MARCH 2016 # 17

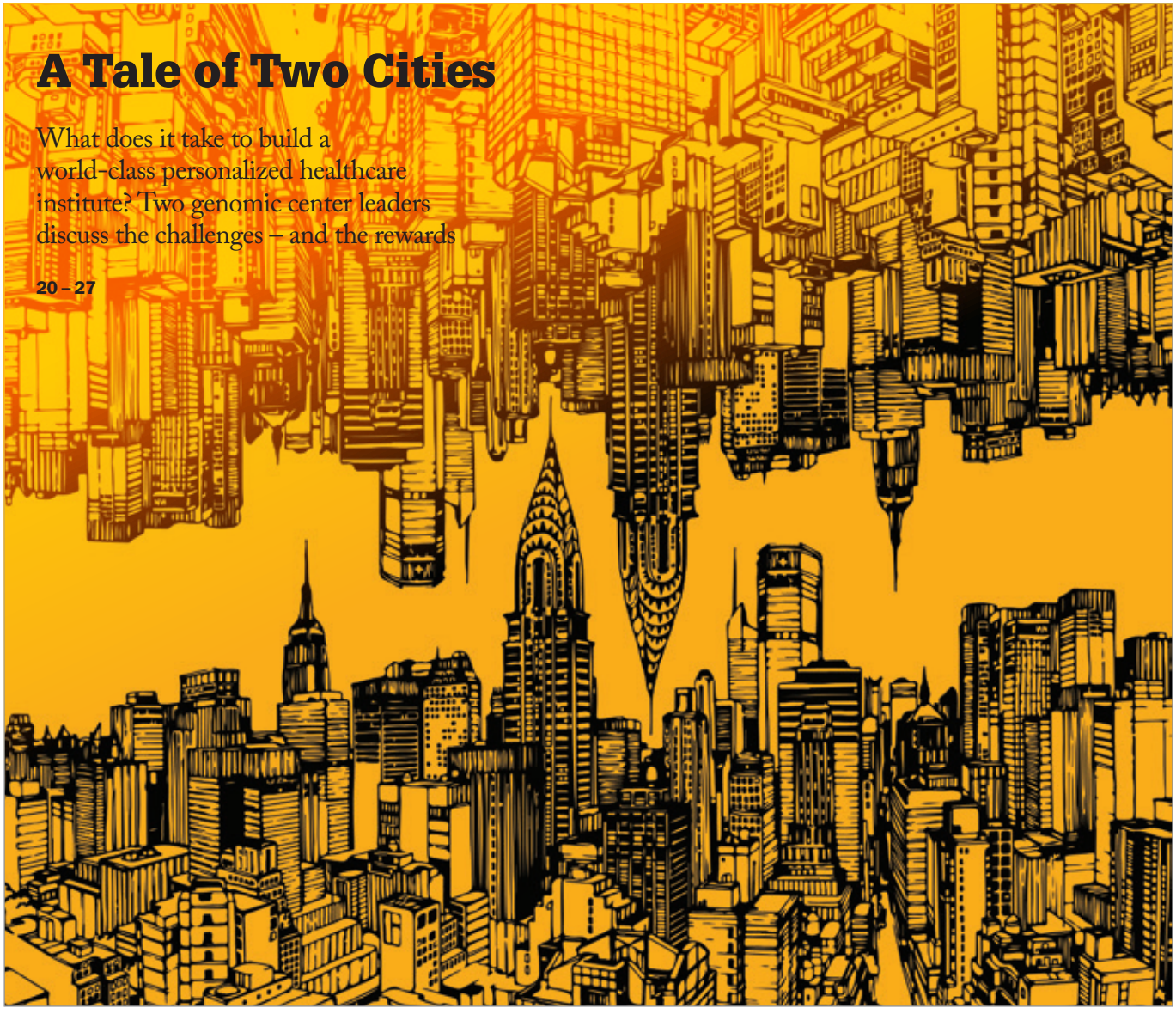
# the Pathologist



## A Tale of Two Cities

What does it take to build a world-class personalized healthcare institute? Two genomic center leaders discuss the challenges – and the rewards





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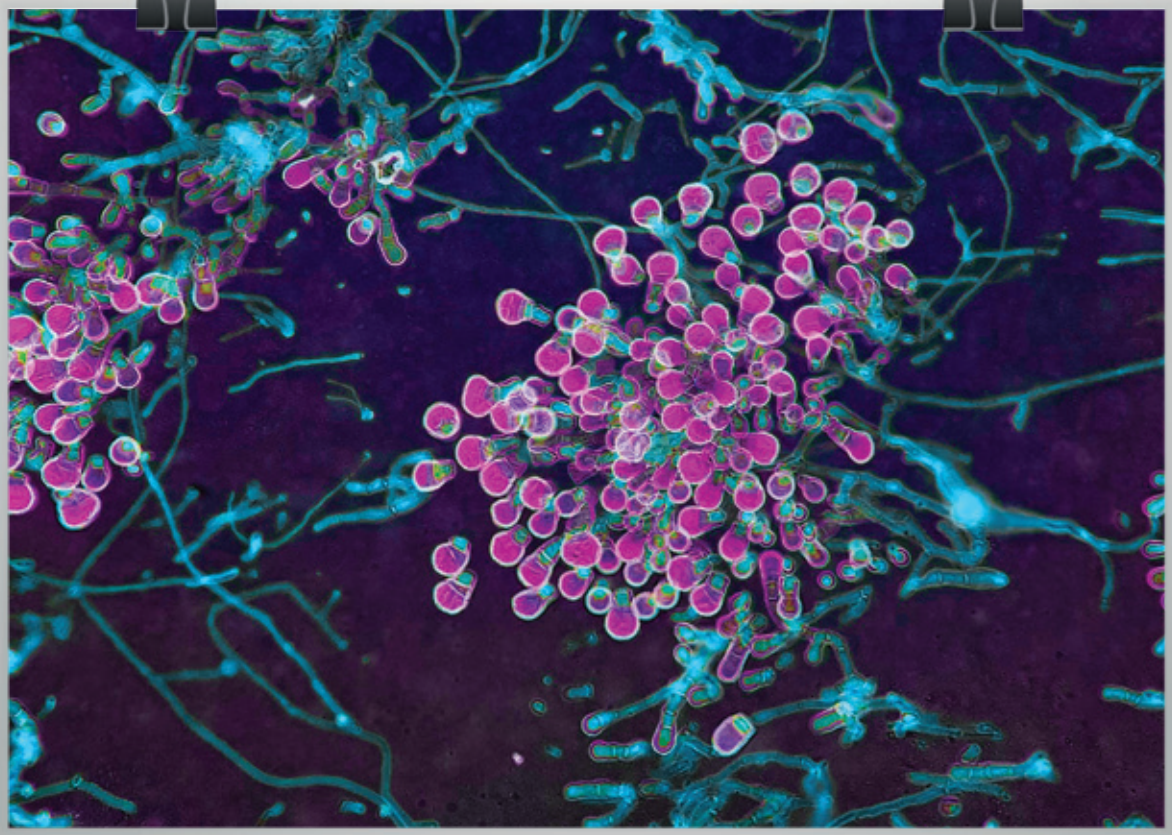
# Image of the Month



This fungus is an *Epicoccum* species, ubiquitous in nature, distributed globally, and commonly found in soil. It does not typically cause infection, but is frequently encountered in patient samples as an environmental contaminant. The conidia (pink) come bursting off a dense mass of conidiophores and hyphae (blue) called a sporodochium. This is a lactophenol cotton blue tape prep viewed through an Olympus BX45 by Eileen Rojas, Clinical Microbiologist at Virginia Mason Medical Center, Seattle, USA, and photographed with an iPhone 5S. This particular specimen came from the inside of a Volkswagen Jetta. The colors have been inverted for additional enhancement.

