK

Fast and effective routes to cancer diagnosis have never been more needed. Thanks to COVID-19, hospitals are burdened by a huge backlog of routine procedures. The scale of the impact of the pandemic on hospital care, and in particular on cancer treatment, is now becoming increasingly apparent.

We need innovative strategies to stratify patients' risk of cancer and to prioritize patients for diagnostic investigations – and here's where technologies incorporating high-level artificial intelligence (AI) could play a key role. AI applications in healthcare have progressed rapidly in the past few years and new, innovative ways of implementing AI are starting to make a real difference within diagnostics. These methods are already being used across the world; for example, AI now assists with detecting lung cancer – one of the most common cancers – from CT scans (1).

AI applications also have an important role in supporting the diagnosis of rare cancers, such as brain cancers – a traditionally difficult task. Patients most often present to primary care with nonspecific symptoms indicative of more probable non-cancer diagnoses. Referring every patient for expensive brain scans is neither possible nor cost effective. The best-performing symptom-based referral guidelines for suspected brain tumor only expect to identify a brain tumor approximately 3 percent of the time (2), so developing translatable technology that can be implemented within the clinic to improve triage for brain imaging is a major unmet need. Because smaller tumors are more often and more easily managed surgically, with less harm to the patient, early cancer detection is a key goal for improving patient outcomes.

Spectroscopic liquid biopsy is an innovative strategy for assessing blood samples – and, because it is quick and cost-effective, it could be a major game-changer in the diagnosis of cancer and other diseases. Blood samples are readily available and convenient for patients, so can be ordered earlier than current diagnostic pathways in the investigation of new-onset nonspecific symptoms. The low-cost technology, based on the interaction of infrared light with molecules present in the patient sample, generates a biological signal which can then be classified using an AI algorithm to detect cancer. In the brain tumor population, this allows the detection of disease within a symptomatic population – identifying which patients need urgent imaging and which do not.

Advances in AI have allowed us to maximize the opportunity that computational approaches offer for the detection of cancer and other diseases. If the technology is harnessed appropriately, spectroscopy-based liquid biopsy and AI have the potential to not just triage patients effectively, but ultimately increase survival rates and improve quality of life.

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FEATURE

Not Just a Sample

Patient–pathologist interactions are vital – and both sides must work together to make the connection

Michele Mitchell is a Patient Adviser and Co-Chair of the University of Michigan Department of Pathology's Patient and Family Advisory Council, Ann Arbor, Michigan, USA

In 2006, I was diagnosed with breast cancer.

I received the tentative diagnosis at work. That day, I went home and told Ray, my husband of less than a year. He agreed to help me in my fight – but, just half an hour later, Ray had a stroke. A month later, I took him off life support. One week after burying him, I began my battle with breast cancer.

I was treated at the University of Michigan's Comprehensive Cancer Center. My treatment plan included a lumpectomy surgery, chemotherapy, radiotherapy, an additional surgery, and years of anti-hormonal "pills." I am now in remission – having completed the final phase of my treatment plan in 2015 – but, like many cancer survivors, I still suffer from the side effects of treatment and I continue to struggle with thoughts of recurrence. A cancer journey is a lifelong marathon, not a sprint.

How it began

Waiting for the results of my biopsy was agonizing. It took two weeks. I couldn't sleep or eat; I worried every hour of every day. Ray was in intensive care. I wondered, if he lived, how would I care for him and manage my own breast cancer journey at the same time? What would my life be like? I wanted to get the tumor out of me. Finally, my oncologist called with the results of my core needle biopsy, which confirmed the ultrasound report from weeks earlier. It was a "malignancy." I heard the words "breast cancer" – and then my mind went somewhere else. I was numb. I had so much on my plate at that point that the news was totally overwhelming.

I still remember the day I decided I wanted to look at my own pathology slides. I already had the honor of being a patient advisor and the co-chair of UMich Pathology's Patient and Family Advisory Council. The chair of the committee, a pathologist, offered to show me my cancer and review my pathology report. At this point, it had been 10 years since my diagnosis and one year since I had completed my treatment plan.

What made me curious? When I was diagnosed, I never saw my pathology report or slides. I was told that my breast cancer had been caught early. What I knew was that I had an invasive ductal carcinoma with no lymph node involvement. It was stage I and small – 0.9 cm. My oncologist said my prognosis looked "good." Over the years, I had done a lot of research on my own – but I still had a lot of questions and looked forward to learning more.

