APRIL 2019 # 53

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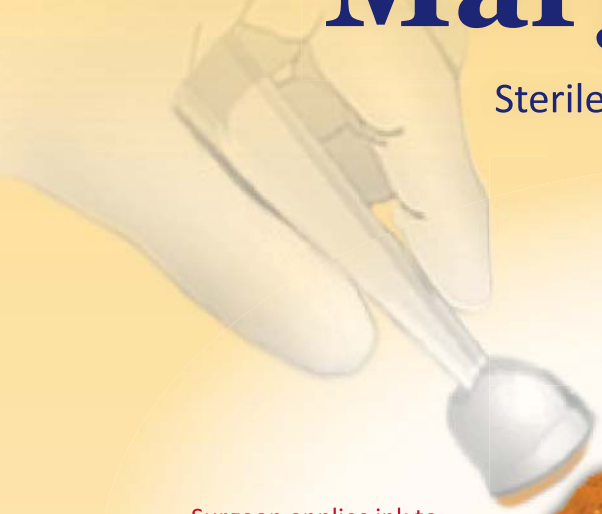
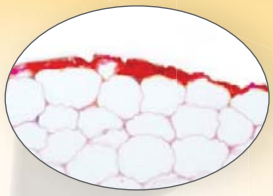
CSI: Cretaceous

Disease and death in the days of the dinosaurs

MarginMarker™

Sterile Ink Kit for Intraoperative Use





Superior

Colors show brightly on slides

Surgeon applies ink to designate each margin

Medial

Lateral

Inks adhere throughout processing

Inferior

Pathology Receives Specimens With Margins Clearly Marked by the Surgeon

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

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American Society of Breast Surgeons 20th Annual Meeting | Dallas, TX | April 30-May 5
American College of Mohs Surgery 51st Annual Meeting | Baltimore, MD | May 2-5

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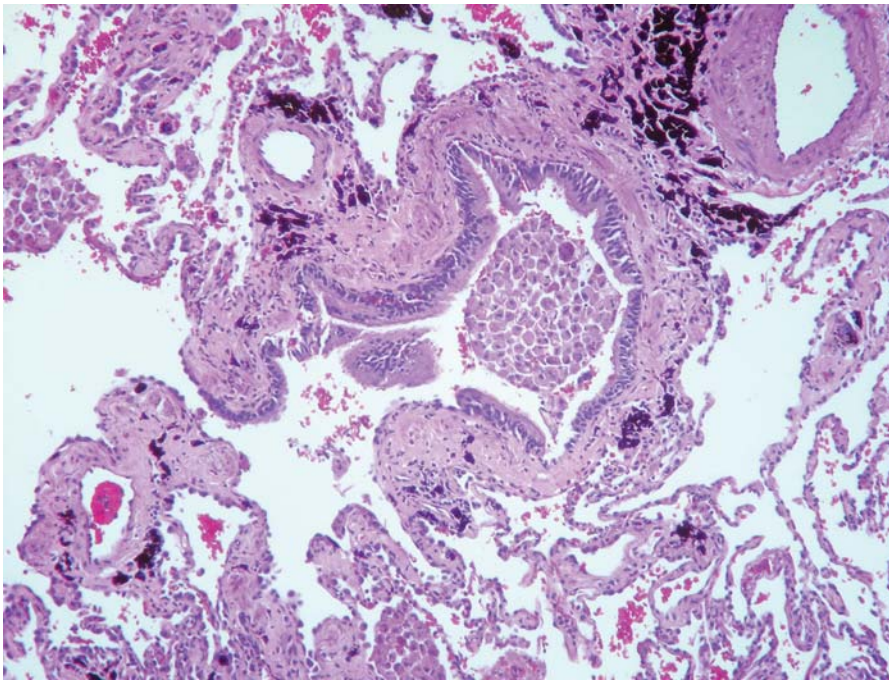
Case of the Month

A male smoker in his 60s presented with a lung mass. Lobectomy was performed following a core biopsy that showed adenocarcinoma.

What is your diagnosis?

- *A* Chronic bronchiolitis
- *B* Respiratory bronchiolitis
- *C* Constrictive bronchiolitis
- *D* Cellular bronchiolitis



Answer to last issue's Case of the Month…

A. WAGR

This photo shows the characteristic features of a Wilms tumor, which may occur as part of several congenital syndromes including WAGR (Wilms tumor, aniridia, genitourinary anomalies, and mental retardation), a syndrome involving mutations of the *WT-1* gene.

To register your guess, please go to http://tp.txp.to/0419/case-of-the-month
We will reveal the answer in next month's issue!

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Across paleontology and pathology, scientists search for ancient soft tissue clues

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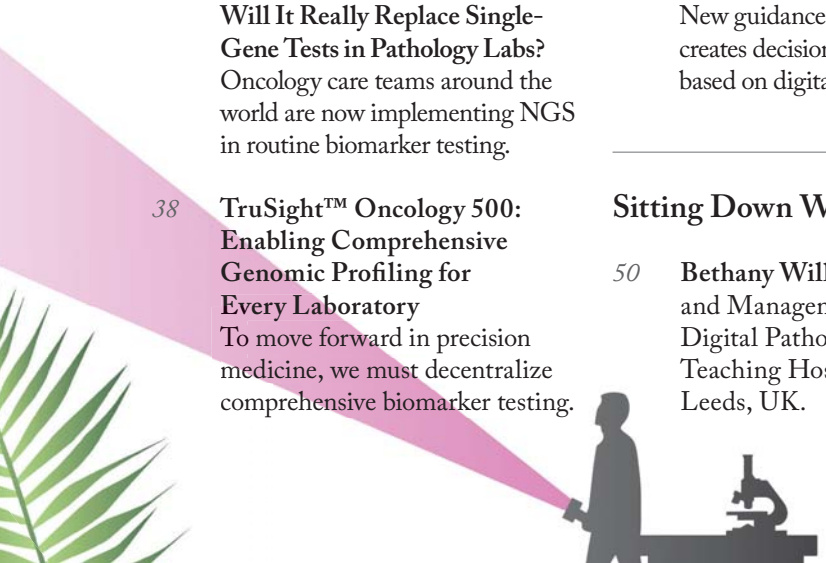

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Ancient Inspiration

From dinosaur diseases to laboratory medicine – a journey of curiosity

"Did dinosaurs get cancer?" My simple question kicked off a less-than-typical dinner conversation back in the early 1990s – but I firmly believed it raised a valid scientific point. After all, modern animals can and do develop the disease, so why shouldn't it have existed in prehistory?

Of course, when I asked the question, Google was unheard-of and children's encyclopedias focused mainly on the all-important questions of how big dinosaurs were, where they lived, and what they ate. That's not to say those factors couldn't have influenced their health, but it seemed no one was able to tell me what I wanted to know.

Now, over two decades later, I not only have my answer, but I've also had the privilege of getting to know people who can address every facet of my question. Of course, there are the modern-day pathologists and laboratory medicine professionals, who generously share their expertise on all kinds of disease. There are paleopathologists who have told me about their work on ancient humans. And there are paleopathologists who work on much older fossils, who have not only assured me that, "Yes, dinosaurs did get cancer," but explained in detail how we can be so certain – to the point where I'm pleased to feature it on the cover of this month's issue of The Pathologist!

It's a story I've heard all over science and medicine – a childhood curiosity or inspiration that led, eventually, to a career in the laboratory. Of course, as children, many of us weren't even aware of the term "pathologist" – so we couldn't have considered it as a career option. And that's something I'd like to see change – but, regardless of whether or not children know the words for such professions, it's important for us to ensure that we welcome and encourage the next generation of potential laboratorians. Some may be inspired by questions that find us on familiar ground (for instance, I was recently asked what blood is made of). Others may want to know about more esoteric subjects… To which end, if you feel the need to shore up your knowledge of dinosaur diseases, enjoy our feature on page 18! Only by fostering natural and open curiosity can we help others see that, in science, all roads eventually lead to the laboratory – and the laboratory itself can lead anywhere!

Michael Schubert
Editor

Upfront

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

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

Email: edit@thepathologist.com

Casting a Broader Net

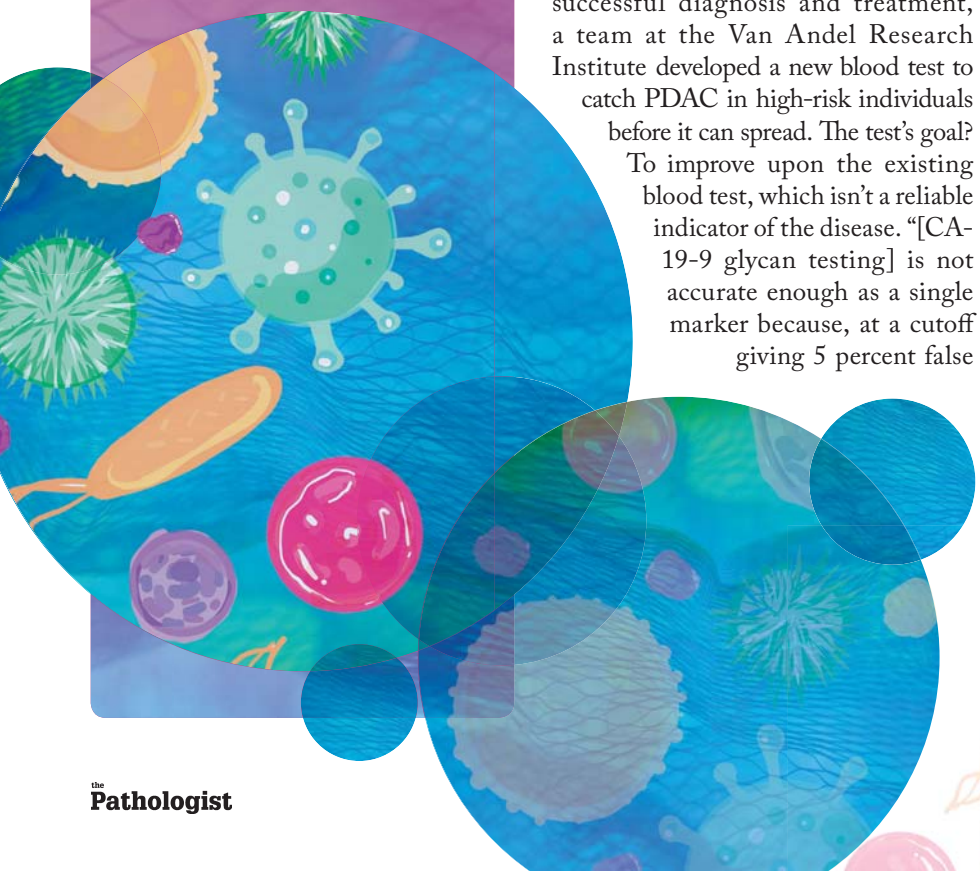
Can a new combination of blood tests for pancreatic cancer improve detection rates for a disease in which early diagnosis is notoriously difficult?

Outcomes for patients with pancreatic ductal adenocarcinoma (PDAC) are poor – only 8.5 percent of patients diagnosed with the most common form of pancreatic cancer survive past five years. That's approximately the same chance of survival as those diagnosed with the same disease 30 years ago… so why haven't we seen any improvement?

The disease lacks early symptoms, hindering early diagnosis, and to make matters even more difficult, PDAC typically spreads aggressively before detection. So, to improve the odds of successful diagnosis and treatment, a team at the Van Andel Research Institute developed a new blood test to catch PDAC in high-risk individuals before it can spread. The test's goal? To improve upon the existing blood test, which isn't a reliable indicator of the disease. "[CA-19-9 glycan testing] is not accurate enough as a single marker because, at a cutoff giving 5 percent false positives, it only detects around 40 percent of cancers," explains Brian Haab, senior author of the study. After realizing that glycans with a similar structure to CA-19-9 could also represent pancreatic cancer biomarkers, the team screened numerous others to identify the sialylated tumor-related antigen (sTRA). Is it a replacement for CA-19-9? Not necessarily; by combining the two, the researchers observed improvements in PDAC detection rate.

"The sTRA glycan is produced and secreted by a different subset of pancreatic cancers than those that produce CA19-9," Haab explains. When combining the two tests and applying a cutoff to give less than 5 percent false positives, the new approach detected around 70 percent of PDAC cases in people with benign conditions of the pancreas (1). This means that labs could spot pancreatic cancer subtypes that might have been missed by the CA-19-9 test alone, casting a broader net on PDAC.

Haab hopes that the new combined panels can improve diagnosis and surveillance for patients with suspected pancreatic cancer. "The aim is to be able to monitor for incipient cancer in people at high risk, such as those with chronic pancreatitis, pancreatic cysts, family history, or new-onset type 2 diabetes." The team are currently working toward real-time prospective studies in a clinical lab before collaborating with clinical partners to offer it as a lab-developed test. If successful, they envisage the test being rolled out widely as a screening tool for high-risk individuals.



Reference

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Unraveling the D-Amino Acid Mystery

Three years after D-amino acids were first identified as possible markers for chronic kidney disease, have they lived up to their potential?

Catching chronic kidney disease (CKD) early is critical – patients have a heightened risk of life-threatening cardiovascular diseases and increasingly progress to end-stage kidney disease. But despite an estimated 850 million cases throughout the world (1), the early referral of patients with CKD remains a challenge due to inadequate tests.

In 2016, we reported on a breakthrough in the quest to identify an effective biomarker for early CKD diagnosis: a team from Osaka University found that levels of D-amino acids could predict progression to end-stage kidney disease (2). However, at the time, first author Tomonori Kimura told us: "the D-amino acid world is a mystery," clouding the potential clinical applications of their discovery. Three years on from their initial research, how close have the researchers come to solving that mystery?

D-amino acids are the enantiomers of L-amino acids and – although only trace amounts are present in humans – are gaining attention as potential biomarkers for a number of diseases. "In our previous study, we saw that the level of D-serine in the blood was well correlated with the estimated glomerular filtration ratio (GFR), which is a key marker of kidney function," says Kimura. In a new study, the team delved deeper into the diagnostic value of D-serine and applied micro-two-dimensional high-performance liquid chromatography (2D-HPLC) to assess its efficacy as an early indicator of CKD.

Their discovery? That D-serine levels in the blood of CKD patients correlated with GFR at a rate equal to or better than existing markers of kidney disease. In addition, the level of D-serine in the urine provided important information on kidney function other than GFR. "To screen for CKD, it is best to monitor D-serine in both the blood and the urine; it can serve as a dual biomarker for both the prediction of kidney function and the detection of CKD," Kimura explains.

Current markers for the estimation of GFR include serum creatinine and serum cystatin C – but both are affected by other factors, such as muscular mass. "One of the main advantages of using 2D-HPLC to measure D-serine is its sensitivity and precision – the ability to potentiate absolute quantification removes any small variations in measurements."

The new research found that the kidney's balance between excretion and reabsorption of amino acids is controlled by chiral selectivity. This makes the reabsorption of D-serine sensitive to the presence of CKD, allowing testing to distinguish patients with the disease from those without. As we take one step closer to unraveling the mystery of D-amino acids, Kimura believes that there is plenty of potential to fulfill: "Because CKD is prevalent in patients with lifestyle-related diseases, such as diabetes, hypertension, and cardiovascular disease, understanding D-serine is likely to improve prognosis and therapy discovery in these, too."

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Circular RNA Makes Its Mark

A new technique trumps other detection methods, expanding circRNAs' significance as potential cancer biomarkers

On the surface, circular RNA (circRNA) looks like a great candidate for a cancer biomarker. These non-coding RNAs have a closed continuous loop structure and, though less abundant than messenger RNAs (mRNAs) in biofluids such as urine and saliva, are highly stable. And that means they can be detected in situations where mRNAs often degrade.

Unfortunately, the two techniques commonly used to profile circRNAs – RNAse R enrichment and ribo-depletion – both have drawbacks. RNase-R enriches circRNAs by degrading linear RNAs, rendering it unsuitable for clinical sequencing. Ribo-depletion preserves both circular and linear RNAs by depleting ribosomal RNA, but requires a large amount (5 μg) of sample to yield reliable results. In search of a more practical solution, a group at Michigan Medicine devised a novel approach using exome capture RNA sequencing.