Do you have an image you'd like to see featured in The Pathologist?
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# Contents



## On The Cover

*Two cities, one focus: to join the personalized healthcare revolution.*

## Upfront



## In My View



## Feature




## In Practice



## Next Gen



## Profession



## Sitting Down With




the Pathologist

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# Perilous Peer Review

*When something seems too good to be true…*



Those of us based in the UK have a tough decision on our hands. Do we stay or do we go? Unsurprisingly, the so-called "Brexit" is dominating headlines – and as decision day looms, the volume and ferocity of the arguments will ramp up. There's already a lot of scaremongering, underhand politics and most frustratingly of all… unanswered questions. Sadly, transparency is not usually associated with government politics, and while listening to yet another government spokesperson on the radio failing to answer the questions posed, I thought about parallels in scientific publishing.

The rules for transparency in the field have tightened a great deal over recent years – and rightly so. As a result, the majority of scientific research papers submitted for peer review are rejected, but a growing number of papers that do make it through are open access – though still not enough, in my view. Nevertheless, I can see this model increasing substantially over the next few years. Certainly, there's room for improvement in the peer review model, but I'd like to think that what we do read is accurate, is based on sound evidence, and is a complete representation of the research (warts and all). Or is it?

The move away from the traditional print-only journal-publishing model has been a boon for science, but has created fear among some publishers; note Elsevier's recent attempts to sue Sci-Hub, which backlashed and instead increased public awareness (1). The new trend towards open access (along with the costs that are passed onto authors to make research freely available), has sadly, however, also created a system that's ripe for misuse. According to a study by Finnish researchers, so-called "predatory publishers" made around $75 million last year (2). As part of their research, they combed a list of discredited journals and they did the math (3). According to the curator of the list, Jeffrey Beall, these are journals that carry fake impact factors, promise a one-week peer review, and overlook plagiarism. Worryingly, the number of papers published by these journals has increased from 53,000 in 2010 to an estimated 420,000 in 2014! Though the Finnish team found the problem to be highly contained in just a few countries (mainly developing nations in Asia, with India accounting for the majority), we can't assume that the predatory publishing trend won't become more widespread. Heated competition makes publishing research increasingly difficult – and given that career progress and funding is often tied to impressive publication credentials, it's easy to see why taking such a risky approach might appeal to some.

So if you're ever tempted by a journal with a fast turnaround "peer-review" process and guaranteed acceptance of your research, it's worth remembering the old idiom: "when something seems too good to be true, it probably is."

**Fedra Pavlou**
*Editor*


*References*

1. *techdirt, "As predicted, Elsevier's attempt to silence Sci-Hub has increased public awareness massively", Accessed March 22, 2016. http://bit.ly/1R9f1tC*
2. *C Shen, BC Björk, "'Predatory' open access: a longitudinal study of article volumes and market characteristics", BMC Med, 13, 230 (2015). PMID: 2642306.*
3. *Beall's List, Accessed March 8, 2016 at https://scholarlyoa.com/publishers/*

## James Nichols

Jim is Professor of Pathology, Microbiology, and Immunology, Medical Director of Clinical Chemistry and Point-of-Care Testing at Vanderbilt University School of Medicine in Nashville, Tennessee, USA. "My research interests span evidence-based medicine, information management, laboratory automation, point-of-care testing and toxicology," he says. He is board certified in both clinical chemistry and toxicological chemistry by the American Board of Clinical Chemistry.

Read Jim's pick of 2015's landmark research publications on page 44.

## Liron Pantanowitz

Liron is Professor of Pathology and Biomedical Informatics, Director of Pathology Informatics, Director of the Pathology Informatics Fellowship Program and Director of Cytology (Shadyside) at the University of Pittsburgh Medical Center, Pennsylvania, USA. He is Editor-in-Chief of the Journal of Pathology Informatics and widely published in the field of digital pathology. Liron's expertise in the application of informatics technology to pathology is in great demand, but his research interests also extend to cytopathology, HIV/AIDS and infectious diseases.

Liron describes his choice of landmark publications in 2015 on page 48.

## Jim Westgard

An internationally recognized expert in quality control, Jim is the inventor of multirule QC, informally known as the "Westgard Rules." He's also co-founder and principal at Westgard QC, Inc., which provides laboratories with technology and training for quality management, as well as an Emeritus Professor in the Department of Pathology and Laboratory Medicine at the University of Wisconsin School of Medicine and Public Health. He continues to work with the University of Wisconsin as a teacher in the Clinical Laboratory Science Program and co-director of an online graduate certificate program in laboratory quality management.

On page 50, Jim discusses quality control and the need to shift the focus from preanalytical, to analytical error.

## Pedro Oliveira

Pedro is a pathologist at Hospital da Luz, Lisbon, Portugal. "Attending a course in molecular biology techniques during my internship changed my life. An enthusiastic young pathologist's talk on cancer impressed me so much that I did a U-turn in my specialization options and chose anatomic pathology over surgery." After many years as a pathologist, Pedro still believes it was the wisest decision of his life, and he never gets tired of telling his story to his residents and students. By the way, the enthusiastic young pathologist who inspired him is Manuel Sobrinho-Simões at the University of Porto, voted #1 in The Pathologist's 2015 Power List!

Pedro makes the case for video microscopy and digital literacy on page 17.

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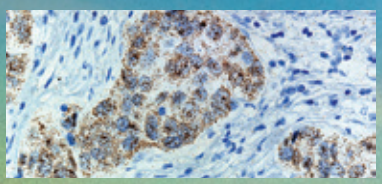

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# Upfront

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

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## Breaking Bad Genetics

**Regulatory RNA-binding protein hnRNP H1 may play a role in the genetics of methamphetamine sensitivity and other substance addictions**

Some of us may have heard people say, "Addiction runs in my family." And it's common knowledge that substance abuse is driven by genetic, as well as environmental factors. It's only recently, though, that a group of researchers from Boston University School of Medicine have identified a gene that demonstrates a causal relationship to addiction – specifically, to methamphetamine sensitivity (1).