Seeing my slides

First, the pathologist showed me "normal" tissue. Next, he put a digital slide of my tumor on the screen. He explained how they stained the slides and determined the tumor's characteristics. My tumor was "luminal A" (ER/PR+, HER2-). He explained how they determined the grade of the tumor. I had never heard of tumor grading before. I thought, "Wait a minute – I had a stage I tumor. What's a 'grade?'" My pathologist explained that the black dots I saw on the screen were cancer cells. He said they count the number of cancer cells to determine the grade of the tumor and that the grade defines the tumor's aggressiveness. I had a grade II tumor, which tend to grow more quickly and are more likely to spread to other parts of the body. My mind raced. My cancer was invasive and there are only three grades – so my breast cancer was more aggressive than I had previously understood.

I was clearly upset by this news, so we stopped for a moment so that I could process the information. The sheer "size of the enemy" is what stays with me from that experience. It has been 16 years and I still wonder whether some of those cancer cells are floating around in my body. Although it was not what I expected, I am grateful for the compassionate way the pathologist handled the visit. The encounter extended a warm touch in a world filled with barcodes, sterile instruments, and starched white coats.

From patient to advocate

I retired from my 25-year career with a large healthcare insurer in 2009. At that point, my desire for patient advocacy work led me straight to the University of Michigan healthcare system. I also serve as an advocate for the American Society for Clinical Pathology and for the American Cancer Society. I am honored and privileged to serve each of these institutions. It feels wonderful to educate and empower patients, move the needle on important issues, and make a real difference in policy, quality, and safety in health care. I view my efforts through the lens of a quote by Ralph Waldo Emerson – "The purpose of life is not to be happy. It is to be useful, to be honorable, to be compassionate, to have it make some difference that you have lived and lived well."

Michele Mitchell poses with a histopathology slide.

"The encounter extended a warm touch in a world filled with barcodes, sterile instruments, and starched white coats."

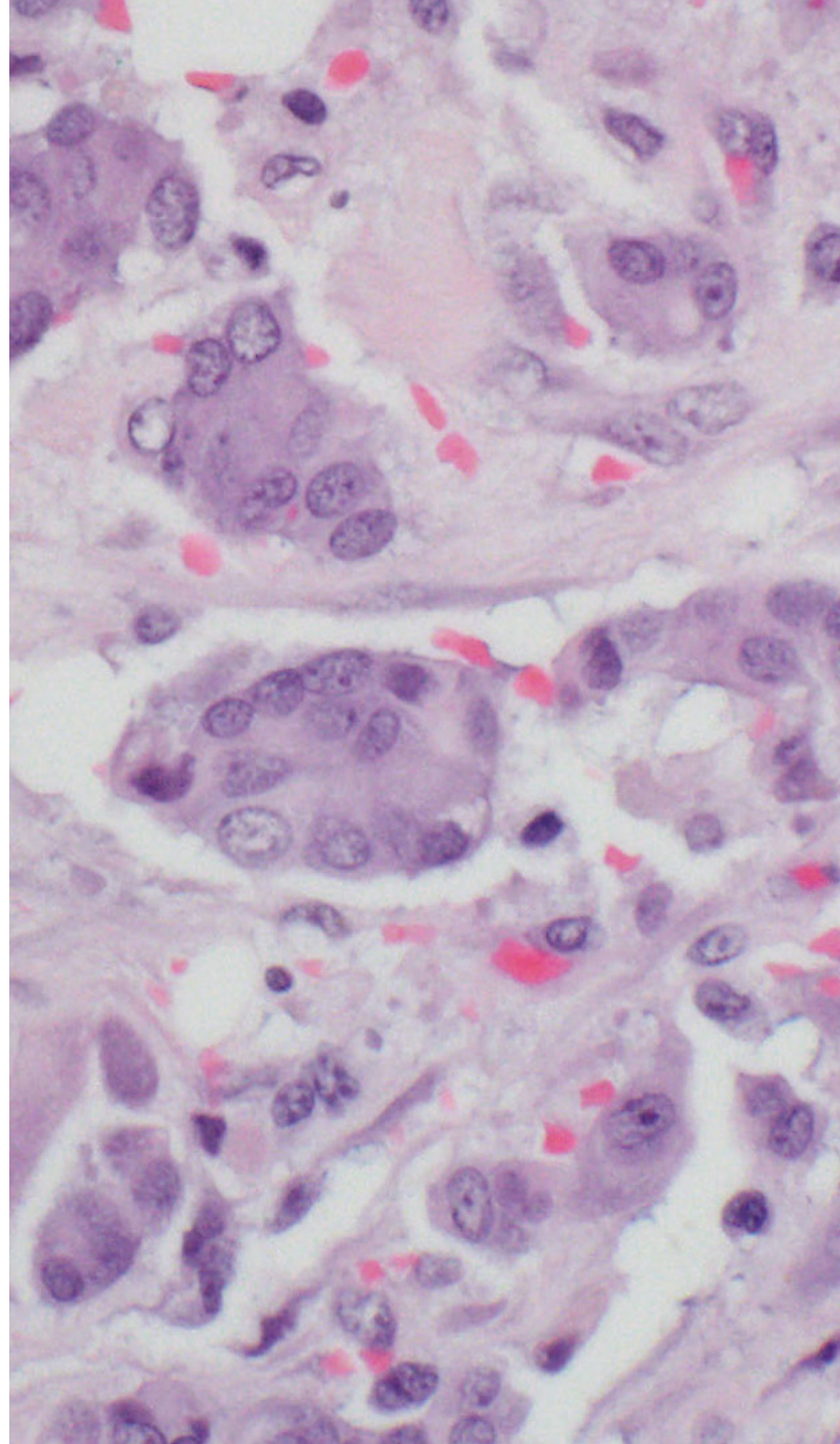
I'm no stranger to advocacy – not just for myself, but for others as well. In 2013, my stepdaughter, Tricia, lost her three-and-a-half-year battle with non-Hodgkin's lymphoma. Tricia had asked for my support on her journey and my help navigating health systems. We tried desperately to get her well – but even experimental treatments failed. She died at the tender age of 23. Next, my parents had open heart surgery 11 days apart. My Dad's lungs never recovered after surgery and he passed away in 2014. My mother's health failed shortly thereafter. She had pulmonary hypertension and congestive heart failure. We lost Mom in 2019. In 2016, my husband, Bill, had a melanoma recurrence and we discovered that he carries a MITF gene mutation that predisposes him to melanoma and renal cancer. In my role as caregiver, I helped family navigate healthcare systems, which can be challenging. My path was made clear; these experiences ignited a fire within me to pursue patient advocacy.