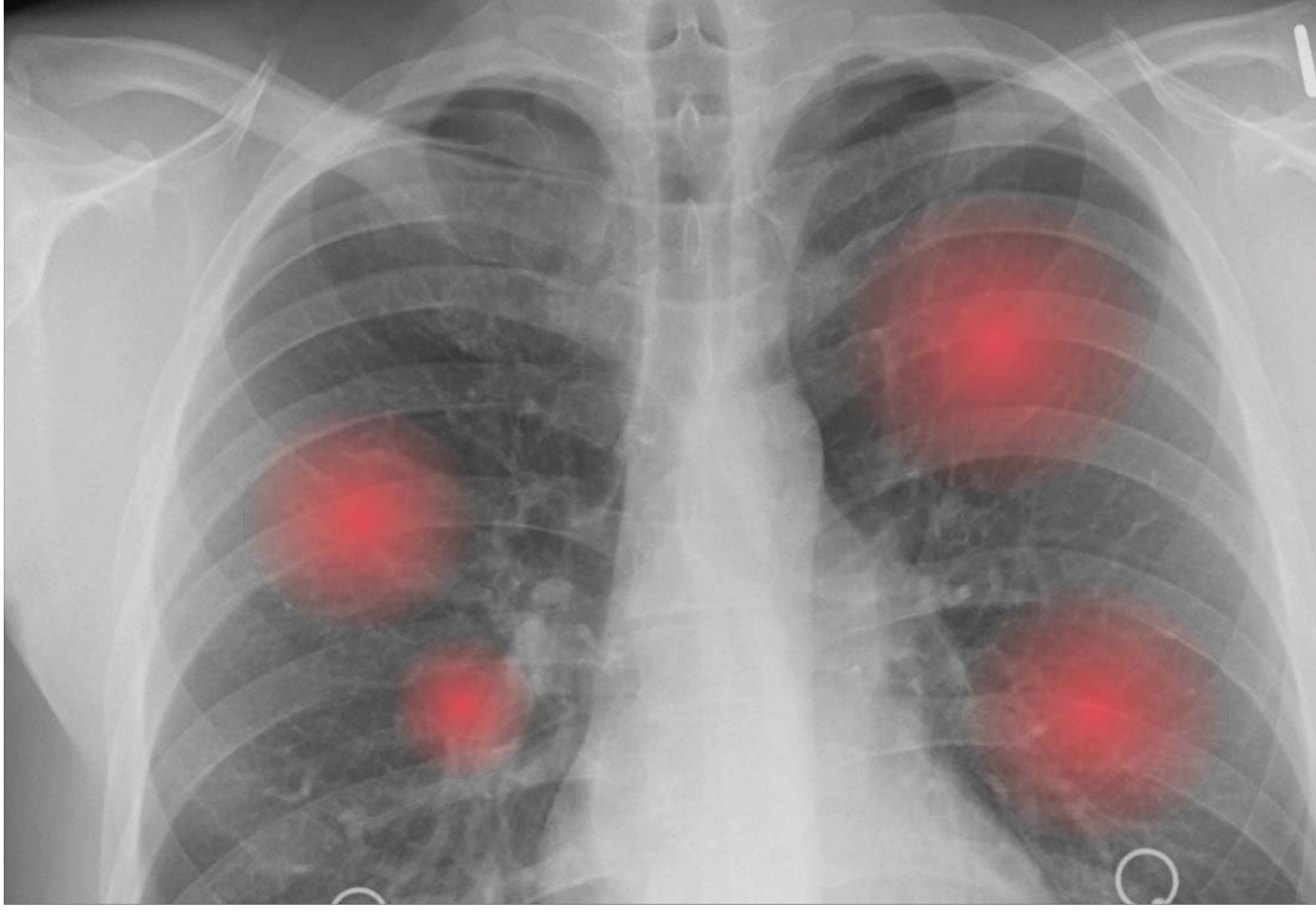
Arul Chinnaiyan, Director of the Michigan Center for Translational Pathology and S.P. Hicks Professor of Pathology at Michigan Medicine, says, "Using exome capture sequencing achieves overall circRNA enrichment comparable to that of the RNase R method, but still keeps all mRNA information intact." The new sequencing method needs only a small amount of RNA to pinpoint circRNAs and has already shown its value in identifying prostate cancer-specific circRNAs in urine samples (1). The team analyzed samples from hundreds of tumors and detected large numbers of previously unknown circRNAs, which they added to a new database – MiOncoCirc – to serve as a resource for future study. "Our resource is the first and largest cancer-focused database of circRNAs curated from clinical sequencing. We hope that it will enable researchers to mine for meaningful cancer biomarker candidates."

Chinnaiyan also hopes that circRNAs, which may be tissue-specific, could be useful as surrogate markers for various types of cancer in future noninvasive tests. "We think this is achievable if we can further refine the list of circRNA candidates and optimize our capture protocol," he says.

The current design of the team's capture panel focuses mainly on coding genes, but they are also systematically investigating the ability of non-coding RNAs to form circRNAs. Another promising direction is to search for circRNAs derived from genomic structural rearrangements, such as gene fusions. With plenty of scope for further research and optimization, will we one day see circRNAs running rings around other cancer biomarkers?

Reference

1. *JN Vo et al., "The landscape of circular RNA in cancer", Cell, 176, 869–881 (2019). PMID: 30735636.*



Out with the Old

Could second generation interferon-γ release assays replace existing tests and transform TB diagnosis through improved sensitivity?

Tuberculosis (TB) testing is big business: tens of thousands of patients who display suggestive symptoms are tested in the UK alone. In countries where TB incidence is relatively low, interferon-γ release assays (IGRAs) are commonly used for diagnosis – but new research has found that these blood tests cannot accurately rule out the disease. In addition, second-generation IGRAs showed much higher sensitivity than existing tests (1), potentially heralding their replacement.

Early diagnosis is essential in suspected TB cases, both to speed patient treatment and to prevent the spread of disease. But a study by Ajit Lalvani, Chair in Infectious Diseases at the National Heart and Lung Institute, and colleagues highlights the issues with current testing. From 845 adults enrolled in the study – including 363 who had TB – the sensitivity readings of the T-SPOT.TB and QFT-GIT tests were 81.4 and 67.3 percent, respectively. "There is a huge unmet clinical need for better diagnostic tests for active TB," Lalvani says; a highly sensitive test that could rapidly rule out the disease soon after initial clinical presentation might be just what the doctor ordered.

In the same patient population, second-generation IGRAs detected TB with 94 percent sensitivity, a substantial increase on currently available tests. "The enhanced sensitivity is conferred by the inclusion of a novel Mycobacterium tuberculosis antigen, Rv3615c, which is recognized by T cells in most TB patients, but not in uninfected individuals," explains Lalvani. "Many patients who do not respond to existing IGRAs have T cells that recognize this new antigen, making it more suitable for use in suspected cases."

Lalvani hopes that more accurate tests will save time and resources, ensuring that patients who require treatment receive it promptly. "Current methods are expensive and time-consuming – in our cohort, 28 percent of patients who were treated for TB didn't actually have positive cultures once the laboratory results came back." Before it can enter the clinic, the new IGRA must go through regulatory approval, but its creators hope to achieve a quality-assured commercial diagnostic kit within two years. "It will transform the diagnostic process in developed countries with low-to-moderate TB burden," says Lalvani, anticipating a future where suspected TB patients have a definitive answer within 24 hours of presentation.

Reference

1. *HS Whitworth et al., "Clinical utility of existing and second-generation interferon-γ release assays for diagnostic evaluation of tuberculosis: an observational cohort study", Lancet Infect Dis, 19, 193–202 (2019). PMID: 30655049.*

In My View

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

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

Contact the editors at edit@thepathologist.com

Act Fast Against Infection

A united front against antimicrobial resistance requires rapid diagnostics

By Matthieu Legrand, Medical Director of the Surgical and Burn Intensive Care Units of St-Louis Hospital, Paris, France

The death toll from antibiotic-resistant infections in Europe is approximately 33,000 per year, according to statistics from the European Centre for Disease Prevention and Control. This number is alarming in itself – and that's even without adding on deaths associated with antifungal-resistant infections, or those that take place in the rest of the world. With such a stark view of our current infectious disease reality, it's clear that there is a need for clinicians and healthcare professionals to take action against antimicrobial-resistant infections. And, with European Congress of Clinical Microbiology and Infectious Diseases just around the corner, now is an especially relevant time to examine the situation from the infection management perspective – from blood draw to pathology.

As a critical care and burn unit anesthesiologist who has a special focus on resistance and laboratory work, I often care for patients with harmful infections, many of which are drug-resistant and some of which even lead to sepsis. I know how crucial the efficiency of the initial infection diagnosis and treatment plan is and how it impacts all of the healthcare professionals involved in a case, regardless of specialty.

Under the current standard of care, clinicians start their patients on broad-spectrum antimicrobial drugs while they wait for positive blood cultures to confirm or rule out a bloodstream infection. These results take one to three days (sometimes longer), and if the patient does have an infection, we then need subsequent testing – and even more time – to determine pathogen susceptibility to specific medications. As a result, clinicians may learn after days of treatment that the initial drugs they used were not effective. In fact, initial empiric therapy is often ineffective in patients with sepsis, where too many delays can lead to death. Furthermore, each unnecessary use contributes to building antimicrobial resistance and requires de-escalation – and clinicians may find that the medication they've selected isn't working because their patient's pathogen is resistant to that therapy, leading to poorer overall outcomes.

Multidrug-resistant bacteria threaten patients in a variety of ways (1,2), which is why it's so important for clinicians to identify and treat them quickly. But, with today's infection diagnosis and treatment process, one of the key issues associated with antimicrobial resistance is the time it takes to receive information vital to selecting the correct treatment. Fortunately, scientific advancements can help diagnose and treat patients more quickly. For example, a study conducted in 2016 on the detection of circulating Mucorales DNA (cmDNA) for the early diagnosis of invasive wound mucormycosis (IWM), "suggests that the detection of cmDNA allows earlier diagnosis of IWM in severely ill burn patients and earlier initiation of treatment. (3)" There is now also lab technology that can rapidly diagnose infectious pathogens in bloodstreams and identify their resistance to specific antimicrobial drugs. In fact, we will soon be able to provide both of these results directly from patients' blood draws in a matter of hours, rather than days. Not only can this

type of innovation help to properly treat resistant pathogens sooner, but it can also inform the research and development of new drugs to sidestep resistance.

At a time when improving clinical outcomes is of the utmost importance, investing in technology that can help fight antimicrobial resistance should be a united priority among healthcare professionals. In addition to saving more lives, such innovations have the potential to streamline workflow for laboratorians and pathologists who would no longer have to rely on the time-intensive blood culture process. They can encourage continuous, dynamic interaction between clinicians and pathologists to guide treatments – potentially reducing inappropriate treatments, antibiotic resistance, hospital stays, and readmission rates. Rapid diagnostics are no longer a whim of the future – and it's the responsibility of clinicians and healthcare executives to recognize that implementing them should be the new standard of care.

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2. *T Vauchel et al., Am J Infect Control, [Epub ahead of print] (2018). PMID: 30503627.*
3. *M Legrand et al., Clin Infect Dis, 63, 1312–1317 (2016). PMID: 27535951.*

Open to the Future

NHS diagnostics must embrace unprecedented openness if AI is to work

By Jane Rendall, Managing Director at Sectra, Ltd., Stansted, UK

There is no way any one vendor could ever hope to develop even a fraction of the potential of artificial intelligence (AI) in diagnosing illnesses. It might sound strange for a representative of a UK National Health Service (NHS) diagnostic technology provider to start an opinion piece by saying what such a provider can't do, but this is a reality that all technology vendors face – and must act on if the NHS is to realize the new "tech vision" the UK's health secretary launched in October 2018. The Department of Health and Social Care's vision argues that artificial intelligence has "huge potential to improve diagnosis." This is absolutely true, but that potential will only be realized if another key facet of the same tech vision – interoperability – extends to the "ologies," and to the imaging technology on which they rely.

Momentum is building for the NHS to move beyond outdated technology, and if traditional IT vendors are to survive they must support the integration of all sorts of innovations into their products, or else suffer the same fate as the fax machine – and sooner than they might think.

Remarkably, although many companies are thinking collaboratively, we still live in an age where some diagnostic med-tech hardware suppliers force hospitals to purchase proprietary software. Now is the time for suppliers to be open, not archaic. Vendors can't block the NHS from accessing innovations just because they weren't the ones to develop them – and the same applies to innovations in the AI space.

Right now, within the NHS and in companies across the globe, people are developing applications and algorithms to tackle real-world problems from detecting deadly diseases sooner to avoiding unnecessary appointments. No single technology vendor could dream up even the smallest percentage of the ideas arising worldwide. Yet, if you were to look across the competitive landscape in NHS diagnostic IT, you would find a plethora of companies obsessed with their own algorithm developments, rather than the wealth of ingenuity they could embrace for their customers.

This isn't about what any one vendor is developing. The key is for suppliers of traditional technologies, such as the electronic patient record (EPR) or the picture archiving and communication system (PACS), to ensure that they are interoperable and that their customers' AI applications can plug into the core technologies on which the NHS relies. Hospitals need their core systems to be open platforms for AI, and my colleagues and I believe fundamentally that this is how suppliers can become – and remain – successful. If a hospital wishes to implement a homegrown application into their PACS, they should be able to. If they want to implement an algorithm developed by another vendor or innovator, following necessary due diligence to ensure that application is safe, they need the ability to incorporate it into the PACS. That should be the ethos of any healthcare technology company, so that their platforms can allow AI to flourish and their customers can take advantage of the newest developments.

Making this work means applying AI to the richest possible dataset. That doesn't necessarily mean sharing data externally, but it certainly necessitates joining together data and imaging across the trust and thinking at the population level. Simply put, enterprise-wide image

management is the only way forward. We must break down the barriers between the ologies so that images from a whole host of diagnostic specialties can support diagnoses and become integrated with information from the EPR.

Standards such as Health Level Seven Fast Health Interoperability Resources can be key in enabling this integration, but require willingness from vendors to collaborate, and from the NHS to think differently about a strategy for digitizing images and information. Only then can AI be used to look for patterns in data – patterns that can identify incidental findings that might show the early onset of a disease not originally investigated, or help to determine how a catastrophic event in the emergency room might be avoided. These are real-world problems, but only through a genuine interoperable and open approach from healthcare systems and their suppliers will they be solved.

A Shared Path to Digital Pathology

Social media platforms can pave the way for the inevitable transition

By Felipe S. Templo, Jr., Staff Pathologist and Director of the Combined Anatomic and Clinical Pathology Residency Training Program, Philippine Heart Center Division of Laboratory Medicine, Manila, the Philippines

The emergence of different social media platforms has changed not only the way we communicate, but also the way we obtain information. Even academic institutions increasingly recognize the power and influence of social media; its practical utility in medical education and training is continually increasing because social media is innovative, accessible, interactive, and evolving. And among all of the medical specialties that may benefit from this evolution, pathology stands out. Why? Because it is so visual.

Platforms like Facebook and Twitter are becoming more relevant as sources of supplementary learning materials, especially among medical students and pathology trainees. They're also gaining prominence as an avenue for continuing medical education among pathology teachers and practicing pathologists, especially with the emergence of Internet-based lectures and webinars, such as PathCast.

In my experience, the diagnostic skills needed to interpret cases viewed on glass slides under a microscope can be slightly different to those needed for cases displayed on computer screen. And yet, digital pathology is rapidly gaining worldwide traction, particularly so in the last few years. Clinicians and surgeons like the idea of viewing pathology reports at their convenience without having to visit the laboratory in person. Patients appreciate access to their own healthcare information via electronic portals. And professional colleagues enjoy the opportunity to consult on cases without relying on the safe transport, storage, and retrieval of fragile glass slides.

It's clear, then, that the pathologists of the future need to hone these new skills – along with the ability to interact professionally on social media. Why are these two things related? Because despite the increasing acceptance of digital pathology, not every laboratory has the desire and the resources to pursue it at this point. So, if the digital transition is inevitable, how can pathologists whose labs have not yet made the move acquire skills?

To bridge the gap between traditional light microscopy and digital pathology, pathologists can use social media for their own education. There are a lot of Facebook groups for laboratory medicine professionals – surgical pathology, cytopathology, electron microscopy, and every kind of subspecialty and interest under the sun. Pathologists at every career stage can join these groups to share and discuss interesting cases. Can't find a group for your subject? You can create your own and tailor it to the training program you want to pursue – and you might be surprised at how many people outside your own institution might share your interests!

Facebook is not the only platform where you might find a professional home. Twitter also has a lot of pathologists who are very active in sharing interesting cases, hosting social media-based journal clubs, and linking to educational or even interactive podcasts and webinars. Pathology is, among other things, a practice of photography – and both Twitter and Instagram feature numerous accounts with excellent microscopic photos. In the process of admiring these pictures and attempting the cases our peers share, we are preparing ourselves to adopt digital pathology – and, by learning to collaborate globally, we are coming together as a community.

Overall, I believe that social media can help us pave the way to accepting digital pathology and incorporating it into our professional life. New technologies will always require a change of perspective, but the communities of other pathologists we can interact with on social media can definitely help us look forward to our specialty's new horizons.

Becoming Our Best Through Continuing Education

From live events to online courses, there are a number of ways that we can learn and evolve to perform at the top of our professional game

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

As pathologists and laboratory professionals, we've dedicated ourselves to saving lives through the study and diagnosis of disease. To perform at the best of our abilities, we must constantly update our knowledge. And to do that, we must participate in continuing medical education. Luckily, there's an expanse of choices before you, and it has never been easier to take advantage of it.