"Both genes and environment can exert independent and interactive influences on an individual's risk for developing an addiction throughout life," explains corresponding author Camron Bryant. "Gene discovery is one important piece of the puzzle in understanding the neurobiological adaptations that confer risk versus resistance to the addictions throughout development and into adulthood." Bryant and his colleagues employed an unbiased, discovery-based approach called quantitative trait locus (QTL) mapping in mice; they sought broad chromosomal regions causally associated with variations in sensitivity to the methamphetamine locomotor stimulant response – a behavior is associated with activation of the dopamine reward circuitry in the brain. "We honed in on a particular region of a chromosome by using a 'fine mapping' approach to identify the smallest possible region necessary for differential drug responding. The region we identified contained only two protein-coding genes, which we mutated to identify the causal factor." The gene Bryant and his colleagues ultimately discovered is called heterogeneous nuclear ribonucleoprotein H1 *(hnRNP H1)*, which codes for an RNA-binding protein that regulates hundreds of genes in the brain.

"An obvious next step is to determine whether or not this novel factor is genetically associated with methamphetamine addiction in humans," says Bryant. "That would strengthen the impact of our findings and could have implications for prescribing psychostimulant drugs like Adderall or Ritalin, which have similar molecular mechanisms of action to methamphetamine." At the moment, the researchers are developing the tools to identify the brain region-specific RNA targets of *hnRNP H1* and assessing its contribution to behavioral traits more closely aligned with addiction, including conditioned drug reward and self-administration of drugs of abuse. They are also extending their findings to other disease models known to be associated with dysfunction of RNA binding proteins. Gene expression analysis indicates a role in dopaminergic neuron development – meaning that *hnRNP H1* may play a role not only in addiction, but in neurological disorders like ADHD, schizophrenia, and Parkinson's disease. *MS*

*Reference*

1. *N Yazdani et al., "Hnrnph1 is a quantitative trait gene for methamphetamine sensitivity", PLoS Genet, 11, e1005713 (2015). PMID: 26658939.*

# Recurrence Ratio

**In patients with early-stage breast cancer, the neutrophil-to-lymphocyte ratio may indicate future risk of recurrence**

With a diagnosis of breast cancer, treatment and remission are only the first step on a long journey. Even patients whose cancer was caught in its earliest stages require long-term monitoring to protect against the return of the disease. But not all patients run the same risk of recurrence, and much like treatment, monitoring shouldn't be a one-size-fits-all strategy. So how can we decide which patients to treat more aggressively, and which to monitor more closely after treatment?

One metric useful in other types of cancers is the neutrophil-to-lymphocyte ratio (NLR) – but its utility in breast cancer has thus far been uncertain. Studies conducted in women of Asian descent, who generally have more favorable odds of survival than those of other ethnicities, have yielded inconclusive results. As a result, a team of researchers from Italy spent 15 years monitoring 300 white women diagnosed with stage I or II breast cancer to determine whether or not a high NLR is associated with poorer disease-free survival. "It has been reported to be able to predict prognosis in a variety of solid malignancies," says Michele Orditura, lead author of the resulting paper (1). "However, evidence is scarce and controversial with regard to breast cancer. Therefore, we sought to determine whether NLR could be useful as a prognostic indicator in early breast cancer."

Based on pre-treatment blood counts, his team stratified patients into low-NLR (≤1.97) and high-NLR (>1.97) groups. At each subsequent checkup (one, three, six, nine, 12 and 15 years after treatment), patients in the low-NLR group showed better disease-free survival than those in the high-NLR group (see Figure 1). The researchers also searched for other factors that potentially influence survival and identified two: a premenopausal state, and the presence of cancerous cells in axillary lymph nodes. Each of these, along with NLR, is independently associated with a patient's risk of recurrence. But what's the mechanism behind the ratio? "We can only speculate on the biological meaning of NLR at the moment," says Orditura. "In simple terms, NLR may reflect both the role of systemic inflammation in favoring development of metastasis and the inability of the immune system to fight cancer cells."

Although this observational study can only highlight the correlation between NLR and recurrence, Orditura hopes that it may one day guide treatment and monitoring plans. "Ideally, NLR evaluation should help physicians choose the most appropriate treatment in the individual patient in order to improve survival." *MS*

*Reference*

1. *M Orditura et al., "Neutrophil to lymphocyte ratio (NLR) for prediction of distant metastasis-free survival (DMFS) in early breast cancer: a propensity score-matched analysis", ESMO Open, 1, e000038 (2016).*

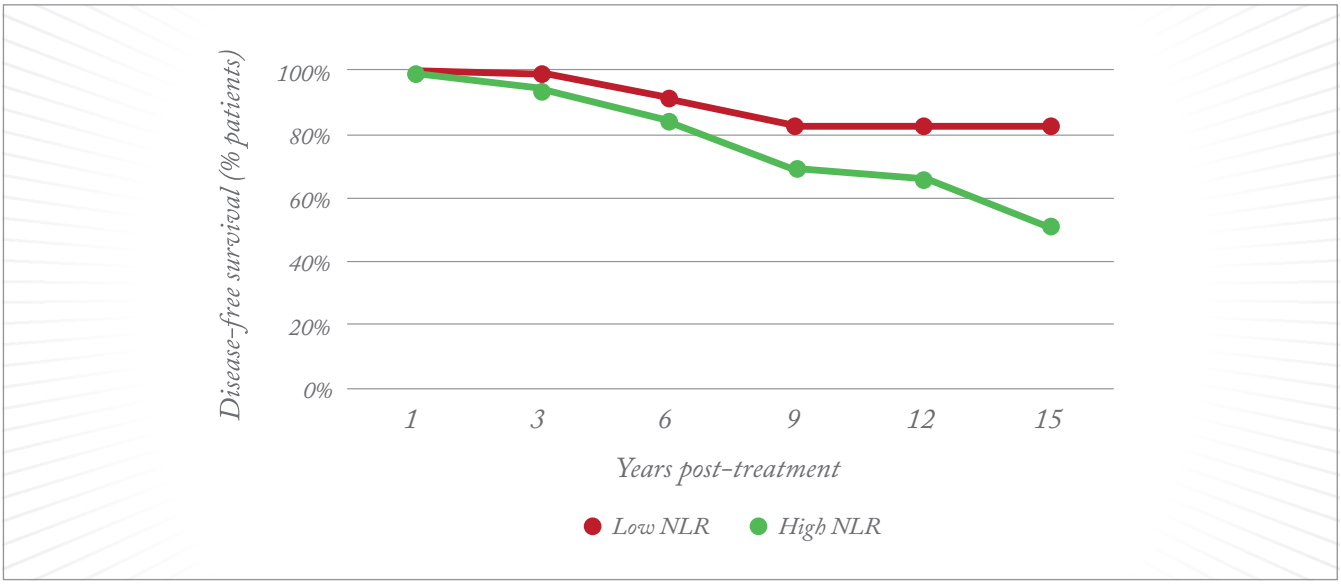
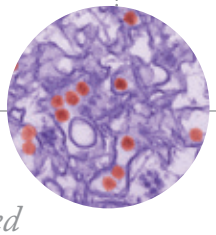



Figure 1. Percentage of patients in the low- and high-NLR groups exhibiting disease-free survival at each checkup after treatment.