You can see how much pathology and laboratory medicine have touched my life – and how much they touch every patient's life.

Advocacy challenges

I think it is important to understand the history of advocacy in the US healthcare system. The Hospital Consumer Assessment of Healthcare Providers and Systems (HCAHPS) survey program over a decade ago highlighted the importance of high-quality patient- and family-centered care. HCAHPS survey results are used in the Centers for Medicare and Medicaid Services Value-Based Purchasing program and affect hospital reimbursement by up to 25 percent – so hospitals are motivated to achieve. Patient- and family-centered care should involve working *with* patients and families, rather than just working for them or doing something to them. I have witnessed some progress over the years to move from doing things on behalf of patients towards doing things in conjunction with patients – but, in my opinion, the pace is too slow.

I would love to reframe the role of councils in healthcare systems everywhere from "advisory" to more action-oriented "transformation" teams. In my experience, hospital staff bring issues to patient advocates, who then provide feedback. I would like to see advocates be given a more active role. I believe this is the next step in the evolution of the movement. Advisors can provide systematic feedback on quality and safety concerns by truly partnering with healthcare systems and organizations – moving from bystander to policymaker. With the advent of personalized medicine, AI, and a more educated patient population, I think patient advocates are ready to make real change.



Michele's cancer.

We're in an exciting and transformational time. Patients have never had more access to information about their health, including options for maintaining or improving their condition. Medical records are available online, as are patient forums, blogs, and websites. With health records now fully digitized and available on patient portals, action can be instant. At the same time – in part because of the rising costs to individuals – increasing numbers of patients are investing in their health to stay well, not just get well. Health trackers are now the norm rather than the exception. Savvy patients are beginning to understand how unhealthy behavior can impact their pocketbooks in the long run. When patients become ill, they are increasingly focused on learning more through online communities. Advanced digital communication technologies enable the delivery of chronic care at home. A patient's laptop or mobile device can be a substitute for the doctor's office through apps and virtual visits – a game-changer during the COVID-19 pandemic.