The American Society for Clinical Pathology is recognized around the world not only as a professional society, but also as a provider of premier educational materials to pathologists and clinical laboratory scientists. This education enables you to learn, advance, and evolve throughout your career. It's quick and easy to maintain your credentials and build your network. All of our education covers innovative, cutting-edge topics such as immuno-oncology, public policy, and pathology informatics.

Live events such as the annual meeting, education courses, Pathology Update, Pathology Update Middle East, and workshops for laboratory professionals provide opportunitiesoffer lecture-based and interactive sessions in specialty and subspecialty areas. They are a great way to refresh your knowledge base, explore new topic areas, and learn about the trends of tomorrow and their practical applications for today. Live events also provide an opportunity for to networking with your peers from around the world.

Our online learning management system provides education you can take anytime, anywhere – at home, at work, or even while traveling. Most courses take less than two hours to complete, so it's a quick, easy way to maintain your credentials. We also offer online certificate programs such as Lab Management University, University of Pathology Informatics, and Leadership Institute. These programs were created to give our members support in their ongoing professional goals.

ASCP Press produces a plethora of reference and guide books, including the Board of Certification Study Guide, The Art and Science of Cytopathology, and Digital Pathology. We also produce two journals (The American Journal of Clinical Pathology and Lab Medicine) and one magazine (Critical Values) that keep members abreast of evolving technologies, applied science, and policy issues relevant to today's bench technologists and practicing pathologists.

Everyone needs to complete their continuing education hours, but our hope is that once you explore ASCP's options, the ease of access and excellence of content will keep you coming back year after year. We want your experience to surpass your expectations.

"[Live events] are a great way to refresh your knowledge base, explore new topic areas, and learn about the trends of tomorrow and their practical applications for today."

Spotting Sepsis Sooner

Using monocyte distribution width alongside white blood cell count raises suspicion of the condition early in identifying adult patients with sepsis or at increased risk of developing sepsis in emergency departments

By Rachel Burnside

Despite advances in modern medicine and acute patient care, sepsis remains the leading cause of death from infection. It's a life-threatening condition that represents a global health care problem; one in 23 hospital patients – over 27 million people worldwide – are affected (1). Sepsis claims more lives than any cancer and currently has a 25–30 percent mortality rate, but it's not just an issue for patients; the health care cost of sepsis in the USA is estimated at US$24 billion each year (2).

That number is on the rise. The incidence of severe sepsis in the U.S. is increasing by 1.5 percent each year – faster than the projected population growth. The number of cases documented in 1995 was 750,000; in 2010, it rose to 934,000, and it is predicted to hit 1,110,000 in 2020 (3). This increase in incidence is due to:

- increased awareness and tracking
- a high incidence of severe sepsis in elderly patients
- a disproportionate increase in the number of elderly Americans
- growing numbers of antibiotic-resistant organisms
- more people living with chronic or terminal diseases, such as diabetes or HIV
- therapies that suppress the native immune system, such as chemotherapy or immunosuppression

Several studies have demonstrated that early intervention and diagnosis are key to reducing sepsis mortality; one well-cited investigation found that there is a 7.6 percent increase in mortality for every hour's delay in antibiotic treatment (4). For this reason, the key challenges for clinical laboratories in the management of sepsis involve striving for earlier diagnosis with reliable tests that accurately assess whether a patient's immune system has started to become septic.

Defining sepsis

In 2016, sepsis was redefined by a panel of experts as "a life-threatening organ dysfunction caused by a dysregulated host response to infection," an explanation that better reflects its clinical presentation. Previously, sepsis was defined solely using systemic inflammatory response syndrome-based criteria. Although initial inflammation is still used as the basis for suspicion of sepsis, we now recognize that many other pathological presentations can also cause inflammation. The new definition is accompanied by a set of criteria summarized in the quick Sepsis-Related Organ Failure Assessment (qSOFA) score, which treats those with sepsis as a subset risk group of infected patients (see Table 1).

Lab tests can be used at a variety of stages in the clinical management of sepsis – to indicate infection and inflammation, to detect the dysregulation of inflammation at the organ level following hypoperfusion, and to mark coagulopathy and organ failure. Numerous biomarkers reflect these stages; however, only a few have sufficient sensitivity and specificity to identify sepsis. Among them, the most widely used are lactate, procalcitonin (PCT), and immune cells.

Laying down a biomarker

Although the role of lactate continues to be debated in the context of sepsis, high levels of lactate can be an early indicator of the condition. The challenge of this approach is knowing what cutoff to apply; a low cutoff to catch sepsis as early as possible might lead to overdiagnosis, which then results in unnecessary antibiotic administration. High cutoffs, on the other hand, risk missing cases and delaying treatment.

Another biomarker currently used is PCT – a peptide precursor of the hormone calcitonin, which helps to regulate calcium homeostasis. PCT concentrations in non-septic individuals are below 0.5 ng/mL, but they increase in multiple tissues throughout the body in response to sepsis. Because PCT levels rise rapidly within six to 12 hours of infection, this test can play an important role in spotting sepsis in the critical first 24 hours. Importantly, neither lactate nor PCT is specific for sepsis and elevated levels of either may be caused by other etiologies. Additionally, a lack of elevated lactate and/or PCT does not eliminate the possibility of sepsis.

Another class of sepsis biomarkers that is typically available more immediately are the body's own white blood cells, including neutrophils and monocytes. These have been shown to exhibit morphological changes in response to infection; for example, when the human THP-1 monocytic cell line is infected with Chlamydia pneumoniae bacteria, infected cells are directly induced to differentiate to macrophages (5,6). Unfortunately, increases in white blood count (WBC) are also nonspecific in patients who present in the emergency department (ED), so a biomarker that is routinely available during initial clinical evaluation could hold the key to alerting providers to the risk of sepsis earlier.

Indication at the earliest stage

Beckman Coulter has addressed this need with the Early Sepsis Indicator (ESId) – which analyzes morphological changes in monocytes. Recent studies have indicated that volumetric increases in immune cells might be useful for dsepsis and, taken together with current standard of care,

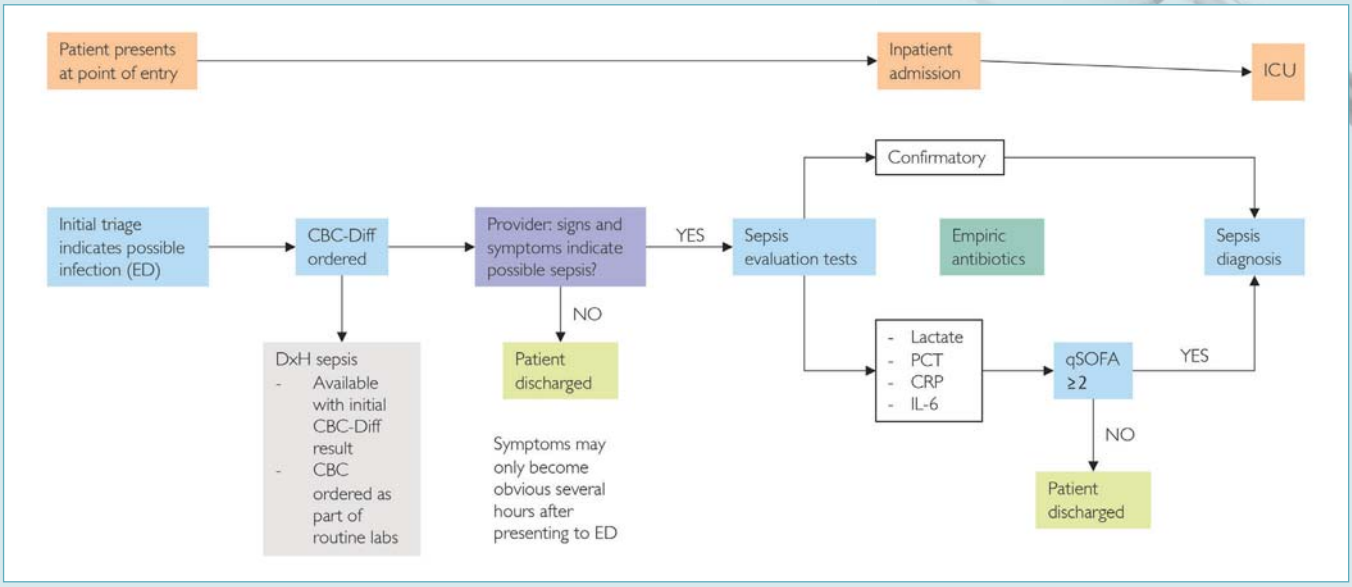

Figure 1. The sepsis patient care pathway.

High respiratory rate	*>22 breaths per minute*	Score: 0 = mortality <1% 1 = mortality 2–3% ≥2 = mortality ≥10%
Low systolic blood pressure	≤100 mmHg	
Altered mentation (Glasgow coma scale)	*<15*	

Table 1. qSOFA criteria for the probability of sepsis mortality.

ESId can augment the suspicion of sepsis or risk of developing sepsis within 12 hours. ESId measures the change in the size distribution of circulating monocytes, known as monocyte distribution width (MDW).

In a prospective cohort study of 1,320 adult ED patients that included 98 with sepsis, the addition of MDW to WBC significantly improved the probability of having sepsis in comparison to WBC alone (7). An advantage of this method over other biomarkers, such as PCT, is its availability to health care providers at an earlier stage of clinical evaluation, when sepsis diagnosis might not ordinarily be considered. It can be offered as part of a routine complete blood count, making it a hematology-based test that can alert ED clinicians in identifying adult patients with sepsis or at increased risk of developing sepsis in the ED.

ED clinicians play a crucial role on the front lines of care for acutely ill patients, and early sepsis detection using MDW and WBC in combination fits neatly into the typical care pathway (see Figure 1). After a patient presents to the ED and the initial triage indicates possible infection, a CBC with differential is ordered. This is where the results of the early detection test might come back indicating possible sepsis, leading to further tests and the application of qSOFA criteria at the inpatient stage. The marker was recently cleared by the FDA and is marketed under the name Early Sepsis Indicator.

The bottom line

Spotting sepsis early is challenging and must be balanced against overdiagnosis to prevent over-administration of antibiotics. No single set of criteria or biomarkers is perfect, so clinicians are best served using a combination of tests and observations to complete the puzzle after reviewing the entire clinical picture. When it comes to sepsis, time equals life – and, given that 80 percent of cases begin outside the hospital (8), it is crucial to recognize and treat sepsis as quickly as possible once patients enter the ED. Early Sepsis Indicator offers a unique approach and will complement other tests that use alternative biomarkers – giving sepsis patients the best chance of survival through the earlier initiation of antimicrobial therapy.

Rachel Burnside leads global marketing for Beckman Coulter's Hematology Business Unit, and has extensive clinical diagnostic laboratory experience, serving as a lab director for over a decade. She completed a Ph.D. in microbiology, immunology and molecular genetics at the University of Kentucky, and an MBA from Duke's Fuqua school of business with a concentration in Health Sector Management.

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CSI: CRETACEOUS

The people who investigate disease and death in dinosaurs

What killed the dinosaurs? You may immediately think of an asteroid impact, a dramatic climate shift, or possibly an alien invasion – after all, the cause of their mass extinction is still somewhat open to scientific debate (although aliens have largely been ruled out). But what about the dinosaurs who died before the Cretaceous-Paleogene extinction event? Were all of their deaths dramatic predator-prey interactions? Did they suffer from cancer, arthritis, or even plain old age?

We've previously explored the unique careers of those who study the pathology of past peoples, whose patients may be hundreds or even thousands of years old (1,2). But just as not all modern pathologists treat human patients, not all paleopathologists investigate the history of human disease. Some study much older – and, in most cases, much larger – patients: the "terrible lizards."

But believe it or not, even the paleontologists and pathologists who study dinosaurs over 100 million years old use many of the same techniques on which modern laboratory medicine professionals rely. "Molecular paleontology" labs feature light and electron microscopes, mass spectrometers, immunohistochemistry platforms, synthetic peptides, and more. Practitioners of this discipline combine a paleontologist's work in identifying and extracting fossil material with the laboratory medicine skills of analyzing the ancient biomolecules preserved in fossilized bone. It's a science that works across interdisciplinary boundaries to yield great strides not only in paleontological studies, but also in the practice of modern molecular biology.

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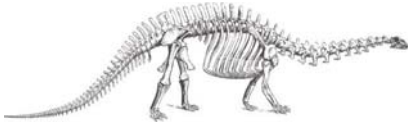
PATHOLOGY *Meets the* King *of the* Dinosaurs

The discovery of soft tissues preserved in *T. rex* bone – and its potential effect on modern pathology

Michael Schubert interviews Mary Schweitzer



What's the most unusual patient you've ever encountered?

Many pathologists and laboratory medicine professionals will recall unusual tumors, rare disorders, or even – for some – a unique veterinary specimen. But how many can claim that their standout examination has been conducted on a dinosaur? And not just any dinosaur, but a true Cretaceous celebrity: a *Tyrannosaurus rex*?

That's precisely the patient Mary Higby Schweitzer dealt with in 1993, when she noticed odd structures within fossilized bone. Faced with the possibility that those structures might be red blood cells – a tissue whose survival from ancient times was previously unanticipated – she delved deeper into the search for soft tissues preserved for millions of years. Ultimately, she was able to identify and isolate collagen, possible red blood cells, protein sequences, and even preliminary evidence of DNA from dinosaur fossils, revealing fascinating new information about how biomolecules can be preserved and how they compare to modern examples.

Fortunately, a Jurassic Park debacle is unlikely – but modern pathology and laboratory medicine still have much to gain from the study of these ancient tissues. We spoke to Mary Schweitzer to learn more…

YOU PRACTICE "MOLECULAR PALEONTOLOGY" – AN UNUSUAL TERM…

Paleontology is the study of "old life." Vertebrate paleontology is the study of old bones, and molecular paleontology is the study of old molecules. It's the study of biological molecules recovered from ancient bone and the methods the discipline's practitioners freely borrow to determine those molecules' endogeneity. We apply well-tested methods to recover these tissues and molecules and then analyze them using many of the same techniques modern pathologists, osteologists, and protein chemists use. It is certainly a far less straightforward discipline, though, because we have to account for chemical changes that have accrued on the molecules within fossilized bone – ones that happy, healthy, modern molecules don't contain. And that means developing new (or modified) protocols for our analyses. We also have to account for the fact that comparative databases consist mostly of mammals, which somewhat limits what we can do with dinosaur tissues.

HOW DID YOU BECOME A MOLECULAR PALEONTOLOGIST?

I never thought I had what it took to be a scientist. I am pretty badly handicapped in mathematics, and chemistry and physics are very much like math. In addition to fearing science, I was also kind of lazy! My first degree was in "educational audiology," which is basically deaf education with a clinical component. I loved it, but I wasn't ready to get the advanced degree I would have needed to practice it. Instead, I got married and had kids – and I decided that, when my kids went off to school, I would get a teaching certificate so I could be where they were in high school. (One of their greatest fears, as I'm sure you can imagine!)

As I was finishing the certificate, I noticed that famous paleontologist Jack Horner was teaching a class. It was mid-year and I couldn't get a job, so I sat in on the class. I guess you could say that my curiosity overcame my fear of science. I continued as a volunteer for a while, but I had a million questions and, finally, Jack ran out of answers, so he told me to go and get my own degree. I signed on for a Master's program, proposed my thesis ideas to my committee and, the day after my committee met, a bone expert sent me back a microscope slide of a *T. rex* bone I was working on. She said to me, "Did you know there are red blood cells in this bone?" But you cannot determine this from morphology alone. After 65 million years in the ground, virtually any chemical reaction is possible. So I proposed some alternatives – for instance, sedimentary structures or pollen – and tried to eliminate those options with data. The rest is history.

NO ONE THOUGHT SOFT TISSUES COULD BE DISCOVERED IN FOSSILS – SO HOW DID YOU DO IT?

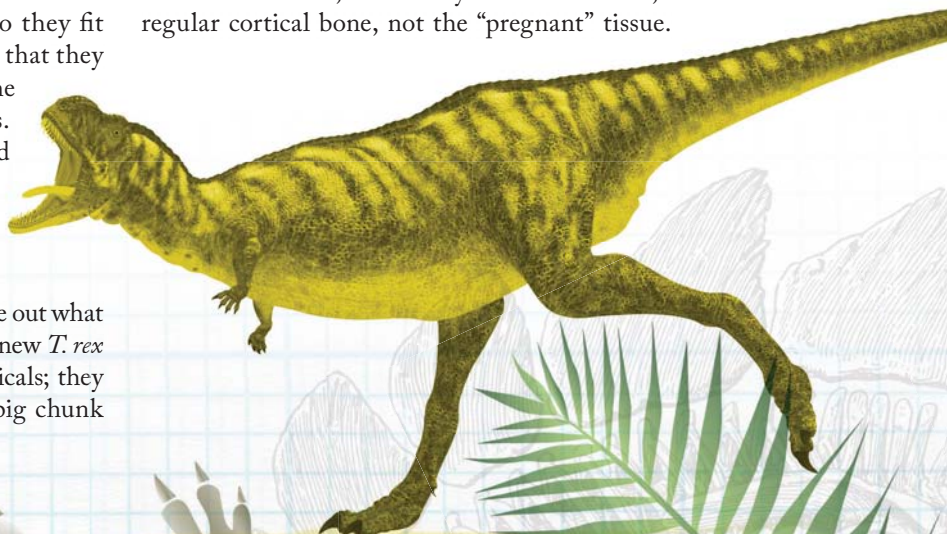
It started with the sighting of those little round structures inside the vessel channels in the *T. rex* bone. Jack said, "What do you think they are?" I said that I knew it wasn't possible, but they were the right size, shape, and location for red blood cells. They were even iron-rich and nucleated, so they fit every criterion. His response was, "So prove to me that they aren't." That formed the basis of my dissertation – the attempt to prove that these weren't red blood cells. In the end, of course, I couldn't disprove it, but I did accumulate a lot of chemical (despite hating the subject!) and other evidence for the preservation of molecular components.