# Tracking the Zika Virus

## What do we know so far?



### Cases of microcephaly in Brazil since October 2015

**6480** *suspected or confirmed*

**2212** *investigations concluded*

**863** *Zika virus confirmed*

*Source: World Health Organization*

## Initiating innovation

*Academic papers published*

**223 from October 2015 to date**
**273 in the last 5 years**
**282 in the last 10 years**

**Source: PubMed**

| *Organization* | *Type* | *Status* |
|---|---|---|
| Inovio Pharmaceuticals & collaborators | DNA | Could start clinical trials by end of year |
| Bharat Biotech | 1 inactivated, 1 recombinant | Preclinical trials to complete this summer |
| Hawaii Biotech | Subunit | Preclinical |
| Replikins | Subunit | Starting preclinical trials |
| Sanofi Pasteur | TBC | Launched a project |
| GlaxoSmithKline | TBC | Assessing its research platform |
| NIAID | 1 DNA, 1 live | Preclinical |


## Diagnosis

| *Viral nucleic acid detection* | *Virus isolation* | *Serological testing* |
|---|---|---|
| • RT-PCR targeting the non-structural protein 5 genomic region<br>• primary means of diagnosis<br>• serum 1–3 days after symptom onset<br>• saliva and urine 3–5 days after symptom onset | • primarily for research<br>• serum 1–3 days after symptom onset<br>• saliva and urine 3–5 days after symptom onset<br>• also possible from infected mosquitoes or inoculated cell lines | • immunofluorescence assays<br>• ELISA<br>• plaque reduction neutralizing test<br>• conducted against anti-Zika IgM or IgG<br>• cross-reactivity with other flaviviruses possible |

*1952*
Zika first detected in humans

*May 2015*
Zika virus confirmed as the cause of an outbreak in Brazil

*September 2015*
Increase reported in the number of infants born with microcephaly in Zika virus-affected areas

*November 2015*
Zika virus isolated in a newborn baby with microcephaly

*February 2016*
- WHO declares microcephaly a public health emergency
- Brazilian scientists sequence the Zika virus genome
- Zika virus detected in the amniotic fluid of fetuses with microcephaly

# A Collaborative Call to Arms

**Thirty-one researchers on three continents have teamed up to call for more research into a potential microbiological cause of Alzheimer's disease**

It's unusual for a scientific proposition to garner so much support that researchers and clinicians from around the world come together to express it. But that's exactly what happened recently in the Journal of Alzheimer's Disease, where 31 specialists from locations as varied as Spain, Finland and the United States co-authored an editorial proposing microbes – specifically, herpes simplex virus 1 (HSV1), *Chlamydophila pneumoniae* and spirochetes – as the major cause of Alzheimer's disease (1).

"We write to express our concern that one particular aspect of the disease has been neglected," the authors said, referencing studies implicating HSV1 and bacterial agents in disease-related changes to the brain. The editorial includes evidence for an infectious component of Alzheimer's disease, including the presence of microbes in the brain, colocalization of pathogen signatures with disease pathology, and the fact that *APOE* polymorphisms affect susceptibility to both Alzheimer's and infectious diseases. The editorial also discusses evidence for causation (such as amyloid beta deposition observed after infecting cell cultures and mouse models with HSV1) and mechanism (such as polymorphisms in the human cholesterol 25-hydroxylase gene *CH25H*, which is selectively upregulated by infection and governs both amyloid beta deposition and Alzheimer's disease susceptibility).

The proposed Alzheimer's disease etiology involves the infectious agents' remaining latent in the central nervous system until they are reactivated by the aging process and the decline of the immune system. Once active, they cause inflammation and neuronal damage that results in dysfunction, amyloid beta induction, and ultimately Alzheimer's disease. But if this is indeed where the disease originates, what can be done? Because of the personal and public impact of Alzheimer's, and because so many therapy trials have failed in recent years, the authors are calling for research into the role of infectious agents – both to uncover the cause of Alzheimer's disease and to explore the potential benefits of antimicrobial therapies. *MS*

*Reference*

1. *RF Itzhaki et al., "Microbes and Alzheimer's disease", J Alzheimers Dis, [Epub ahead of print] (2016). PMID: 26967229.*

# Personalizing Stroke Prevention

## C-reactive protein levels and genetic variations are linked to increased risk of a second ischemic stroke

The humble C-reactive protein (CRP) test is used for a wide range of assessments, including infections, autoimmune diseases and chronic inflammatory conditions. But it's possible that the test has just added another function to its arsenal: identifying patients at risk of a second stroke.

"We were actually interested in biomarkers in general that might be useful in prediction of stroke recurrence," says Stephen Williams, first author on the paper recently published in Neurology. "In this study, we tested six biomarkers loosely classified as 'pro-inflammatory.' By taking this unbiased approach, we did not expect any one biomarker to be more useful than any other. It just so happened that CRP came up positive." Elevated CRP levels are a known risk factor for incident vascular disease – so it makes biological sense that not only high levels of the protein, but also variations in the CRP gene, are linked to an increased risk of recurrent ischemic stroke.

Brad Worrall, associate medical director of the Stroke Service and director of the Acute Stroke Intervention Team at the University of Virginia Health System, says, "Stroke recurrence risk is generally assessed based on the mechanism of the stroke and the associated risk factors. Our current interventions do work, but the CRP tests (both circulating CRP and the genetic test) might allow us to select those individuals that are at especially high risk." Williams believes that combining the genetic information from his study with circulating CRP levels would be the most powerful approach for the clinic. However, he cautions that, before a broad recommendation can be made, follow-up studies addressing the clinical utility of the tests will be needed.

"Currently, genomic data is not broadly used in day-to-day clinical evaluations, and we hope that this study will aid in shifting that practice toward a more integrated approach," Williams says. "Genomic testing is coming – and coming fast. In the very near future, we will need laboratory medicine professionals to be able to execute these genetic tests and clinicians to be knowledgeable as to how to interpret them." And the researchers haven't halted their efforts – they're currently investigating additional biomarkers, assessing the utility of metabolomics data, and trying new methods of using gene expression to explore the underlying biology of atherosclerosis, the biggest risk factor for stroke. If the group's follow-up studies go as hoped, ischemic stroke patients may one day learn their risk of recurrence from a simple blood test. *MS*