The era of the passive patient is over – and consumers of healthcare will increasingly demand better tools, personalized treatment plans, and multidisciplinary care teams.

A double-edged sword

Patients have a right to their own health information but often need assistance understanding and interpreting it. There is a vast amount of information – and, unfortunately, misinformation – out there. Patients need help to synthesize this information and I think doctors and health systems need to take on an expanded role. Trusted clinicians should provide reputable sites to search, engage in more outreach, and preemptively educate their patients. I love the phrase, "Nothing about me without me." However, patients must also take more responsibility for their own care. They need to help their providers by engaging in the prescribed treatment plan, asking the right questions, and partnering with their providers in shared decision-making.

The 21st Century Cures Act, which was signed into law in 2016 and updated in December 2020, includes provisions for making pathology reports and laboratory results immediately available to patients via electronic portals to ensure timely access to health information. The College of American Pathologists provides some guidance for how to handle publishing test results, including this note on the new rule: "Pathologists should not delay the release of laboratory and pathology results until the ordering clinician's review (1)."

Some pathology practices have responded with a great deal of anxiety; others have risen to the opportunity by rethinking the way they provide results to patients. Some practices have added disclaimers on the patient portal; others have added links to reputable sites on the resources tab so that patients can find trusted information.

Most cancer patients never meet or interact with the pathologist responsible for evaluating their tissue samples to determine the type and stage of their disease. Yet pathologists can impart knowledge about test results and better prepare the patient to participate in their own care. There is a growing movement in pathology to create opportunities for pathologists and patients to interact in what is referred to as "pathologist–patient consultations" or "pathology educational clinics." I hope that this unique approach to personalized medicine takes off and is offered at many health institutions.

Tips for Institutions

Lessons from UMich on increasing the patient's role in pathology and laboratory medical care

- Patient advisors have been added to patient safety meetings, in which participants perform root cause analyses and examine lessons learned when things go wrong.
- The team has completed work on a video project to show what pathology is all about. The 20-minute video takes viewers behind the scenes of Michigan Medicine's pathology labs with a former leukemia patient now in remission.
- Patient advisors attend the Department of Pathology Quality Council and participate on quality panels.
- The pathology department has participated in patient experience expos in which members spread the word about the impact of pathology on diagnosis and treatment.
- The group is currently working on developing a pilot patient education consultation program focusing on two disease groups: breast cancer and diabetes.
- We are addressing the Cures Act by adding a disclaimer to the patient portal and reliable resources for patients to access regarding pathology reports and lab results.

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"You can see how much pathology and laboratory medicine have touched my life – and how much they touch every patient's life."

The case for consultations

I think pathologists can offer patients a different perspective on their condition. Showing a newly diagnosed patient their tumor offers them an opportunity to understand how the diagnosis was made and to learn some of the science behind the diagnosis. The pathologist can clarify the diagnostic process, enhance the patient's overall understanding of the disease, and answer any questions – ideally without inducing "information overload." There are trailblazers out there doing this work already. For example, Lija Joseph at Lowell General Hospital, who has been conducting pathologist–patient consultation services for breast cancer patients, recently held a webinar that included a step-by-step masterclass in successful patient interactions.

Even for more experienced patients, the ability to ask questions can help decisions about a course of treatment or a clinical trial. Moreover, a conversation with a pathologist can help them understand how their treatment plan is working and offer them some feeling of control over their disease process. Knowledge is power.

There is also plenty of data on how interactions with patients can help pathologists. A recently published article indicated that 86 percent of pathologists were interested in meeting their patients (2). Pathologists identified several benefits, including increased job satisfaction through meaning and purpose. Some have described these encounters as a reason to get up in the morning and called them "the right thing to do." In addition, it may increase motivation to complete the less interactive parts of their work. Other positives include creating a more multidisciplinary work environment; pathologists have unique expertise and including them in the care team could reduce clinician burden. Pathologists are often referred to as "the doctor's doctor" – but they can be much more than that. A pathologist can also be the patient's doctor and a vital part of their care team.