When I took a job at North Carolina State University, I wanted to repeat those studies to see if I could figure out what was going on. Jack sent me a box of fragments from a new *T. rex* discovery, that had not been treated with any chemicals; they were "fresh out of the ground." I pulled the first big chunk

> "JUST BECAUSE IT LOOKS LIKE SOMETHING, AFTER 70 MILLION YEARS, THAT DOESN'T MEAN IT IS."

out of the box, turned to my technician, and said, "Oh, my gosh! It's a girl, and she's pregnant!" I believed I was holding medullary bone – the estrogen-dependent reproductive tissue birds produce during lay. In an effort to determine whether or not this new tissue I saw really was what I thought, I went to the literature to see how bird medullary bone was studied and found that they always removed the mineral phase of the bone to study the organization of collagen in medullary bone, which is different to that of other bone types. We didn't think that would work in a fossil of this age, because conventional wisdom states that organics don't preserve – so if we removed the mineral phase, we assumed there would be nothing left.

I told my tech to do just a short etch to reveal the pattern of the collagen fibers that had once been there. The process went on longer than I had intended… Imagine our surprise when, not only did the bone not completely dissolve (as it should have if we had been right about the organics' degradation), but it actually left a stretchy, fibrous matrix like modern bone! We repeated the process because I thought it had to be wrong – a fluke – but no, it was very consistent. Then, I tried it on regular cortical bone, not the "pregnant" tissue.

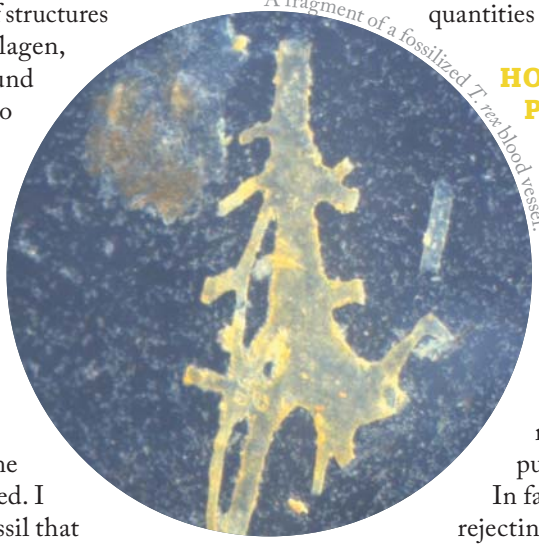


That's when we observed the vessels and cells. But we had to remember an important lesson in paleopathology: just because it looks like something, after 70 million years, that doesn't mean it is. With that in mind, we turned to chemical and molecular methods to satisfy our curiosity (did I mention I hate chemistry?).

WHAT FEATURES MAKE A FOSSIL A GOOD POTENTIAL SOURCE OF SOFT TISSUE?

After our initial findings, we applied our methods to a time point study, looking at bony remains from fossils that spanned multiple ages, taxa, depositional environments, and continents. We looked for the presence of structures morphologically consistent with collagen, blood vessels, and cells – and we found soft tissues were present in one-half to one-third of all fossils we examined. It does seem, though, that it was better to be buried in sandstone than in mudstone, in terrestrial versus marine environments, and to be buried rapidly and deeply. So those were our starting points when looking for a new dinosaur.

Since that time, I have become convinced that the full answer is a lot more complicated – and, for the time being, I don't know everything involved. I think that, to start, you should have a fossil that demonstrates unusual preservation – articulated, preserved with skin, or preserved in an environment that yields other pristine fossils. Then, you just have to look…



A fragment of a fossilized *T. rex* blood vessel.

But how have these soft tissues remained intact for so long within fossils when they degrade so quickly under laboratory conditions? I think you have to account for the unique environment that exists in vertebrate bone. Bone mineral is microcrystalline, with a huge surface area and a strong affinity for organics. In fact, researchers have even purified DNA on apatite columns. Additionally, the way bone is structured makes it difficult for microbes to access the internal-most parts. I also think iron, as a reactive oxygen species, has a lot to do with preservation (1,2), and iron is released into the local environment in large quantities when a *T. rex* drumstick degrades.

HOW DID YOU HANDLE PUSHBACK FROM RESEARCHERS WHO DOUBTED YOUR CONCLUSIONS?

It was (and is) really hard sometimes. With everything we publish, we try to set rigorous standards and never overstate our data. We never publish unless we can repeat our results three times, and we never ever publish using just one or two methods. In fact, I even had a reviewer recommend rejecting one of my papers because there was "too much data!" But there are some who will not be convinced with data – and, rather than accept the evidence we provide, say they don't "believe" our data or challenge our methods. Antibodies, for instance, can cross-react with structures containing epitopes of a similar shape to their target proteins. As a result, I've been told that they can yield "false positives," which is not quite the case, and which risks their being devalued as a molecular paleontology technique. Rather, these types of inaccuracies are the reason we use controls!

I was taught that, if I wanted to challenge work in the peer-reviewed literature, I had to come up with an alternative explanation that cohesively fit all of the published data and explained the conclusions equally well, and then come up with additional data that better supported our alternative than the original hypothesis. Generally speaking, our challengers have not applied this standard. Typically, they fail to acknowledge many of the

"TO START, YOU SHOULD HAVE A FOSSIL THAT DEMONSTRATES UNUSUAL PRESERVATION… THEN, YOU JUST HAVE TO LOOK!"

WHERE WERE THESE FOSSILS FOUND?

Canada

United States of America

China

WHAT TECHNIQUES ARE USED?

- Careful excavation
- Short etch
- Morphology
- Imaging
- Biochemistry
- Novel techniques

WHAT CONDITIONS PRESERVE SOFT TISSUE BEST?

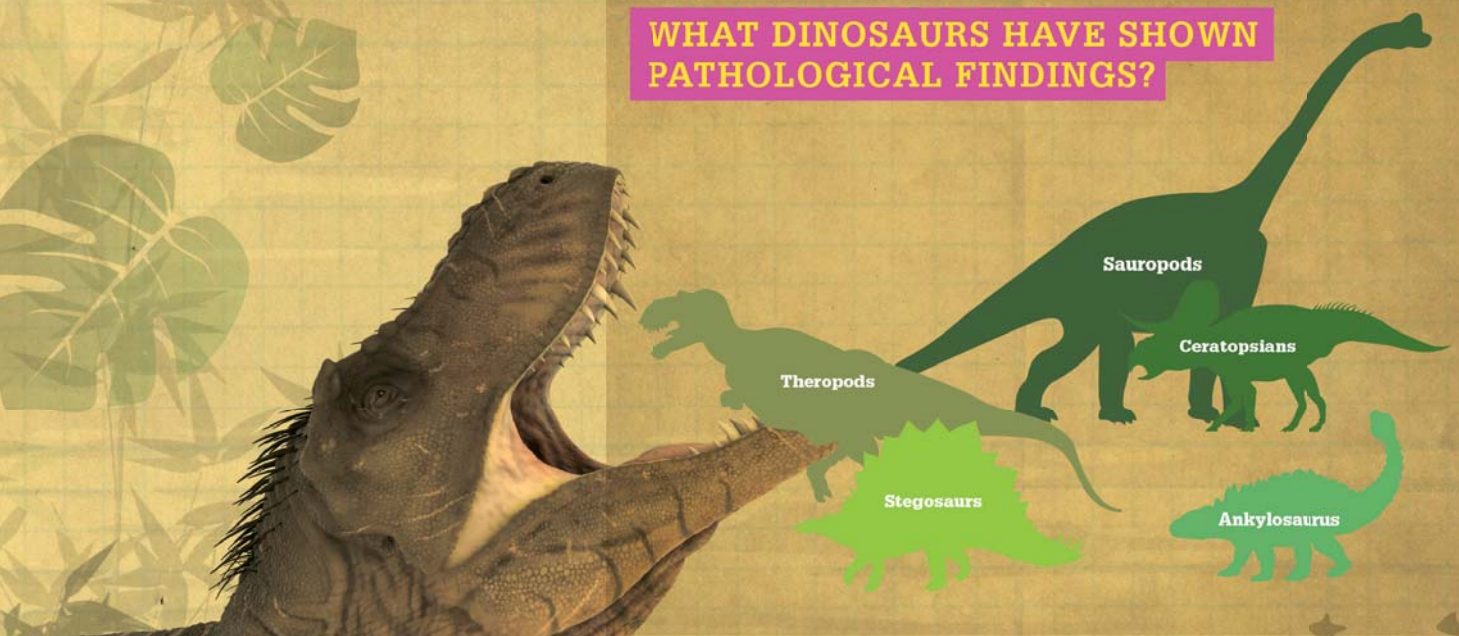
Sandstone

Terrestrial environment

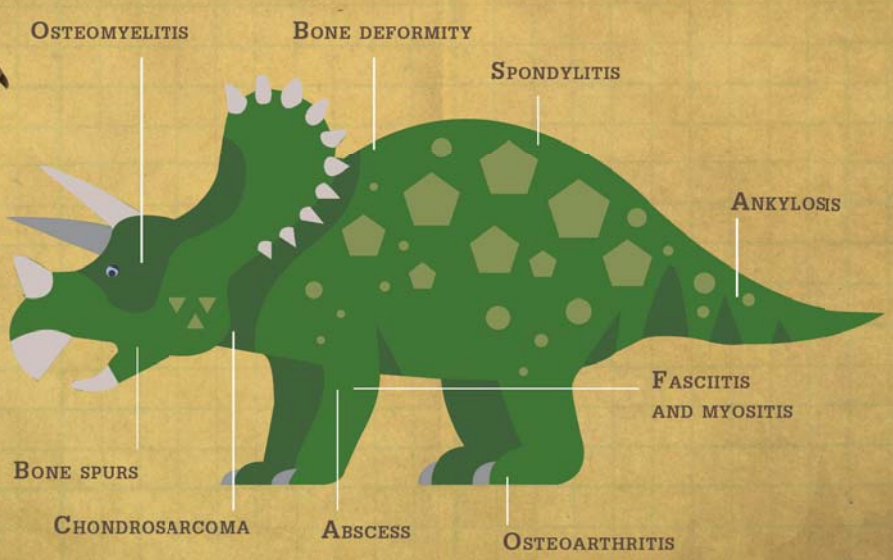
Rapid burial

Deep burial

WHAT DINOSAURS HAVE SHOWN PATHOLOGICAL FINDINGS?



WHAT PATHOLOGIES HAVE BEEN FOUND?



Sue, the famous *T. rex*, exhibits signs of possible gout, avulsion injury, and five other pathologies. Credit: Connie Ma

The skull of a *Vagaceratops irvinensis*, which exhibits evidence of possible chondrosarcoma, osteoma, and bony lesions. Credit: D. Gordon E. Robertson

"EVIDENCE OF THE POSSIBLE PRESENCE OF DNA IN FOSSILIZED BONE IS, TO ME, JUST ONE MORE PIECE OF EVIDENCE THAT PROTEINS COULD PERSIST."

lines of evidence we produce, addressing only the one that they try to disprove. Or, when they purport to "test" our hypotheses, they use methods different to those that we used. I don't think that is a valid trial.

So how do I handle it? I yell and stomp around and whine to my friends and get really snarky for a day or two – and then I try to see the value in the criticism. Ultimately, I go out and try to produce more data. Even the very worst reviews contain something I can learn from.

YOU'VE FOUND EVIDENCE OF DNA IN DINOSAUR FOSSILS. WHAT COULD WE POTENTIALLY LEARN FROM THAT?

Evidence of the possible presence of DNA in fossilized bone is, to me, just one more piece of evidence that proteins could persist – rather than having proteins be a step toward the eventual goal of DNA recovery. I'm told that this is a rather unusual attitude! DNA is not the focus of our research, nor is it the end goal. DNA is not what natural selection operates upon; rather, it is the expression of DNA as proteins. Now, of course, if we have DNA we can predict protein sequences and go looking for them. However, the DNA sequences don't say much about what the end product – the functioning, three-dimensional protein – may look like. And that folded, convoluted, final protein is what determines its function.

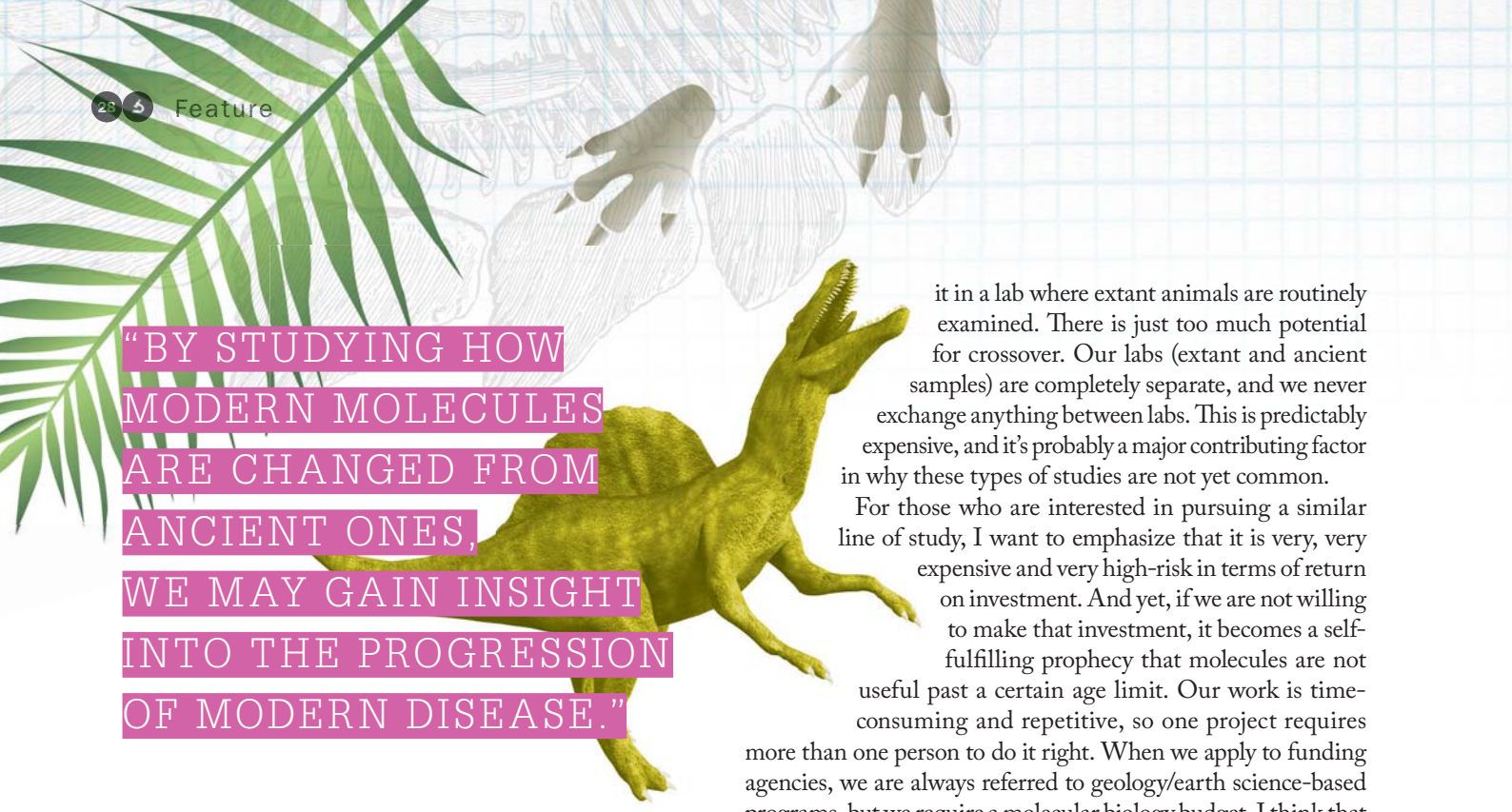
It is predicted that DNA has a shorter half-life than proteins. I don't put much weight on such predictions, but if I am really interested in non-avian dinosaurs, it doesn't make a lot of sense to start with DNA because of its predicted lack of longevity. However, if DNA is there, then proteins surely should be – just as, if proteins are there, we should be able to see the persistence of lipid-based compounds, which are predicted to last longer still. In fact, we recently published evidence of blubber in an ichthyosaur fossil (3), based on its resemblance to other lipid-rich fossils and the presence of potential fatty acid-derived moieties.