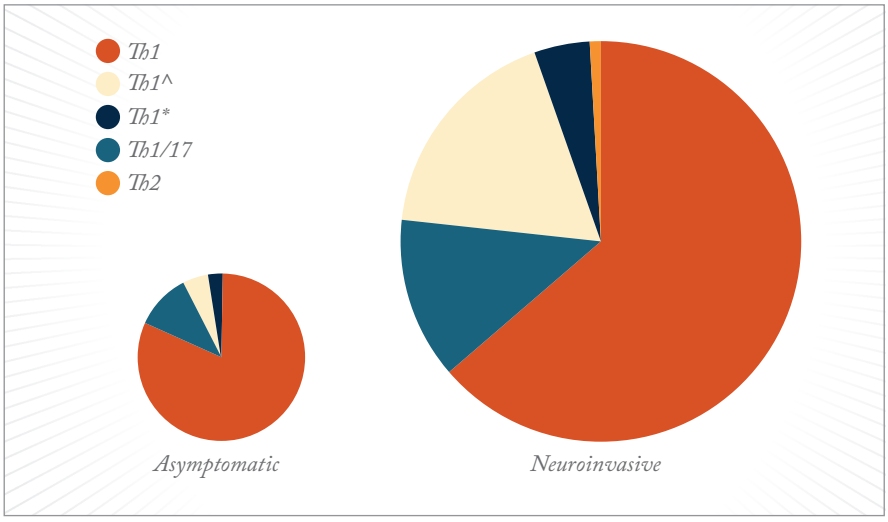
*Reference*

1. *SR Williams et al., "Shared genetic susceptibility of vascular-related biomarkers with ischemic and recurrent stroke", Neurology, 86, 351–359 (2016). PMID: 26718567.*

# Reaction Rights and Wrongs

## Research into patient response to West Nile virus has uncovered some unexpected results...

Every few years, the mosquito finds itself in the news for its impressive ability to transmit pathogens to humans. Though the list of mosquito-borne diseases is a long one that includes malaria, encephalitis and the currently infamous Zika virus, one that regularly makes its presence known is West Nile virus (WNV). This flavivirus, although




Differences in magnitude and characteristics of WNV-specific responses between asymptomatic infections (left) and neuroinvasive disease (right).

Credit: James et al.

asymptomatic in about 80 percent of those infected, causes flu-like symptoms in others – and worse, one in 150 patients experiences severe neuroinvasive disease with a fatality rate of about 10 percent (1). But what makes some patients respond poorly to infection, while others experience no symptoms at all? Eddie James and his colleagues at the Benaroya Research Institute at Virginia Mason Medical Center thought the answer might lie in patients' immune responses.

"This was actually a case where our initial results were not what we expected," James says. "Several of our first subjects with severe infections were elderly and we expected them to have impaired T cell responses, but instead we saw the opposite – many more WNV-specific T cells and a more functional response. That observation led us to a new hypothesis that the virus was triggering exaggerated immune responses and causing problems in subjects who had worse outcomes." The researchers compared blood samples from 24 donors with asymptomatic infections and 16 with neuroinvasive disease (2). Patients in the latter group had higher numbers of WNV-specific CD4+ T cells, which are crucial for viral protection and clearance from the central nervous system. Not only that, but the cells behave differently to those of asymptomatic individuals.

The information has applicability beyond WNV. "In an earlier publication on H1N1 infections (3), we noticed that subjects hospitalized due to influenza infection had exaggerated influenza responses. In cases like that, it could be beneficial in some cases to use low-dose steroids or more targeted drugs to dampen the immune response." The team hope to explore the issue further using more common viruses, like influenza, as a model system to find out whether the potential for exaggerated response can be detected beforehand – and perhaps one day lead to a clinical test. *MS*

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1. *WebMD, "West Nile Virus Questions Answered". Available at: http://wb.md/1S4g3au. Accessed March 16, 2016.*
2. *EA James et al., "Neuroinvasive West Nile infection elicits elevated and atypically polarized T cell responses that promote a pathogenic outcome", PLoS Pathog, 12, e1005375 (2016). PMID: 26795118.*
3. *J Yang et al., "CD4+ T cells recognize unique and conserved 2009 H1N1 influenza hemagglutinin epitopes after natural infection and vaccination", Int Immunol, 25, 447–457 (2013).*

# Making Scents of Alzheimer's

## A unique odor signature in the urine of Alzheimer's mouse models may one day lead to simple tests for the disease

Between animals that can detect cancer and tuberculosis (1) and humans who can smell Parkinson's disease (2), it seems clear that – when it comes to detecting disease – there's something in the air. Until now, it has been a challenge to detect that unique "something," but a new study from the Monell Chemical Senses Center has reported evidence of an identifiable odor signature for Alzheimer's disease (3). What makes these findings particularly strong is that they were demonstrated by two independent tests: both a bioassay and a chemical assay showed alterations in the urine of mice overexpressing the protein associated with Alzheimer's disease in man.

"We believe that the mice in the bioassay are responding to a unique pattern of volatiles," says Bruce Kimball, one of the study's authors. "Likewise, we approached the chemometric portion of the study in the same manner – looking for patterns of up- and downregulated odorants." It's not that new compounds appear in the urine of Alzheimer's mouse models; rather, the same compounds exist in mice with no disease, but because they exist in different concentrations, they emit a unique odor. "Our results suggest that the odor signal may be present well before any pathological changes occur," adds Kimball, although he notes that this first study did not monitor the progression of the disease.

Could this lead to a simple Alzheimer's test in humans? Perhaps one day, but right now the work is still at the proof-of-concept stage. "If an alteration is identified in the human population, the desired result would be some sort of chemical confirmation – be that by chromatographic analysis at a laboratory or perhaps even some sort of urine dipstick." Kimball believes that the next step is to examine urinary volatiles in humans, but we're much more variable than APP mice – meaning that a huge number of samples will be required to investigate even one disease. And Kimball doesn't want to stop at just one. "We currently think that all inflammatory processes result in alteration of body odors, and much work is required to evaluate the specificity of such alterations," he says. "There is much to be done!" *MS*

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3. *B Kimball et al.,"Alterations of the volatile metabolome in mouse models of Alzheimer's disease", Sci Rep, 6, 19495 (2016). PMID: 26762470.*

# In My View

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

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

*Contact the editor at fedra.pavlou@texerepublishing.com*

## Organization Overhaul

### Breaking down the clinical laboratory's walls to make way for the future of lab organization

*By Stacy G. Beal, Clinical Pathologist, Associate Medical Director of Microbiology, Clinical Chemistry, and Point of Care, University of Florida Health Shands Hospital, Gainesville, Florida, USA*

Clinical laboratories are undergoing a substantial revolution. Within a few decades, the laboratory will be nearly unrecognizable. Where we have physical and theoretical walls that separate different areas of the lab, such as chemistry from microbiology, hematology from urinalysis, and bacteriology from virology, the labs of the future will no longer be in these silos. The virology laboratory offers one of the best examples of technology driving these barriers down.

While virology labs previously performed laborious and time-intensive cell cultures, many turned into molecular labs, with polymerase chain reaction (PCR) as the mainstay of the diagnostic techniques. It was necessary to separate these labs into three areas: pre-PCR, where patients' samples were processed and nucleic acids were extracted; PCR, where the actual amplification of nucleic acids took place; and post-PCR, where further analysis of the amplicons was performed. This separation minimizes the risk of contamination from one PCR reaction to another; since so few nucleic acid strands are necessary to begin an amplification process, contamination was a very real and dangerous risk.