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FOUNDATION

Determining Microsatellite Instability: The Ideal Method

Seeking cancer diagnostic and therapeutic guidance with microsatellite instability testing

Richard Hamelin is Research Director (Retired) at Inserm, Paris, France

Microsatellite instability (MSI) in tumors is an approved biomarker for breakthrough immune checkpoint inhibitor therapy (1), with polymerase chain reaction (PCR) testing currently the most direct, accurate, and cost-effective measurement method. In 2017, the US FDA granted the first tissue-agnostic approval for pembrolizumab – a monoclonal antibody that blocks immune suppressing PD-1/PD-L1 receptor interactions, or "immune checkpoints" – for patients with unresectable or metastatic MSI or DNA mismatch repair-deficient (dMMR) solid tumors. Moreover, December 2020 saw the European Medicines Agency (EMA) adopt a new indication for pembrolizumab as a first-line treatment for metastatic colorectal cancer based on MSI or dMMR biomarker status (2).

These approvals were based on clinical trial data demonstrating that dMMR or MSI was able to predict treatment response across 12 different solid tumor types, including colorectal cancer (3, 4, 5). Given the compelling therapeutic rationale for measuring dMMR and MSI and the fact that MMR deficiency has been identified in up to 20 percent of different solid tumors (3), the benefits of detecting these biomarkers accurately and affordably are clear.

There's an indelible biological link between dMMR and MSI (6). The MMR system of proteins recognizes and repairs DNA base pair mistakes, insertion and deletion errors (indels), and DNA damage that occurs during replication and recombination. Mutations in genes encoding MMR proteins can cause DNA mismatch repair defects throughout the genome, including within microsatellites – widely distributed stretches of DNA composed of short (up to six base pairs) motifs repeated up to 50 times.

Just like gene coding sequences, microsatellites can accumulate errors from dysfunctional DNA repair, including base-base mismatches and small indels that differ from the inherited microsatellite (7). MSI – the accumulation of these errors – reflects overall tumor genetic instability, whereas indels in coding sequence microsatellites may lead to frameshift mutations. In tumors driven by dysfunctional DNA repair, genetic instability is responsible for the increased tumor mutation burden that drives immune cell infiltration into the tumor microenvironment (TME) (5, 6). As a survival strategy, tumors can inhibit immune cell activation in the TME by engaging immune checkpoints. MSI and dMMR can signpost tumors that are more likely to respond to immune checkpoint inhibitor therapies (3).

"If we only employ one method for detecting MSI and dMMR, it must be the most direct, accurate, and efficient method out there."

The MMR system includes a number of proteins including MLH1, MSH2, MSH6, and PMS2 (8) – and dMMR is often assessed in tumor cells by the absence of immunohistochemical (IHC) staining for any one of these four major proteins (9, 10). MSI is detected either by PCR amplification of tumor DNA or by next-generation sequencing (NGS) methods (8, 11, 12); however, neither approach is optimized for MSI detection and not all platforms use the most sensitive marker panels. NGS is also expensive and not all NGS biomarkers have been clinically validated. Concordance between IHC and MSI assessment of tumors is high (approximately 90 percent), tempting pathologists to rely on one method alone (9, 13) – but, if we only employ one method for detecting MSI and dMMR, it must be the most direct, accurate, and efficient method out there.

IHC detection of dMMR

IHC detection of dMMR is based on specific antibody recognition of MLH1, MSH2, MSH6, and PMS2 in tumor cell nuclei (10), with the absence of an IHC signal indicating dMMR. IHC protocols are simple, rapid, inexpensive, and require minimal specialized instrumentation.

A distinct advantage of IHC for dMMR detection is that it reveals the identity of mutated MMR genes by lack of IHC staining – using specific antibodies against the wild-type proteins. However, IHC staining doesn't cover all MMR genes (12), requires a tissue sample large enough to perform four separate incubations, and may be unreliable (10). For example, tumors from patients exposed to preoperative chemotherapy or radiation therapy are more difficult to assess using IHC due to artifactual loss of MSH6 protein expression (14).

The major caveat with IHC dMMR detection is the potential disconnect between MMR protein antigenicity and function (9, 10). Some missense mutations involving only a single nucleotide can lead to nonfunctional MMR proteins that are nevertheless recognized by antibodies, resulting in a false negative result (15). In addition, MMR gene mutations can code for unstable truncated proteins that can stain with the IHC test sample before degrading in the remaining tumor tissue. This leads to false negatives in up to 10 percent of samples (5, 16). On the other hand, false positives can be caused by missense mutations that lead to the loss of antibody recognition without compromising protein function (9, 10). Clearly, equivocal IHC results must be verified by follow-up or tangential PCR MSI testing.

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