WHAT TECHNIQUES DO MODERN PALEONTOLOGY AND LABORATORY MEDICINE SHARE?

As far back as the 1950s, paleontology started to borrow from modern osteology by investigating fossil bone at the microscopic level. This was pioneered by Donald Enlow and colleagues, but not really followed up on until the 1980s, when Armand de Ricqlès, Kevin Padian, and Jack Horner brought it to the forefront. Now, most paleontological studies involve histology in one form or another.

Molecular analyses are off to a rather slow start. I think, though, that the broader community is beginning to see the power of molecular methods and the added information they provide, not only for elucidating phylogenetic relationships, but also for demonstrating function and evolutionary mechanisms. In the future, we hope to make de novo sequencing more efficient for what we do. One of our big problems, as I said above, is that most of the databases out there for molecular studies are mammalian – and mammals are not a great comparator when searching dinosaur sequences.

I believe strongly in the power of interdisciplinary research – including collaborations between paleobiologists and laboratory medicine professionals. There is so much to learn that no one person can learn it all. I am deeply honored to work with the colleagues I have across the board, and very humbled that they, for a moment, put aside some of their own projects to follow up on this crazy idea that molecules don't necessarily always have a time limit. My goal for work produced in my lab is that it be as robust and well-documented as possible. The reason I have never published a paper using just one method (nor will I ever) is that all methods are limited. The multiple methods we use are a great cross-check; they test different aspects of a molecule and validate its presence in ancient tissues. Each method we use contributes another piece of information about the functioning molecule, and thus the functioning organism. Large interdisciplinary studies require a lot of trust, though, so it's good to be familiar with the people you work with and their reputations. I have worked with some of the best people out there. They think of stuff I haven't; they employ methods I can't; and I have been deeply honored to work with great teams.



"BY STUDYING HOW MODERN MOLECULES ARE CHANGED FROM ANCIENT ONES, WE MAY GAIN INSIGHT INTO THE PROGRESSION OF MODERN DISEASE."

DO YOU HAVE ANY ADVICE FOR LABORATORY MEDICINE PROFESSIONALS INTERESTED IN THIS KIND OF WORK?

I think one of the huge potentials of the work we are trying to do is its applicability to medicine. Co-evolution of diseases/pathogens and hosts is a huge new area of study, but most are based upon the distribution of disease vectors among living hosts. They don't really address (except by inference) questions like when and how these diseases started, when they invaded new lineages, or how they have been modified to become less virulent. Perhaps a disease plaguing us now had its start in ancient hosts. Can we probe those skeletal elements to identify them?

In addition, by studying how modern molecules are changed from ancient ones, we may gain insight into the progression of modern disease. We have found that iron-mediated crosslinks are key to the preservation of these tissues and molecules, but of course they are pathological – or lethal – to living organisms. But we know the molecular makeup of happy, healthy blood vessels, and now we have an end product. Can we find a pathway? And, if so, can we perhaps find a way to interfere with the process? Of course, if you want to pursue this line of inquiry, you cannot do it in a lab where extant animals are routinely examined. There is just too much potential for crossover. Our labs (extant and ancient samples) are completely separate, and we never exchange anything between labs. This is predictably expensive, and it's probably a major contributing factor in why these types of studies are not yet common.

For those who are interested in pursuing a similar line of study, I want to emphasize that it is very, very expensive and very high-risk in terms of return on investment. And yet, if we are not willing to make that investment, it becomes a self-fulfilling prophecy that molecules are not useful past a certain age limit. Our work is time-consuming and repetitive, so one project requires more than one person to do it right. When we apply to funding agencies, we are always referred to geology/earth science-based programs, but we require a molecular biology budget. I think that we must rely more and more on private funds to do this kind of work. I particularly struggle with the ethics of recruiting students, although I love to work with them – I truly believe that they are the future. But if I still have so much trouble with funding after 25 years, can they make a career doing this? Perhaps therein lies the benefit of interdisciplinary research. If molecular paleontology and modern laboratory medicine team up, who knows what we could learn?

Mary Schweitzer is Professor of Biological Sciences at North Carolina State University, Research Curator at the North Carolina Museum of Natural Sciences, Research Associate of Paleontology at Montana State University's Museum of the Rockies, and a Visiting Professor of Geology at Lund University, Sweden.

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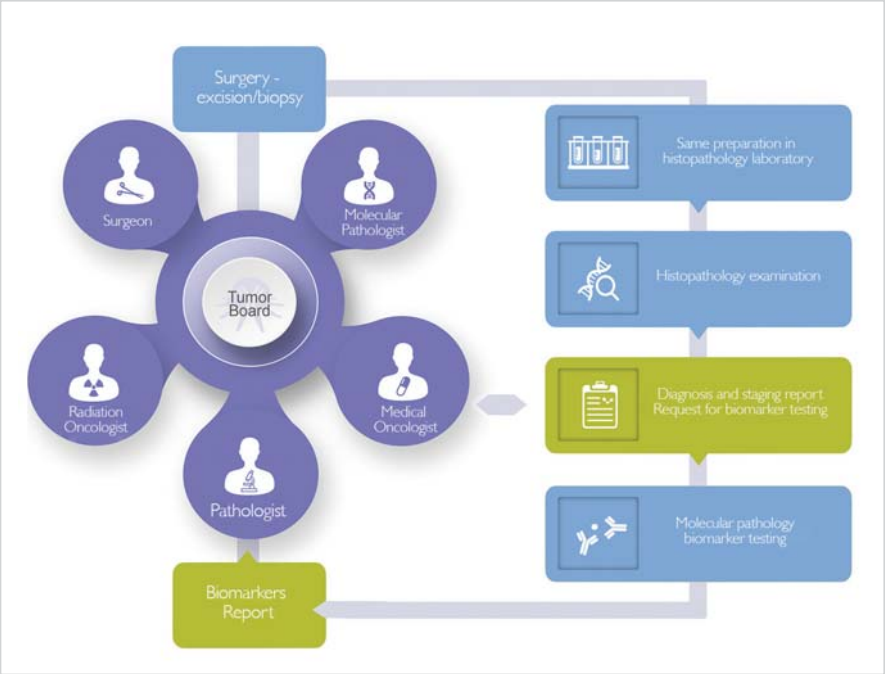
Next-Generation Sequencing: Will It Really Replace Single-Gene Tests in Pathology Labs?

We asked oncology care teams from both sides of Atlantic Ocean whether – and why – they are implementing NGS in routine biomarker testing



NGS in-house enhances collaboration between care team members.

"Like many in the field of cancer diagnostics, we're starting to substitute next-generation sequencing for a lot of our traditional tests," says *Matthew Hiemenz, Assistant Director of the Center for Personalized Medicine at Children's Hospital Los Angeles (CHLA).* It's a shift well worth making, he continues. "With NGS, you can multiplex hundreds of different DNA mutations and RNA fusions in a single test. That's incredibly helpful in terms of diagnosing challenging lesions and maximizing your chances of finding a useful prognostic indicator or treatment target."

Hiemenz is not the only laboratory medical professional making the move. *Tabetha Sundin, Molecular Diagnostics and Serology Scientific Director at Sentara Healthcare*, says, "We moved to NGS for non-small cell lung carcinoma testing, which allowed us to bring many of the single-gene tests together. Now, we can look at DNA mutations and structural variance, such as copy number variance and fusions, all in one assay. That saves us time, tissue, and cost."

With that latter observation, Sundin is highlighting some of the most significant benefits NGS has to offer. A more comprehensive approach like NGS can offer a shorter turnaround time, consume less of a precious sample, and open up new opportunities for collaborative care.

Giulio Settanni, Pathology Department, Negrar Hospital, Calabria, Italy, says, "A single NGS platform provides multiple answers to multiple clinical requests, leading to deep and accurate results in a very short time. The heterogeneity of the diseases treated in a molecular pathology lab makes NGS the best way to approach each molecular analysis. The cost per analysis has also dropped in recent years and nowadays, in my opinion, NGS represents the most cost-effective technology for DNA sequencing in pathology."

It's about saving time

Hiemenz says that moving to internal NGS testing has yielded significant time savings in his laboratory. "The average time to result with our reference labs is around two weeks. At CHLA, we can typically provide a result in one week." And the effect on patient care is huge, especially in cases where clinicians need urgent results. "We had a young patient in intensive care with a large head and neck tumor. It was inoperable, so we needed to know what to do as soon as possible. Because our testing was in-house, we were able to expedite it – and, in less than a week, the child was enrolled in a promising clinical trial. Rapid NGS testing made a big difference to that patient's care, so it's valuable to have that flexibility."

Scott Cross, Hematologist/Oncologist at Virginia Oncology Associates, has seen patients' frustration at long wait times firsthand. "Typically, I would inform my patients that we had diagnosed their cancer, but needed further testing to be sure that we were providing them the best possible therapy. We would often tell people that it would take two weeks or more to get results – which was always difficult." No patient who has already waited for a chest X-ray, a chest CT, a PET CT, and a biopsy wants to add yet another delay before beginning treatment for a life-threatening disease.

Sentara now conducts NGS in-house, and Cross says that one of its biggest benefits is in reducing time to results. "It removes a layer of trying to track down the specimen, which improves the turnaround time. Additionally, if the patient has any questions or issues, it's very easy for them to pick up the phone, speak with the professionals who have ordered and conducted the tests, and get answers immediately."

Settanni agrees. In his opinion, time savings is one of the clearest advantages of NGS. Why? Because it allows users to determine multiple different molecular markers at the same time, moving patients swiftly toward diagnosis and treatment.

It's about saving tissue

"Tissue conservation is a major point of focus for our laboratory and every laboratory out there," says Sundin. "There's never enough. We're doing more and more biomarker tests for these patients, and every test means another section of tissue removed from the block. We want to go into that block as few times as possible so that we can conserve tissue for when it's really needed."

Cross agrees. "Prior to having NGS available locally from Sentara, it was not uncommon for us to need to send a tumor specimen to two or three separate locations to obtain molecular diagnostics. So we needed to obtain a large amount of sample up front – but it's not always easy to obtain a larger specimen without putting the patient at risk for other complications," says Cross. "Having the opportunity to do multiple tests on a small amount of sample under our own roof has certainly cut down on the number of occasions on which there's not enough tissue."

Hiemenz adds, "We ask for as much tumor sample as we can get, but in-house NGS allows us to test samples on the low end of tumor percentage. We input 20 ng samples, but we're able to process amounts lower than that as well." And for patients with difficult-to-access tumors, in-house NGS testing can mean the difference between missing an important mutation and discovering it.

"A good NGS analysis is strictly related to the quality of the preanalytical phase," says Settanni. "We set the histology quality standard to permit subsequent high-quality NGS. In this way, our failure rate has dropped to less than 3 percent, even with samples containing low numbers of cells."

Encouraging collaborative care

As diagnostic technology advances, so too does the knowledge required to corral the available information to make the best decisions for each person. Hiemenz explains, "We discuss every single patient in a multidisciplinary conference. By having this bidirectional communication about each case, we're able to help the oncologists understand the prognosis for a given cancer type or even enroll a patient in a clinical trial." Previously, such a back-and-forth could be expected to introduce potentially dangerous lag time – but not now, says Hiemenz. "Because we're in constant contact with the clinicians, they help us prioritize the cases that are really important. As soon as those results come off the sequencer, we get the variants, write and issue the report, and talk to the clinicians by phone."

"We're able to obtain information much more rapidly and reliably by having NGS available in-house."

The increased information yield of NGS testing also prompts more dialogue between specialists. "I think it has enabled us to build a stronger relationship with the oncologists," says Hiemenz. And Cross agrees: "Local NGS has made it very easy to pick up the phone, and it also helps you to know that the people performing the tests are doing a good job and that the results can be trusted. We're able to obtain information much more rapidly and reliably by having NGS available in-house."

Sundin and her colleagues use in-house NGS for reflex testing on lung cancer. "That has vastly improved our relationship with oncology," she says. "They no longer consider us a barrier – instead, we're a partner in patient care." It's a phenomenon that Tim Triche, Co-Director of the Center for Personalized Medicine at Children's Hospital Los Angeles, echoes. "Pathologists and oncologists have forever had to learn how to speak to one another," he says. "I need to know what's important to the oncologist, and the oncologist needs to know what information I have that could help them manage the patient."

With the introduction of NGS, oncologists must now learn aspects of genomics that they may never have expected to need. That's why Triche and his colleagues established special tumor boards that run every other week. "We have three different classes – brain tumors, liquid tumors, and solid tumors – in which we share information." The groups have been highly effective because, instead of just sharing a written report, each specialty has the opportunity to ask questions of the other. "Everybody walks out of the conference far better informed than they were when they walked in, and it translates immediately into practice. I think that has been one of the most surprising and rewarding aspects of introducing in-house NGS. The utility of molecular testing for both clinicians and patients is profoundly enhanced when the oncologists can literally walk into the laboratory and say, 'Can we go over this together?"'

For Hiemenz and his colleagues, the benefits of NGS cannot be overstated. "I remember the first time I saw actual reads from NGS," he says. "I knew looking at my computer screen that this was going to have a huge impact, and we're still at the very beginning of what we can do. There is so much left that we can imagine – and, one day, NGS will help us achieve it."

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Improving Our Early Warning System for AML

A more sensitive approach to monitoring acute myeloid leukemia relapse after bone marrow transplant

By Amanda Winters

Acute myeloid leukemia (AML) can be a devastating disease – but it's not without treatment options; for example, allogeneic bone marrow transplantation (BMT), which replaces a patient's cancer-producing hematopoietic stem cells with ones that produce healthy cells. But even though it's meant to conclude a patient's cancer care, even BMT is not a guaranteed cure: 40–50 percent of patients who receive a transplant still relapse (1).

Patients who undergo BMT do not typically receive any additional therapy to prevent relapse. If a patient's body does not accept the graft and the transplant fails, they will likely also respond poorly to any alternative treatment. However, using more sensitive methods to detect relapse earlier – even before it appears using standard clinical measures – might provide patients with more effective options with respect to chemotherapies and targeted agents.

At a Glance

- *Bone marrow transplants can be a curative therapy for acute myeloid leukemia, but a significant proportion of patients still relapse*
- *Chimerism testing is routinely used to monitor relapse in transplanted patients, but a more sensitive, effective method is needed*
- *Droplet digital PCR measures the concentration of bone marrow–derived DNAs containing AML-associated mutations to offer a reliable approach to relapse monitoring*
- *The technique enables physicians to detect signs of relapse sooner, giving them more time to plan treatment regimens for their patients*

Our current approach

At the moment, the gold standard method for early detection of relapse post-transplant is chimerism testing. In this method, we analyze short tandem repeats in the patient's bone marrow to identify the cells' origin and calculate the proportion of donor marrow to recipient marrow. A higher-than-expected percentage of patient-derived bone marrow is an early sign of graft failure and an early indication of leukemia relapse. These clinical diagnostic tests usually take place one, three, and 12 months after transplant – but beyond this test, we have to depend on non-specific symptoms (such as weight loss, fatigue, and muscle weakness), which are less precise measures of cancer recurrence.

There is a paucity of research on the effectiveness of chimerism testing in monitoring AML following a BMT. The research that does exist has produced mixed results; whereas some studies have confirmed that chimerism testing predicts relapse, others have not (2). In fact, one study found that chimerism

"As ddPCR proves its effectiveness in monitoring AML following BMT, it can potentially begin to serve as an adjunct to chimerism testing."

testing yields a false positive rate of over 10 percent, which could expose patients to unnecessary toxicity from treatments, and a false negative rate of over 40 percent, which leaves patients without a course of treatment that could improve their outcome (3).

A recent study highlights two potential reasons why we don't yet have a firm grasp on the sensitivity and specificity of chimerism testing. First, a significant subset of the few existing studies focus only on pediatric patients. Second, those that do address adult patients use a small or heterogenous population that includes people with several different subtypes of leukemia (2). The lack of a consistent body of literature makes it difficult to assess the test's appropriateness for monitoring AML relapse after transplant. Therefore, although chimerism is the gold standard method for predicting relapse to AML following a BMT, we cannot say with certainty that the technique is effective in every case.

An alternative way to detect relapse in AML is by minimal residual disease (MRD) testing. MRD is defined as leukemic cells that persist in the body following therapy at levels below the limit of morphological detection. Researchers have found that patients with MRD following treatment are at a greater risk for relapse and survive for a shorter amount of time (4).