Now manufacturers offer entirely closed systems. This means that once a patient's sample is in the reaction cartridge, it remains sealed through all the extraction and amplification steps, substantially reducing the risk of contamination. Post-amplification analysis also occurs within the instrument, and an associated computer software program delivers a result, which minimizes the need for specialized expertise and allows moving these assays out of molecular labs and into other laboratory sections. Breakthroughs such as these require us to change our way of lab organization.

Similarly, many PCR platforms are random-access, so the sample can be placed on the instrument at any time – if there is an open place for it. Since these samples require very little up-front processing (no separate nucleic acid extraction steps), there is no need to batch these in groups in order to maximize workflow efficiency, as was done in the past. In addition, many platforms include all of the controls and reagents needed in each disposable cartridge, so you can perform the tests as they arrive in the lab.

*"Within a few decades, the laboratory will be nearly unrecognizable."*

*"I believe that future labs will no longer carry distinctions between different areas, such as chemistry, virology, bacteriology, and urinalysis."*

This leads to another aspect of laboratory management: hours of operation. In the "olden" days of batch testing, manual steps and long turnaround times, it made sense to operate labs only during business hours. The idea being if a test takes 10 hours, why perform it stat? Getting a result in 10 hours is probably not much better for patient care than getting a result in 20 or 30 hours. However, with these new molecular tests, where results can be available in as little as 30 to 60 minutes, it doesn't make sense to wait until Monday morning to run a test that could have produced results which could have been acted upon Friday at 6 pm. The commonly used molecular test for *Clostridium difficile*, a highly contagious diarrhea-causing bacterium, exemplifies this approach. Random-access PCR testing enables extremely rapid, highly sensitive and specific diagnosis of this organism, allowing treatment and infection control practices to start almost as soon as the symptoms begin.

In summary, today we have molecular tests that are random-access, very fast, closed systems that require minimal technologist hands-on time, almost no analysis, and contain onboard reagents and controls. What does this resemble in our current lab environment? These platforms fit perfectly into what we now consider our core lab, and I believe that future labs will no longer carry distinctions between different areas, such as chemistry, virology, bacteriology, and urinalysis. In my view, as more and more testing continues to become entirely automated using PCR or other methods, we need to be ready to completely overhaul our approach to laboratory organization.

# Real-Time Vision

**Video microscopy sessions enable lively and enjoyable discussions of case presentations**

*By Pedro Oliveira, Pathologist at Hospital da Luz, Lisbon, Portugal*

Digital technology is revolutionizing microscopy for pathologists (1). Now we can capture optical images electronically and display them in their digital format directly on a PC monitor. And, because the image is projected onto a screen, there is no need for complicated optics otherwise necessary for direct human eye observation. Although based initially on commercially available microscopes coupled to video cameras and monitors, rapid technological evolution is enabling manufacturers to deliver sophisticated systems with faster real-time digital image reconstructions as well as whole slide scanning. And the cost of these new devices is becoming more attractive to hospital pathology departments, so naturally, use of them is increasing.

I have no doubt that for case discussions and residents' sign-outs, pathology departments worldwide will soon replace their traditional multi-head microscopes with video microscopy, which will not only help with training, but will also rise in importance at multidisciplinary meetings and conferences, in particular when used with large, high-definition screens. The direct, live image feed from the glass slide on the microscope

*"I have no doubt that for case discussions and residents' sign-outs, pathology departments worldwide will soon replace their traditional multi-head microscopes with video microscopy."*

*"In my view, in the near future, we shall see the end of "digital illiteracy" in pathology."*

makes these meetings more interesting and proactive. Not only that, but they allow a more accurate representation of the work of the pathologists, so I see PowerPoint slides being redundant during these multi-team meetings too.

To help educate pathologists about the utility of these digital developments, the European Society of Pathology (ESP) has introduced video microscopy session masterclasses held at its Brussels headquarters and at its annual meetings.

As Chair of the video microscopy uropathology sessions during the Lisbon (2013) and more recently Belgrade (2015) annual meetings of the ESP, my overall impression is positive. Discussions are now much more interactive and informal; presenters can easily show an area of a slide to support a diagnosis or quickly respond to questions by literally showing the answers on screen! This is difficult to do using just a homemade PowerPoint presentation. Moreover, the feedback from residents and trainees attending these sessions is extremely encouraging, and they appreciate the more realistic format. The only drawback, which is minor, is that some presenters still need to master the new technologies, but this will come with more time spent using it in their daily routines. The current chair of the ESP Uropathology Working Group, professor Antonio Lopez-Beltrán (University of Cordoba, Spain), wrote in a society newsletter, "[…] slide seminars and video-microscopy sessions should be entrusted to young European pathologists with interest in the field, as a way to stimulate their active participation in ESP activities and foment their interest in uropathology."

In my view, in the near future, we shall see the end of "digital illiteracy" in pathology. Our residents and junior specialists already digitize their personal lives, so getting to grips with digital pathology technologies will be an easy task for them. They in turn will become our teachers in this rapidly emerging new world of pathology that we have the pleasure to be a part of.

*Reference*

1. *I Armstrong-Smith, "Facing the digital future of pathology", The Pathologist, 1, 16–25 (2014).*

# Critical Decision Support

## Liquid chromatography-tandem mass spectrometry has a pivotal role in diagnosing intoxicated patients

*By Jennifer Colby, Assistant Professor of Pathology, Microbiology and Immunology, Associate Director of Clinical Chemistry, Vanderbilt University Medical Center, Nashville, Tennessee, USA*

Imagine your lab receives a call from an emergency physician regarding a patient who has just presented to your hospital. A witnessed seizure prompted the patient's visit to the emergency department, but the patient has no reported history of seizures. Their mental status is altered, though, and they are becoming increasingly agitated. The patient's friends say that they take citalopram for anxiety and occasionally smoke marijuana. The emergency physician wants a comprehensive drug screen to determine if the patient's condition is the result of an ingestion. But what sort of testing would you recommend?

Comprehensive drug screening, as requested by the physician in the vignette, can take many forms. It should include as many relevant compounds as possible, and in the case of an acutely poisoned patient, the results should be available quickly.

*"The technique has several features that make it attractive for comprehensive drug screening."*

In most laboratories, a comprehensive drug screen would involve running a patient's urine sample through a battery of immunoassays, which are attractive because they are fast and compatible with automated analyzers. However, immunoassays have two limitations when assessing poisoned patients. First, they're only available for a small fraction

*"Imagine your laboratory could perform comprehensive drug screening using both immunoassays and LC-MS/MS."*

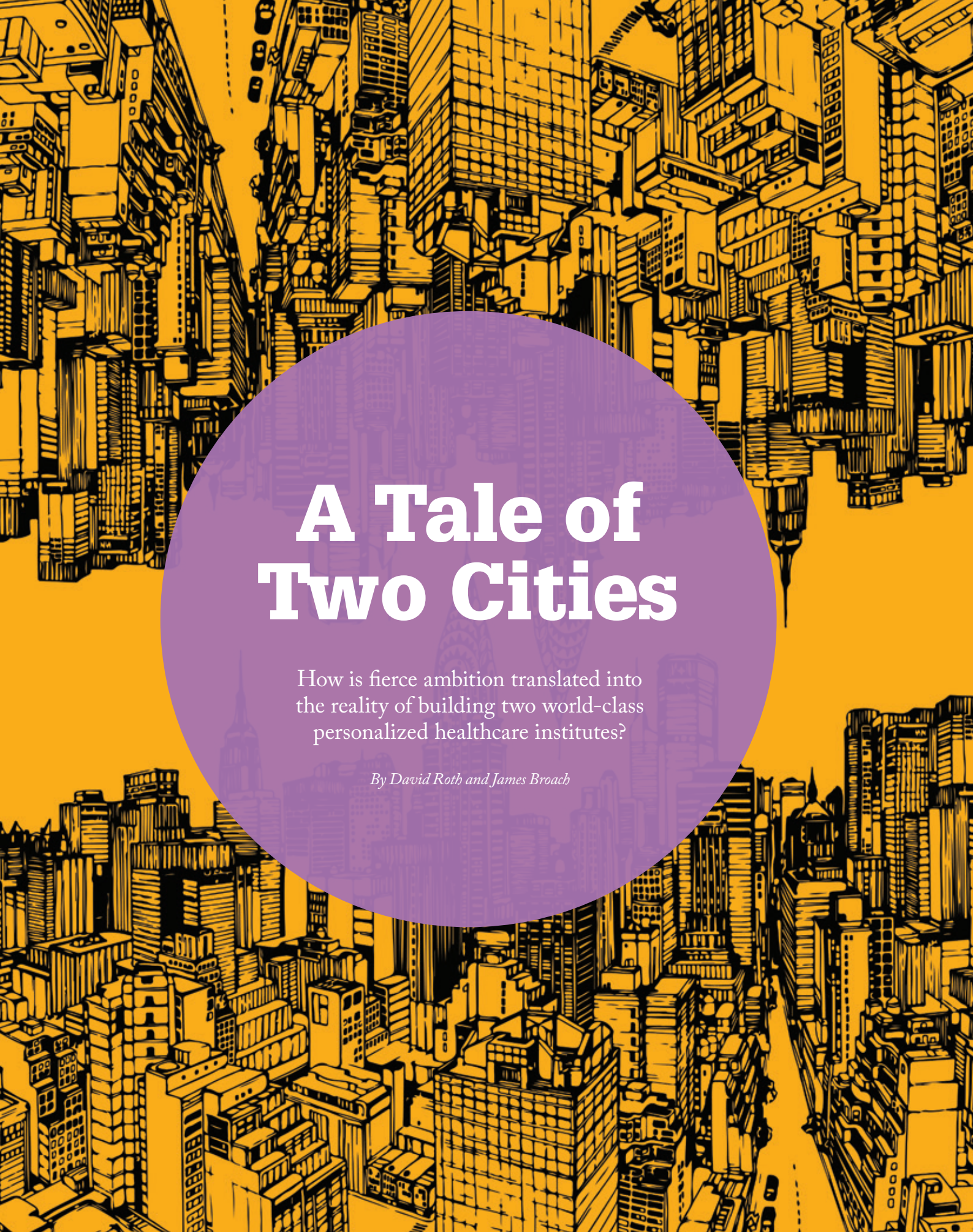
of toxicologically relevant compounds. Second, most immunoassays are designed for urine samples, and although urine is the specimen of choice for monitoring drug use, it has a more limited utility for assessing acute intoxication.

One of the most promising alternatives is liquid chromatography-tandem mass spectrometry (LC-MS/MS). The technique has a lengthy history in the clinical laboratory, and is often used as the gold standard method for quantitative analyses. Many laboratorians will be familiar with LC-MS/MS as a confirmatory technique for urine toxicology testing, but it can also be used to measure a wide variety of clinically relevant substances. LC-MS/MS methods are typically laboratory-developed tests, which allow the laboratory to include as many compounds as desired and to establish which sample type(s) to accept. The disadvantages of LC-MS/MS are that most methods involve manual sample preparation and somewhat complex data processing, both of which require experienced personnel and can prolong turnaround time.

Despite these disadvantages, the technique has several features that make it attractive for comprehensive drug screening. One is the ability to analyze serum or plasma samples, which can provide more useful information on acute intoxication than a urine sample. Another is the ability to include many different types of drugs. This means the laboratory can customize the comprehensive screen based on regional drug use, and can include over-the-counter and "designer" drugs that would not be detected by immunoassays. Turnaround time can be reduced to approximately three hours by choosing a minimalist sample preparation protocol, having well-trained staff, and having a dedicated LC-MS/MS available for testing.

Returning to the opening vignette… imagine your laboratory could perform comprehensive drug screening using both immunoassays and LC-MS/MS. So you recommended that the clinician order both types of testing. Results from the immunoassays performed on the patient's urine were made available to the treating team in under an hour, and were negative. Although this is valuable information, it doesn't explain the patient's condition. Results from the LC-MS/MS screen were available in three hours, and showed diphenhydramine in the patient's serum and urine samples. This over-the-counter antihistamine has anticholinergic effects and can cause seizures at high doses, so the team decided to administer a cholinergic agent, and the patient's clinical picture improved immediately.

As illustrated here, an effective comprehensive drug screening program can provide critical decision support for a hospital's emergency department and improve patient care. Thoughtfully designed, LC-MS/MS-based tests can provide valuable information in a clinically relevant timeframe, and when used in conjunction with immunoassays, serve as an all-round solution for rapid toxicology screening.



# A Tale of Two Cities

How is fierce ambition translated into the reality of building two world-class personalized healthcare institutes?

*By David Roth and James Broach*

*It was the best of times,*
*it was the worst of times,*
*it was the age of wisdom,*
*it was the age of foolishness,*
*it was the epoch of belief,*
*it was the epoch of incredulity,*
*it was the season of Light,*
*it was the season of Darkness,*
*it was the spring of hope,*
*it was the winter of despair...*

Many experts in genetics and genomics believe that some – or all – of the above reflections are true of today's personalized medicine. It's easy to see how some of them apply. With technology advancing rapidly and the cost of sequencing dropping precipitously, we're certainly experiencing the best of times in terms of our ability to explore the mysteries of the human genome. But our ability to store and interpret data still lags behind, and the more we hype the "personalized medicine revolution," the more patients expect miracles that simply aren't achievable. So how can we approach precision medicine from a balanced perspective? How can we optimize our resources and abilities to best serve our patients?

Precision medicine is growing at an astounding pace – in the last decade, over 100 prominent new personalized diagnostic and treatment products have become available, and the Personalized Medicine Coalition has expanded more than tenfold. Even the President of the United States has launched a new Precision Medicine Initiative that funds not only grants, but the assembly of patient cohorts and the establishment of better regulatory standards. It's clear the concept is catching on, so how can research and clinical laboratories get on board? We spoke with the leaders of two major genomic research centers to ask about their institutions, the challenges they've faced and continue to face, and how others can make the move to personalized medicine and contribute to the next evolution in healthcare.