The most common methods for detecting MRD are flow cytometry and quantitative PCR (qPCR). Multi-parameter flow cytometry can be used for detecting aberrantly expressed proteins associated with AML, but this technique has not been quantitatively or qualitatively standardized (5) and, depending on the case at hand, it is only sensitive down to 0.1 percent, the clinical flow cytometry-based threshold for MRD (4,6). qPCR, on the other hand, is used to detect AML-associated genetic abnormalities. It is more sensitive than flow cytometry – in some cases, down to 0.01 percent (4). This measure, however, yields a relative result that must be compared with a standard curve, meaning that AML-associated mutations cannot be quantified in absolute terms. Instead, the accuracy of the result depends on the accuracy of the standard curve (7).

A new hope

At the 2018 American Society of Hematology Annual Meeting, my colleagues at the University of Colorado and I presented data on a highly specific and sensitive new assay for detecting AML-associated mutations in post-transplant bone marrow samples. Our goal? To determine MRD and predict relapse with greater certainty (8). The new assay, based on droplet digital PCR (ddPCR), can detect, track, and quantify cancer-related mutations in the blood down to a 0.01 percent variant allele frequency, which is at least equal in sensitivity to qPCR. In other cases, ddPCR has been shown to reach a sensitivity of 0.001 percent (9).

Why is ddPCR is so sensitive? It partitions a blood or tissue sample into thousands of separate PCR reactions, which renders the method less sensitive to the PCR inhibitors that often plague qPCR, while also eliminating the need for a standard curve (10). To execute a ddPCR reaction, a sample is divided into 20,000 nanoliter-sized droplets, each of which contains no more than a few copies of DNA. The PCR reaction is run to endpoint separately in each droplet, amplifying the template molecules. Importantly, though, only the template molecules that contain the target sequence – for instance, an AML-associated mutation – will be amplified. When this happens, the droplets fluoresce, and we can directly count the number of target sequences in the sample. Finally, the data is analyzed using Poisson statistics to determine the concentration of target DNA in the original sample.

Unlike chimerism testing, a ddPCR-

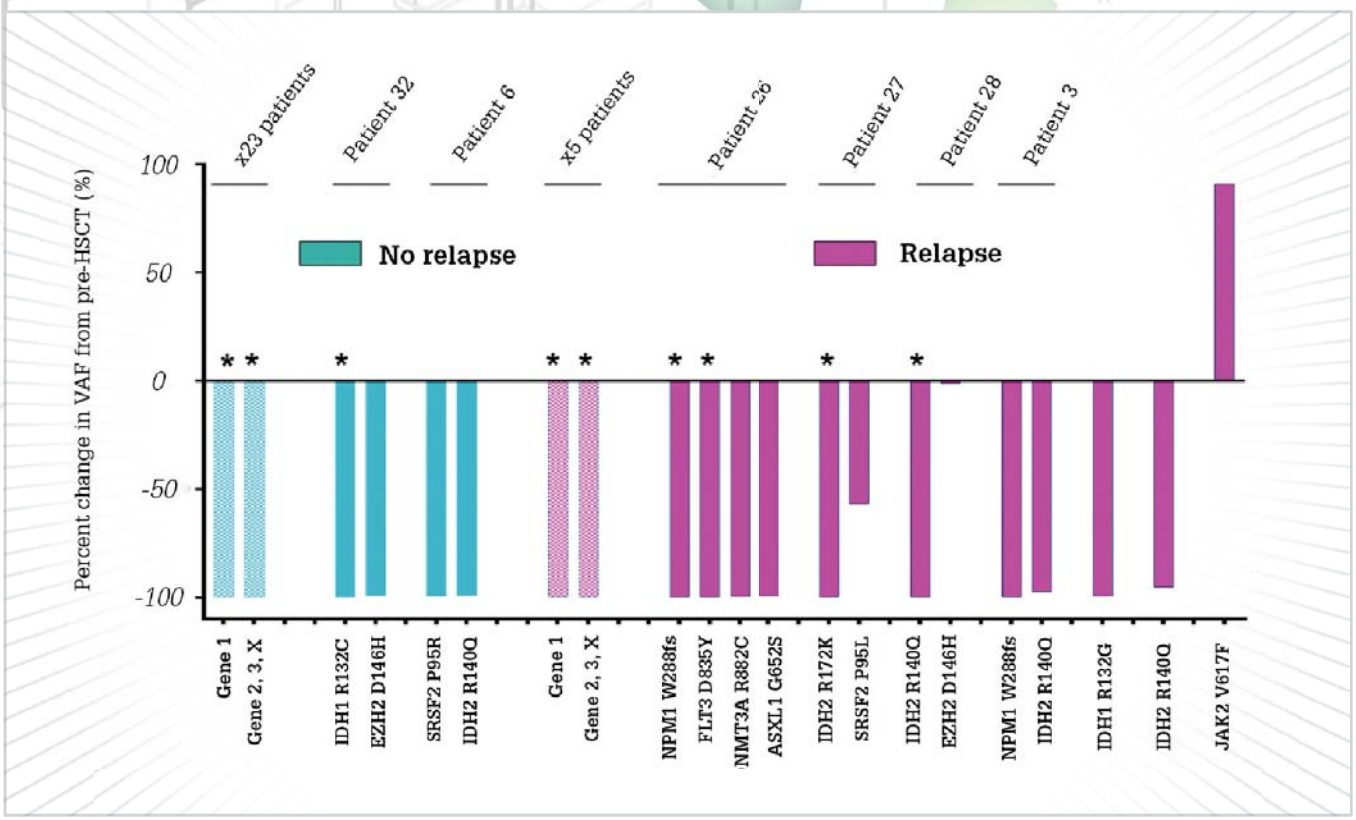

Figure 1. ddPCR measurements of MRD largely differentiate between patients who relapse following BMT and those who do not. VAF = variant allele frequency. Asterisks denote mutations that became undetectable by ddPCR (VAF – 100%). 23 patients were MRD-negative after BMT and did not relapse (blue hatched bars). Six of these patients were MRD-negative prior to BMT and remained MRD-negative after BMT. Five patients were MRD-negative after BMT and still relapsed (red hatched bars). The rest who relapsed after BMT had one or more mutations detected by ddPCR. All of the patients who relapsed were MRD-positive prior to BMT. Patient 6 died from transplant-related causes shortly after his day 28 assessment.

based liquid biopsy directly quantifies the presence of cancer in the bone marrow and indicates if the patient is progressing towards relapse – well before any clinical symptoms or even morphological signs of disease appear.

In our study, we tracked 21 cancer-associated mutations in bone marrow-derived DNA among a cohort of 36 patients (see Figure 1). These mutations appear in 5–20 percent of patients with AML. We examined samples taken during patients' bone marrow testing, which meant we could quantify mutation levels at one, three, and 12 months. We found that our ddPCR-based liquid biopsy (8) could predict relapse-free survival and overall survival at one month after a BMT (see Figure 2) and, although our sample size was small, we also saw signs that ddPCR might be more sensitive than chimerism testing – a finding that warrants more investigation to confirm.

Although we initially tested our assay on samples from a biobank, we are now examining ddPCR's ability to monitor relapse in AML patients who are currently on targeted therapies and haven't received a BMT. We are already seeing some evidence that our ddPCR-based assay can predict relapse before it happens, and we are hoping to publish those results soon.

As ddPCR proves its effectiveness in monitoring AML following BMT, it can potentially begin to serve as an adjunct to chimerism testing. Physicians can order a ddPCR-based liquid biopsy at regular intervals – for instance, once a month – to enable closer monitoring of MRD after BMT. Furthermore, ddPCR's sensitivity allows physicians to detect signs of relapse earlier, opening the door to more effective treatment options that will hopefully yield better outcomes for patients. Although more research must be done to directly compare ddPCR with chimerism, it's likely that ddPCR could play a significant role in surveilling patients with AML who have received BMTs, and will give physicians greater confidence in their treatment decisions.

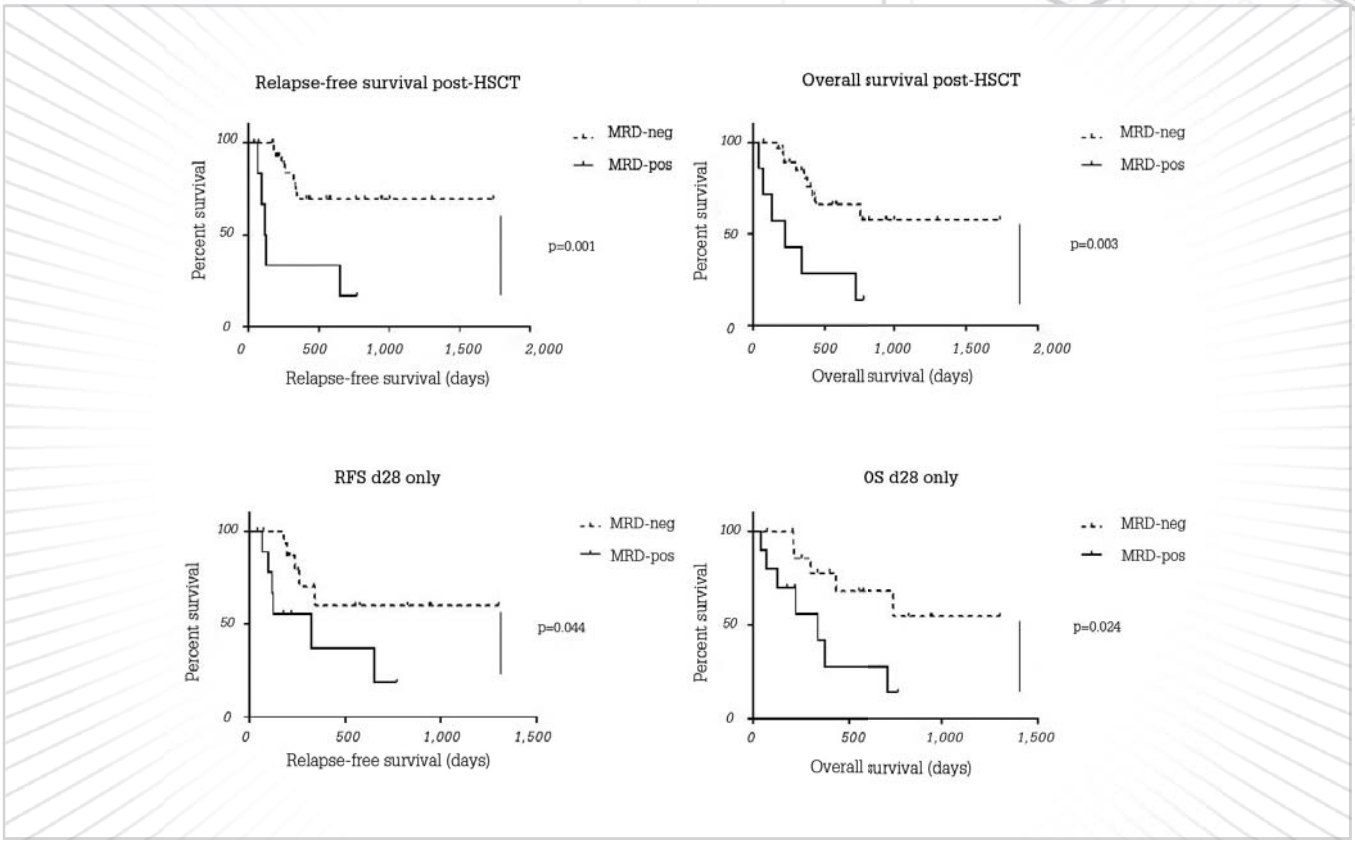

Figure 2. MRD status following BMT, as measured using ddPCR, strongly correlates with clinical outcomes. Relapse-free (A) and overall (B) survival of patients broken down by MRD status following BMT, irrespective of post-BMT day. Relapse-free (C) and overall (D) survival of patients broken down by MRD status specifically at day 28 after BMT. P-values <0.05 were considered significant.

Amanda Winters is an Instructor in the Department of Pediatrics at the University of Colorado Denver and a pediatric oncologist in the Center for Cancer and Blood Disorders at Children's Hospital Colorado in Denver, USA.

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TruSight™ Oncology 500: Enabling Comprehensive Genomic Profiling for Every Laboratory

The next step in precision medicine lies in decentralizing comprehensive biomarker testing

Table 1. CGP content consolidates multiple assays into one

TruSight Oncology 500		
DNA	*Key variant types*	*Example biomarkers*
	SNVs	KRAS G12D
	Indels	EGFR exon 19
	CNVs*	BRAF V600E
	Key biomarkers	*Example biomarkers*
	MSI	MSI-high
	TMB	TMB-high
TruSight Tumor 170		
RNA†	*Key variant types*	*Example biomarkers*
	Fusions	ALK ROS RET NTRK
	Splice variants	Met exon 14

**CNV calling will be available in 2019 with an Illumina software upgrade.*

† The products to evaluate DNA and RNA variants consist of the TruSight™ Oncology 500 DNA panel and the TruSight™ Tumor 170 RNA panel (PN: 20028215, 20028216, 20032626 & 20032627).

In the fight against cancer, the future lies in immuno-oncology. This powerful treatment strategy bolsters the immune system's ability to target and destroy cancerous cells – but despite its promise, not all patients who undergo this treatment will benefit. Why? Because not all tumors respond equally to immunotherapy. The solution lies in leveraging biomarkers that can assist in distinguishing responders from non-responders to thereby recommend appropriate immuno-oncology approaches for patients who are likely to benefit.

Key immuno-oncology biomarkers include tumor mutational burden (TMB), which measures the number of nonsynonymous mutations within the coding region of a tumor genome as a surrogate of the likelihood of neoantigen presentation by the tumor, and microsatellite instability (MSI), a Food and Drug Administration (FDA)-approved pan-cancer biomarker that can identify tumors for treatment with immune checkpoint inhibitors. When combined with detailed mutation analysis, these markers contribute to an unprecedented level of personalization.

The personal touch

Biomarkers can yield clues to tailor the best treatment for each individual – but biomarker testing carries challenges of its own. In lung cancer, for instance, pathologists often apply a battery of iterative single-gene tests: *EGFR, ALK, ROS*, and more. And, as molecular pathology becomes increasingly advanced, multiple biomarkers are needed – TMB, MSI, and fusions, including *NTRK* with the added complexity of multiple unknown fusion partners. Guidelines from the European Society for Medical Oncology (ESMO) now include TMB in the recommended biomarker tests for non-small cell lung cancer (1). But testing each of these markers sequentially requires additional time and money. It also calls for a significant amount of tissue, which presents a challenge for most lung cancer biopsies.

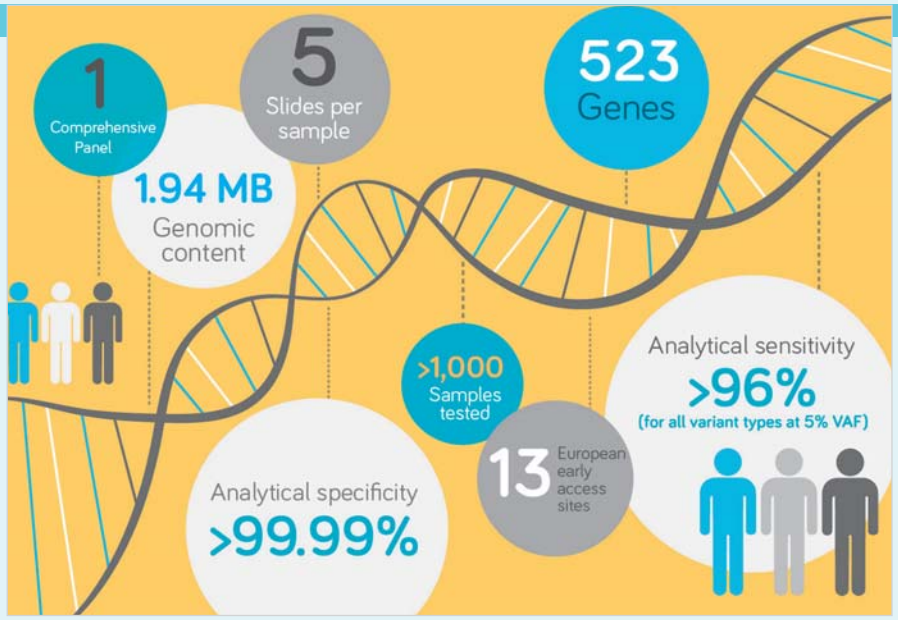
Sequential testing is not the only – or the optimal – approach to personalized lung cancer treatment. Comprehensive genomic profiling (CGP) through the use of next-generation sequencing (NGS) can achieve the same results and more – while taking less time and using less precious tissue. Performing NGS on limited tumor material also can minimize the impact of intratumoral heterogeneity.

NGS is being used to examine multiple biomarkers simultaneously, allowing users to identify potential driver alterations and treat patients in accordance with the characteristics their tumors display. NGS is key to the advancement of CGP, but there remains a need for a commercially available, standardized solution that enables laboratories to perform their CGP testing on-site.

Enabling CGP Using TruSight Oncology 500

TruSight Oncology 500 (TSO500) is currently on the market as a research use only (RUO) product that analyzes hundreds of current and emerging cancer-related biomarkers (see Table 1). These include key immunotherapy markers like TMB and MSI, which TSO500 examines using 1.94 MB of genomic content, a tumor-only workflow, and sophisticated software algorithms to yield results similar to whole exome sequencing. When bundled with TruSight™ Tumor 170 (TST170), this DNA + RNA assay† targets 523 genes to also assess small variants, splice variants, and fusions, with hybrid-capture chemistry that ensures high sensitivity and fewer sample dropouts.

TSO500 launched in January 2019 following the completion of an early access project in collaboration with 13



TruSight™ Oncology 500 – By the Numbers

leading European cancer centers.

- University of Birmingham, UK
- Institute of Pathology, University Hospital Cologne, Germany
- Institute of Pathology Erlangen, Germany
- Heidelberg Institute of Pathology, Germany
- Technical University of Munich, Germany
- Institut Gustave Roussy, France
- Radboud University Medical Center, The Netherlands
- Uppsala University, Sweden
- Jessa Hospital Hasselt/University Hospital Gent, Belgium
- University Hospital Lausanne (CHUV), Switzerland
- Hospital 12 de Octubre, CNIO & CIBERONC, Spain
- European Institute of Oncology - IEO, Italy
- Medical University Vienna/CeMM, Austria

Notably, of the 13 participating institutions, 11 participated in an inter-laboratory reproducibility assessment. This inter-laboratory reproducibility assessment demonstrates the robustness of TSO500 with observed Standard Deviation (SD) in the range of 1.5 for TMB values around 5 mut/Mb and near 3.0 for TMB values ranging from 30–80 mut/Mb (data to be published in the coming months). The study provided proof-of-concept that this assay can be reliably performed in decentralized laboratories. To scale for future worldwide deployment, users need a commercially available solution – and the minimal variation between sites in this broad study indicates that, with TSO500, it's a feasible goal.

A promising outlook

Feedback from the TSO500 early access sites was enthusiastic. Andrew Beggs, Reader in Cancer Genetics and Surgery at the Institute of Cancer and Genomic Sciences at the University of Birmingham, presented some of his laboratory's first results during the satellite symposium at the ESMO Immuno-Oncology Congress in Geneva. Beggs stated, "TruSight Oncology 500 has a number of significant advantages. It allows us to explore tumor samples of limited scope in a much more advanced way than we've previously been able to do."

Illumina's CGP product roadmap includes CE-IVD certification, initially for TMB and subsequently for other biomarkers. Given the potential benefits of liquid biopsy, Illumina entered into a multi-year collaboration using TSO500 with the Frederick National Laboratory for Cancer Research (FNL) to further explore clinical utility (2). Because liquid biopsy is still in its infancy, it requires additional validation to more fully demonstrate clinical utility. As we move down the path to regulatory approval and future in vitro diagnostic tests, we have begun to set a pioneering standard for accurate and reproducible testing.

"TruSight Oncology 500 has a number of significant advantages. It allows us to explore tumor samples of limited scope in a much more advanced way than we've previously been able to do."
- Andrew Beggs
University of Birmingham

† The products to evaluate DNA and RNA variants consist of the TruSight™ Oncology 500 DNA panel and the TruSight™ Tumor 170 RNA panel (PN: 20028215, 20028216, & 20032627).

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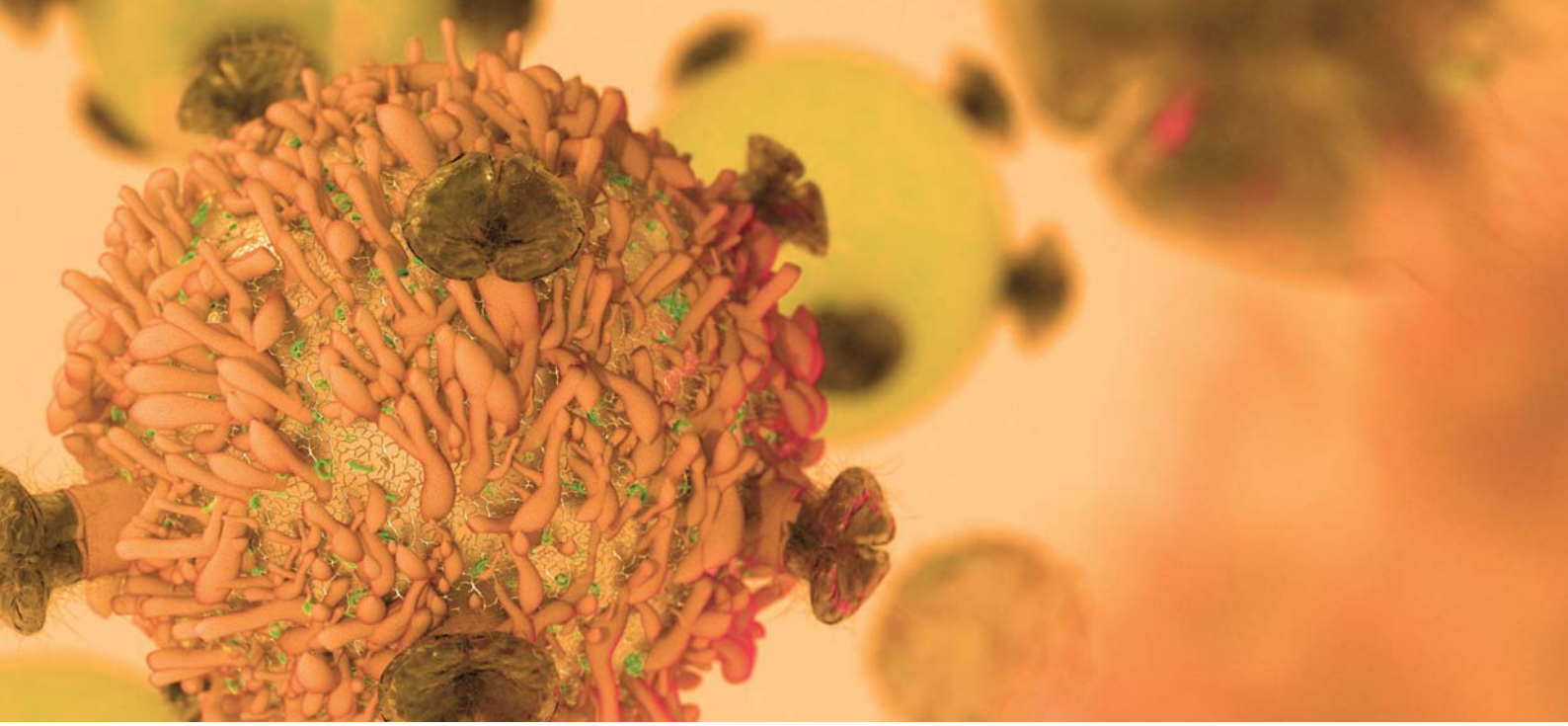

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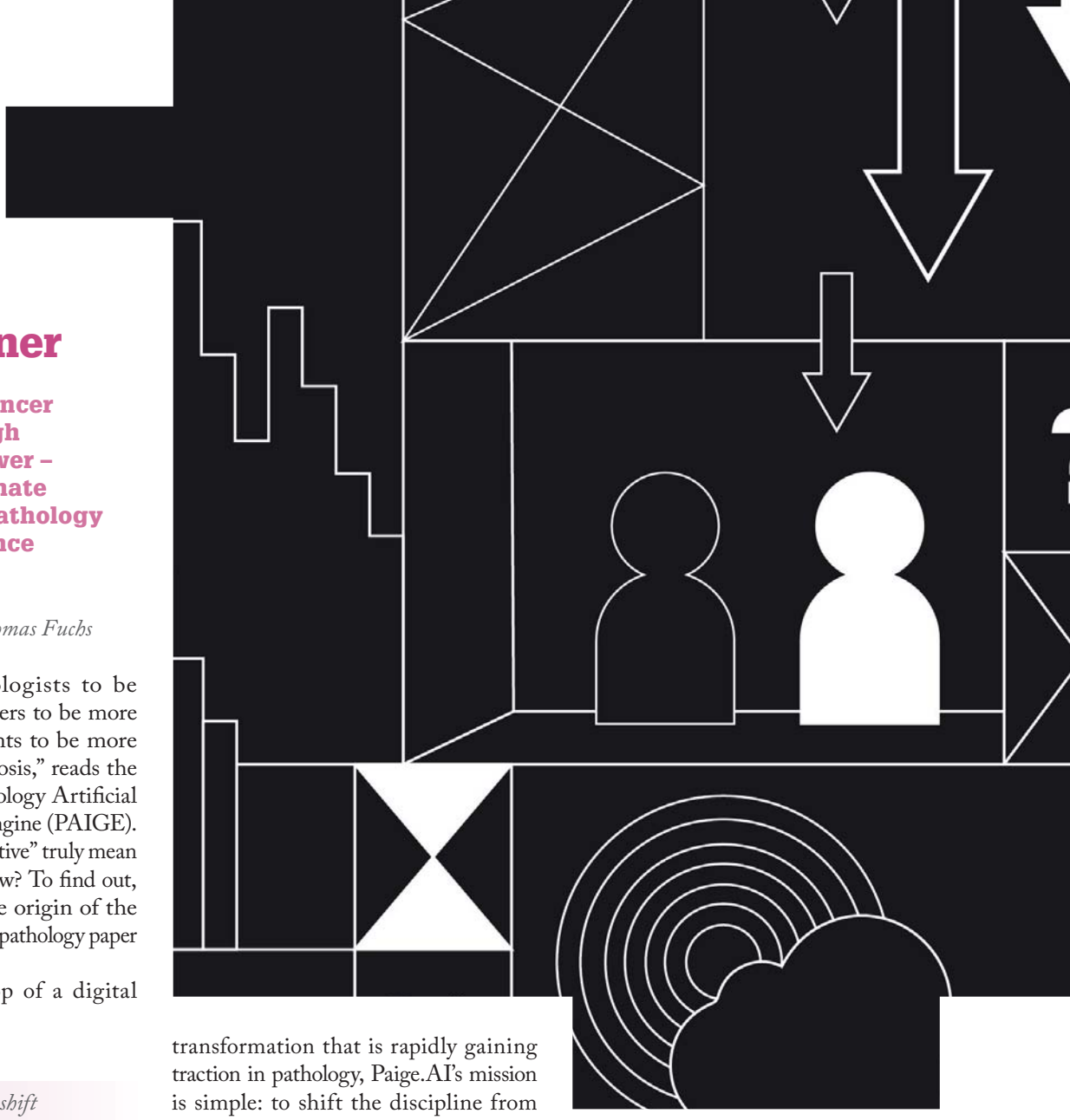
A Real PAIGE-Turner

Revolutionizing cancer diagnostics through computational power – exploring the ultimate ambitions of the Pathology Artificial Intelligence Guidance Engine

By Luke Turner, with Thomas Fuchs



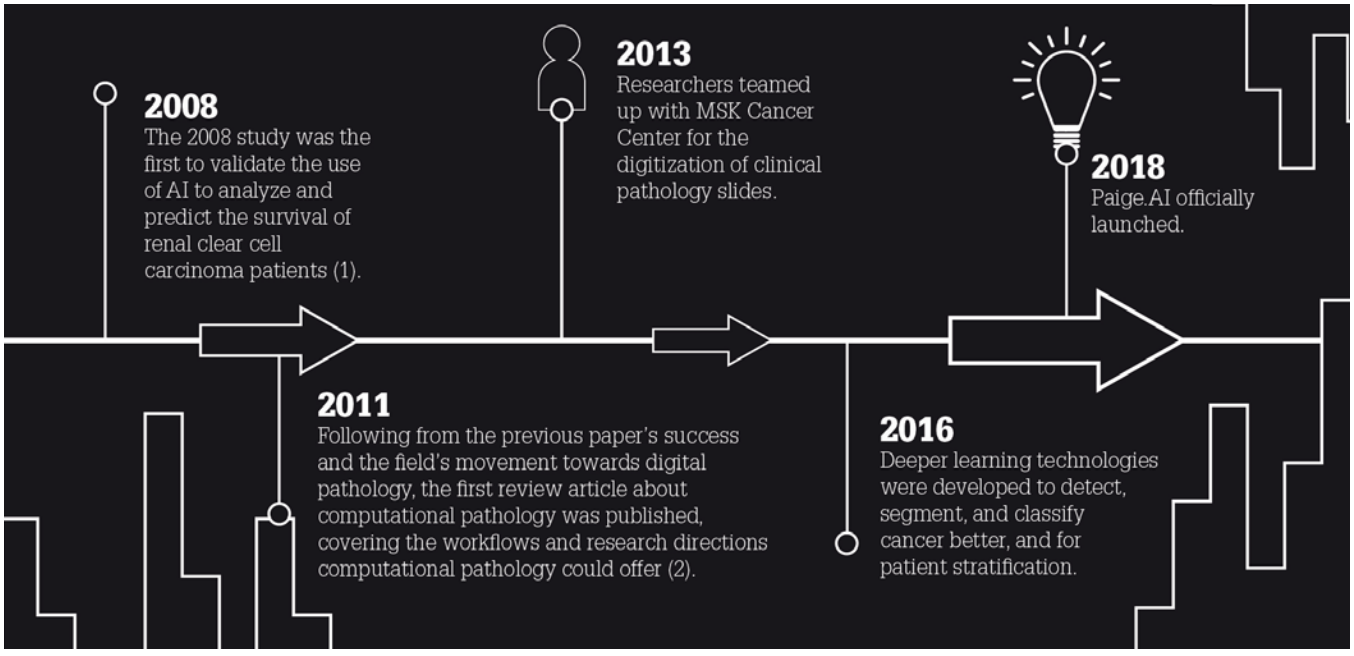
"PAIGE helps pathologists to be more effective, researchers to be more quantitative, and patients to be more confident in their diagnosis," reads the splash page for the Pathology Artificial Intelligence Guidance Engine (PAIGE). But what does "more effective" truly mean in a pathologist's workflow? To find out, we have to dive into the origin of the venture – a computational pathology paper published in 2008.

Against the backdrop of a digital transformation that is rapidly gaining traction in pathology, Paige.AI's mission is simple: to shift the discipline from a qualitative to a quantitative one. To improve the quality of image processing in pathology, Paige.AI, in partnership with the Memorial Sloan Kettering (MSK) Cancer Center, is digitizing vast numbers of slide images to develop decision support systems for pathologists.

"We have two baskets of applications. One is aimed at clinical pathology uses, where we take tasks that pathologists already carry out and help them to be faster, more robust, or more reproducible," says Thomas Fuchs, founder of Paige.AI. "The other part relates to integrating pathology data with other important healthcare data to develop new diagnostic approaches and treatment paradigms, which involve processes that pathologists could not complete without computational pathology and PAIGE. For example, if you notice a strange lesion or some unusual morphology in a patient's sample, at the moment, you can only query your own brain or ask someone for a second opinion if they are available." What PAIGE aspires to achieve is an image retrieval technique that marks the region in question and searches through an entire archive of cases to identify patients with similar tissue morphology. Based solely on an image (rather than text), the search would allow pathologists to learn which treatments those patients received and what their outcomes were, leading to better treatment selection for current and future patients.

At a Glance

- *Paige.AI is striving to shift pathology from a qualitative field to a quantitative one by digitizing vast number of slides and creating decision support systems*
- *Applications include clinical uses to save time for pathologists and an image retrieval technique to identify similar cases*
- *Collaboration with Memorial Sloan Kettering (MSK) Cancer Center facilitates the digitization of 30,000 slides per month, now being ramped up to 100,000 to develop effective algorithms*
- *Paige.AI has recently been granted Breakthrough Device designation by the FDA, boosting its quest for faster and more accurate diagnosis*



Information taken from paige.ai/about

Broad aspirations

Only last month, Paige.AI announced that it has been granted Breakthrough Device designation by the US Food and Drug Administration (FDA). As what appears to be the first-ever such designation for artificial intelligence (AI) in cancer diagnosis, this represents a significant milestone and underlines Paige.AI's ambition to deliver effective diagnostic technologies for cancer patients that surpass those already available. It will enable timely access to Paige.AI's technologies for patients and health care providers alike by accelerating the development, assessment, and review of breakthroughs. "We are honored to achieve this designation by the FDA, which demonstrates the groundbreaking nature of our technology," says Fuchs. "We see it as the next step to producing leading clinical-grade AI in computational pathology, combining vast amounts of high-quality data with unique deep learning architectures to deliver better patient care."

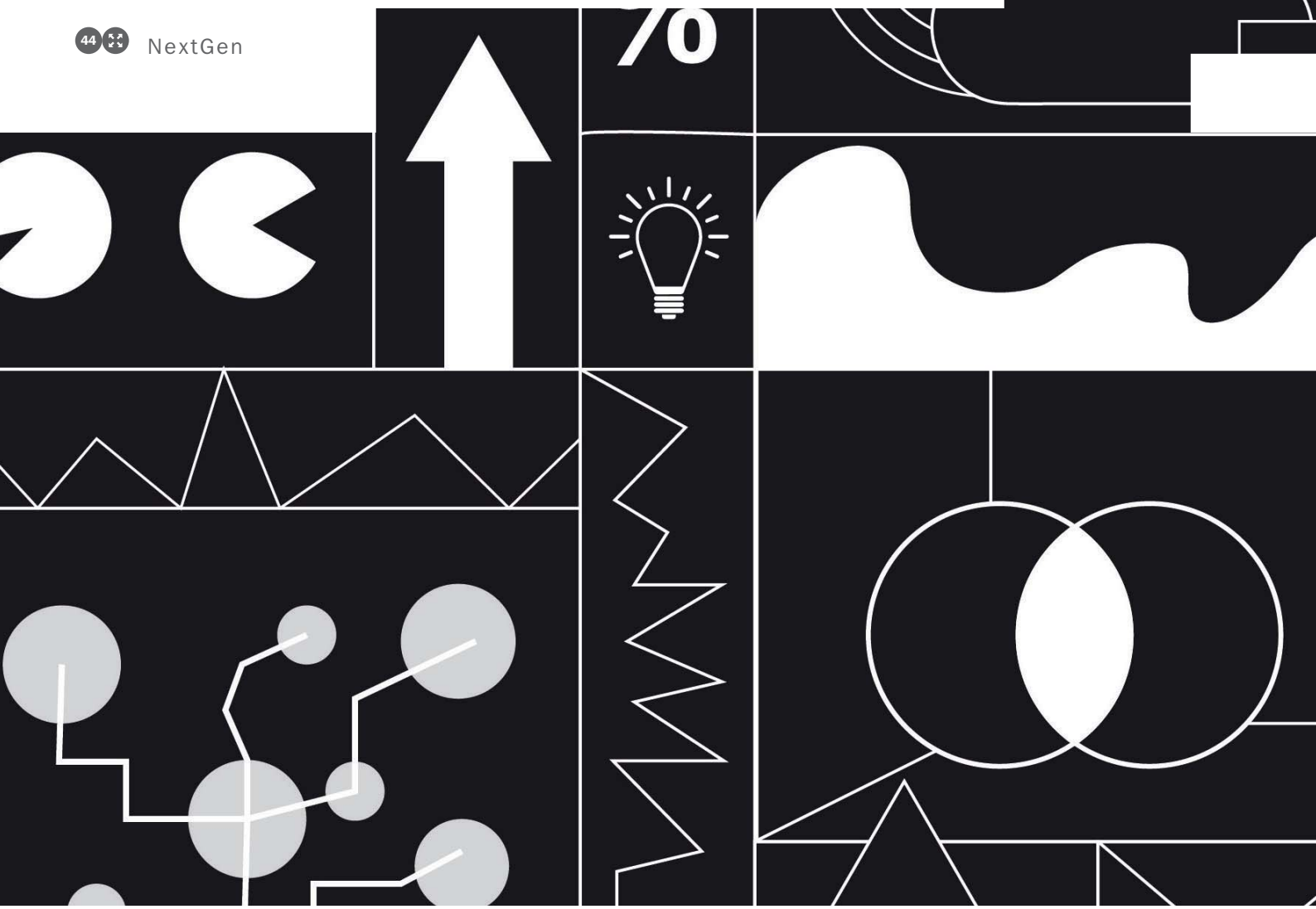
One of the ambitions of the project is to create powerful predictive technologies that span multiple cancer types – a goal MSK facilitates by providing a platform to prospectively scan a large number of slides.

"You can search for patterns that enable you to predict where mutations might arise, and ask whether some mutations lead to different growth patterns."

"We can easily multiplex from one organ type or task to another. We are, of course, focused on the most prevalent types of cancer – such as prostate, breast, and lung – but we're also applying this to the more rare types, such as cholangiocarcinoma and pancreatic cancer. MSK enables this because it is a specialty hospital and we screen tens of thousands of patients to collect a significant amount of data."

Another benefit of the PAIGE project is the sheer wealth of information that is being digitized – data on treatment, survival, and disease recurrence in patients. Fuchs believes that this will prove crucial to future research and education. "Because we have all the required licensing for the correlative information, we have been able to start building technologies that go beyond the image domain. You can search for patterns that enable you to predict where mutations might arise, and ask whether some mutations lead to different growth patterns by using this huge bank of data."

He recognizes that the biggest hurdle in terms of developing the project is implementing PAIGE within the



clinic. "This is something at which big corporations usually fail, and I think that the crucial element to achieving clinical relevance is truly understanding pathology and its detail," he says. The typical pathology department is a network of well-oiled machinery, in which the loss of a few seconds over a certain step can culminate in large disruptions. And this is where the collaboration with MSK strengthens PAIGE's practical potential. "I believe that the AI revolution will emanate from centers like ours, where you have not only the data and machine learning knowledge and experience, but also an abundance of domain experts who can test and optimize the technology in the clinic."

To provide this seamless transition into the clinical workflow, PAIGE's slide viewer software is vendor-agnostic, so it is not tied to the products of a specific manufacturer. The advantage of this approach is that hospitals don't need to replace long-established systems to use PAIGE, and the technology can be installed in laboratories with a variety of different workflows. Fuchs thinks that this will be instrumental to clinical adoption. "It's important to work with the pathologists and institutions whose methods are already ingrained, rather than rushing in and trying to replace everything, which is the strategy that big companies often take. This is only possible if you have that larger set of key opinion leaders who form an integral part of the whole initiative."

The changing face of pathology

Not much has changed in pathology over the last 150 years, so Fuchs can forgive those who question the large-scale changes

"The typical pathology department is a network of well-oiled machinery, in which the loss of a few seconds over a certain step can culminate in large disruptions."

"The widespread adoption [...] will have huge potential benefits for remote areas and those that suffer from a lack of pathologists."

associated with the switch to using AI in the lab. He believes that there is absolutely no danger of pathologists' being replaced by the technology. Rather, it will allow them to reduce the amount of time they spend on repetitive tasks and increase the time they can devote to crucial aspects of the job. "Nobody wants to spend their time counting nuclei. When there are hundreds of slides that need meticulous examination, a machine could easily complete the task, leaving pathologists to think about the statistics instead of creating them."

Another benefit of embracing PAIGE in the laboratory relates to the current shortage of pathologists in many locations. With demand continually increasing as more and more cases require expert diagnosis, the use of computational methods offers an attractive opportunity for workloads to be managed more effectively. "Pathology will look completely different in 10 years. It will be much more diverse and will include algorithms that aid with not only the imaging side, but also the genomics," says Fuchs.

Clinical convenience

The widespread adoption that results from these changes will have huge potential benefits for remote areas and those that suffer from a lack of pathologists. "Imagine a small hospital somewhere in the Midwest that has a patient with a strange or rare type of cancer. They won't be able to send every case to MSK, but imagine if they could use PAIGE – which has been trained by the best pathologists at MSK – to analyze these slides." The project has ambitious targets and strives to have international impact. MSK has strong ties with Nigeria, and the people behind PAIGE are in discussions with Indian pathologists and Chinese cancer centers to optimize their machine learning algorithms for the global stage. The concept of uploading an image from a small rural village and getting a meaningful result back quickly is an enticing prospect.

PAIGE's slide viewer was rolled out institution-wide at MSK in 2017 and is the single entry point for pathologists and cancer researchers there. But how close are we to experiencing PAIGE in clinics around the world? "We are very close with our slide viewer and with our initial disease modules, as evidenced by our recent Breakthrough designation by the FDA. We expect to roll out beta versions of the slide viewer and initial disease modules with partner hospitals and commercial labs later this year, and aim to start selling them in 2020," said Fuchs.

MSK currently scans up to 30,000 pathology slides each month; however, they are ramping up this input to an ultimate goal of 100,000 slides per month. Fuchs' present focus is on digitizing the 25 million slides in the MSK archive to create the single largest digital dataset in pathology, but he recognizes that progress will be slow and steady. In the future, when PAIGE works with more cancer centers, the digitization rate of pathology slides will increase.

One thing is certain – Paige.AI has launched an ambitious project that in no way hides its aspirations to change the face of cancer diagnostics for pathologists and patients alike.

Thomas Fuchs is Founder and Chief Scientific Officer at PAIGE.AI, and Associate Faculty Member at Memorial Sloan Kettering Cancer Center.

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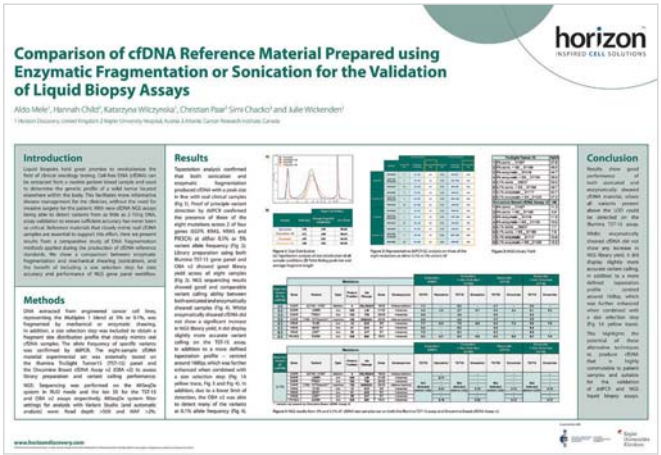
A comparison study of cfDNA reference material preparation methods


Comparison of cfDNA Reference Material Prepared using Enzymatic Fragmentation or Sonication for the Validation of Liquid Biopsy Assays

Introduction

Methods

Results

Conclusion

The growing field of liquid biopsies has huge potential to transform the clinical oncology space. In order to realize the full potential of this emerging technology, sequencing labs need to ensure accuracy by validating a range of challenging new techniques.

This includes the ability to extract cfDNA from blood samples, sequence it at new levels of sensitivity (down to 0.1 percent limit of detection) and establish effective bioinformatics pipelines. Reference materials that closely mimic real cfDNA samples are critical to support this effort.

We have investigated the use of sonicated and enzymatically sheared cell-line derived DNA as alternative methods to create the most commutable cfDNA reference material for the validation of liquid biopsy assays

Download your copy to see the full investigation and our results
https://app1.horizondiscovery.com/comparison-cfdna-reference-material-enzymatic-fragmentation-vs-sonication

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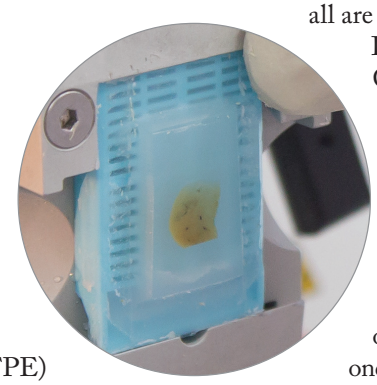

Formalin-fixed paraffin-embedded (FFPE) tissue samples are a standard clinical sample type that serve as an invaluable repository of information for oncology research. With recent advances in next-generation sequencing (NGS) technology, genomic information preserved in these samples can now be recovered to yield important insights.

Targeted NGS is increasingly used to assess the genomic alterations in oncology. While there are several commercially available amplicon-based target enrichment technologies, not all are created equal.

In this technical note, the expert team at SOPHiA GENETICS compared the performance of Paragon Genomics' CleanPlex® OncoZoom® Cancer Hotspot Panel to three other commercially available amplicon-based cancer hotspot panels using DNA of varying quality and quantity extracted from FFPE tissue samples.

The CleanPlex OncoZoom Cancer Hotspot Panel allows for rapid detection and characterization of somatic mutations across the hotspot regions of 65 oncogenes and tumor suppressor genes. The panel is powered by Paragon Genomics' CleanPlex technology which utilizes a proprietary background cleaning chemistry to remove nonspecific PCR products to produce NGS libraries with the highest coverage uniformity.

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http://info.cepheid.com/strat4-test

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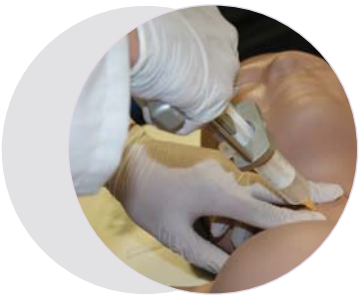

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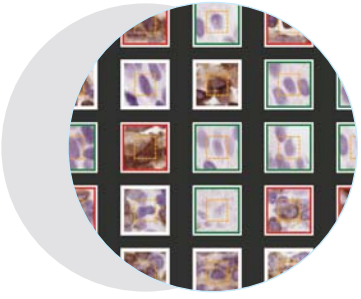

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Leading by Example

Sitting Down With…
Bethany Williams, Leadership and Management Fellow in Digital Pathology at Leeds Teaching Hospitals NHS Trust, Leeds, UK

How did you find your way into digital pathology?

I only chose medicine as a career at the last minute. My original choice was fine art preservation and restoration! Art is my great passion, so I was naturally drawn to histopathology – the assessment and interpretation of complex images, rendered in aesthetically pleasing shades of pink and purple! Making a diagnosis on a histology slide mirrors the way one assesses a work of art – identifying and responding to features and patterns and pulling this together with contextual information to reach a conclusion. What I love about digital pathology is how accessible and engaging these diagnostic images become when they are viewed on a large, high-resolution screen, rather than through the keyhole view of your own light microscope. Branching out into a leadership and management role as a pathology trainee allowed me to gain a new perspective on how pathology services are planned and delivered, and how digital pathology could help address some of the issues we are facing at the moment – particularly mismatches in capacity and demand for histology case reporting.

Can you tell us a little about your world-first fellowship?

I am fortunate to live and work in Yorkshire, where Health Education England offers a fantastic leadership scheme for specialty registrars and allied health professionals from all medical specialties. Successful candidates can take a year out of their training to develop and deliver a quality improvement project and receive formal training in management and leadership skills. My supervisor created the world's first leadership fellowship in digital pathology at Leeds, and I was charged with planning and delivering our pilot project for primary digital diagnosis in breast specimens. This role has since been taken up by others here and elsewhere, so there is a growing community of junior doctors and allied professionals across the world who have experience in digital pathology deployment. We ourselves scaled up to 100 percent slide scanning deployment in September 2018, and I am currently overseeing the training and validation of our remaining pathologists who don't yet diagnose digitally.

In the course of my leadership fellowship, I also developed the research interests I am now pursuing in my PhD fellowship: the patient safety aspects of digital pathology, and the training and validation of doctors for primary digital diagnosis. Much of my work seeks to answer very pragmatic questions about digital adoption: is it safe? Why should our laboratory go digital? How do I know when I am safe to diagnose digitally? How do I build a business case for digitization? It's very exciting to focus on questions that could fundamentally change how we perform routine work.

"I believe pathology is undergoing a complete rebrand."

What advice do you have for others who want to pursue a similar path?

Think carefully about what you really want out of your career, as well as the environments and situations in which you thrive. You are unlikely to find a "perfect" fit with what you want to do, so be prepared to be adaptable (I was once asked to select digital slide images to adorn the toilet cubicles of the new Royal College of Pathologists headquarters – most unexpected!). If necessary, create your own opportunities. Established leadership schemes for junior doctors are excellent, but thin on the ground; if you have the enthusiasm and the inclination, you can collaborate on or develop your own quality improvement projects.

When I was asked what I do for a living, I used to shy away from mentioning that I was a pathologist, thinking it would preface a look of disgust or a lengthy discussion about Silent Witness. In fact, as pathologists, we are privileged to work in a field that people find genuinely fascinating, and I am now more than happy to satisfy that curiosity.

How do you see the future of pathology?

I believe pathology is undergoing a complete rebrand. Digital pathology emphasizes the fact that we are, first and foremost, an imaging specialty. Bringing our work environments and practices into the 21st century will make pathology a more visible and appealing career option for medical undergraduates.

The other great advance we are going to see (as the use of digital images for primary diagnosis becomes more widespread) is the development and implementation of augmented intelligence applications to support our diagnostic work. Deputizing some of the more onerous tasks (counting, quantifying, searching) to background AI will leave pathologists more time to concentrate on refining diagnoses and contextualizing them with patient metadata. This hybrid approach to diagnosis is likely to improve the reproducibility of certain aspects of the diagnosis and improve some of the time pressures placed on pathologists.

As a woman who has worked less than full time in an attempt to balance the demands of family and career, I am particularly excited about the opportunities digital pathology offers for flexible and remote working. I see the technology as a great enabler and leveler, supporting the needs of parents, carers, and those with long term health issues to optimize the hours they can put into their training and careers.

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Klebsiella pneumoniae group
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Proteus spp.
Pseudomonas aeruginosa
Serratia marcescens
Staphylococcus aureus
Streptococcus agalactiae
Streptococcus pneumoniae
Streptococcus pyogenes

Atypical Bacteria (qualitative)
Chlamydia pneumoniae
Legionella pneumophila
Mycoplasma pneumoniae

Viruses (qualitative)
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Influenza B
Parainfluenza virus
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