# YOUNG BUT POWERFUL

Penn Medicine's Center for Personalized Diagnostics is on a mission to improve patient care using the power of genomics

*By David Roth*

I was in college, planning to become either a veterinarian or a professional musician, when I first encountered cloning. My mind was blown by the incredible promise of this new technology – and right away, I decided to become a physician-scientist. From then on, I became totally focused on research – specializing in pathology, choosing the most research-oriented residency I could find, and then working for the National Institutes of Health (NIH) on a problem that provided me with many years of scientific inspiration: how DNA rearrangements in developing lymphocytes occur normally, and how aberrant rearrangements cause lymphoid malignancies. That inspiration carried me through the next two decades of my scientific career, until, in 2011, I moved to the University of Pennsylvania.

I was invited to Penn for two reasons: to chair the Department of Pathology and Laboratory Medicine there, and to start up Penn's next generation sequencing (NGS) diagnostics by setting up a new Center for Personalized Diagnostics (CPD). The center, a joint effort between my department and the Abramson Cancer Center (ACC), just turned three years old and has rendered diagnoses on almost 5,000 clinical cases. It's also inspired us to move deeper into personalized medicine – and the first step in that direction is Penn's new Center for Precision Medicine. Its main role is to help us design, test and implement precision medicine-based clinical care pathways.

## Pushing for precision

At its most basic, "precision medicine" means giving the right therapy to an individual patient at the right time. Many would argue that physicians have been practicing individualized medicine since the beginning of the profession, and I agree. Doctors have always used the best tools available to diagnose and treat their patients. What has changed is our level of accuracy – so I think the term "precision medicine" is apt.

My experiences as a medical student 30 years ago planted the seeds of my work in precision medicine. At that time, we weren't doing a great job with patients who had advanced cancer. I spent a few weeks with a group that was trying a new therapy for a very difficult disease. The results were so dismal that it was clear we couldn't treat cancer effectively without knowing much more about its biology. That realization drove me into pathology, and I've worked on the mechanisms underlying cancer ever since. In 2006, my lab genetically engineered mice that rapidly developed lymphomas with incredible levels of genomic instability. We disabled some of the regulatory controls associated with antigen receptor gene assembly in developing lymphocytes, and every animal developed lymphoma within a few weeks. To understand the mechanism, we needed to characterize the genomic alterations in the tumor cells – so we began to develop NGS technologies. As the results came in, I grew convinced that these technologies would soon unlock many of cancer's mysteries.

As we were conducting our research, others were doing the same, and a group of scientists working to sequence various kinds of cancers began to publish. Perhaps their most striking finding, and one that was repeated over and over again, is that cancers with the same histopathological features – such as adenocarcinoma of the lung – could have many different kinds of genetic lesions. This suggested that a particular morphologically defined

cancer was not one, but many different diseases, and that they could be treated individually. I realized that the ability to make these diagnoses routinely could be a game changer – and that was my key motivation to set up the Center for Personalized Diagnostics.

## The nature of the beast

Getting the right diagnosis has always been critical in medicine. But with the tools we have now, especially recent advances in genomic diagnostics, we can be incredibly precise. Instead of treating, for example, adenocarcinoma of the lung as a single disease with an unknown cause, we can now recognize many different subtypes of this disorder, each bearing its own molecular signature. Importantly, we can often relate that signature to a physiologically important alteration of a gene that causes cancer (a "driver mutation"). Even better, in many cases we now have specific drugs that target the altered pathways. We can identify the targets (precision diagnostics) and hit them with "magic bullets" (targeted therapeutics), causing minimal collateral damage to normal tissues. In fact, physicians can even engineer a patient's own immune system to specifically attack their tumor cells – an approach that has recently cured some leukemias.

At this point, precision medicine is most advanced in the field of cancer care, but its concepts will be important in a wide variety of other disorders. After all, the principles are the same for every disease – find out exactly what's wrong, and then provide carefully tailored tools to fix it.

## Bumps in the road

Three challenges in particular have been on my mind recently:

- One is applying existing knowledge to devise and institute clinical care pathways based on principles of precision medicine. This will require substantial changes in the way we deliver care for particular disorders, and we will need to collect large amounts of data along the way to continually evaluate whether or not we're really improving upon the standard of care.
- At the other end of the pipeline, devising new diagnostics, biomarkers, and therapeutics will require collection of many kinds of data from individual patients (known as "deep phenotyping"). This is expensive and cumbersome, and integrating data from disparate sources can be difficult. Harmonizing data coming from different institutions is equally difficult, so we'll need to develop interoperability standards for easier collaboration. These areas are a focus of the President's new Precision Medicine Initiative, so the future looks promising!
- Finally, we must be careful not to overpromise. The technologies are exciting, and some of the promise has already been realized. I think precision medicine as a field is on pretty firm footing. But it's easy for enthusiasm to get out of control, and there is an awful lot of commercial activity in this field. I remember the excitement (and the flurry of tech startups) that surrounded the completion of the Human Genome Project. At the time, many people thought cures for cancer were right around the corner. Needless to say, there was some disappointment. A decade and a half later, these dreams are beginning to come true.

## Creating a center

To sum it all up in a sentence: creating the center was exciting, difficult, at times scary, and it turned out to be the most fun and rewarding activity of my professional life.

My initial vision was for the CPD to have a tripartite mission: clinical care, research, and education. Given the pressing need for molecular cancer diagnostics, our first clinical care priority was to develop NGS-based diagnostics for use in tumor samples. Given the financial constraints and the expectation that the laboratory would need to rapidly become financially self-sufficient, we wanted to focus on clinically actionable results, and we wanted to deploy quickly. That's why we opted to deploy just two relatively small panels: one for solid tumors and a custom leukemia panel.

We were able to build and validate the tests, which are based on the Illumina MiSeq platform and supported by a custom bioinformatics workflow, quite rapidly – the clinical lab opened just a year and a half after we conceived of the project. Along the way, we also developed a curriculum in genomic pathology for residents and fellows, as well as offerings for the medical students. The research mission has lagged behind because of our initial focus on clinical development, but now that the clinical lab is functioning well, we are turning our attention back to science.

The lab was designed to be financially self-sustaining after startup, which served as a constraint (and a guideline) for test development. We developed targeted panels for use in somatic cancer testing because those tests provide clinically useful results and, ultimately, allow us to be reimbursed. Even more critical than money, though, was forging collaborations between departments. From the very beginning, the CPD was a collaboration between my department and the ACC. We had many meetings with clinicians and other key stakeholders in which we worked out our strategy – the scope and nature of the tests to be offered, reimbursement paradigms, the reporting of results, and so on. The process was very time-consuming, and sometimes it took a lot of effort to get everyone on the same page, but it paid off. Our partnerships with clinicians

## Penn Medicine's Center for Personalized Diagnostics

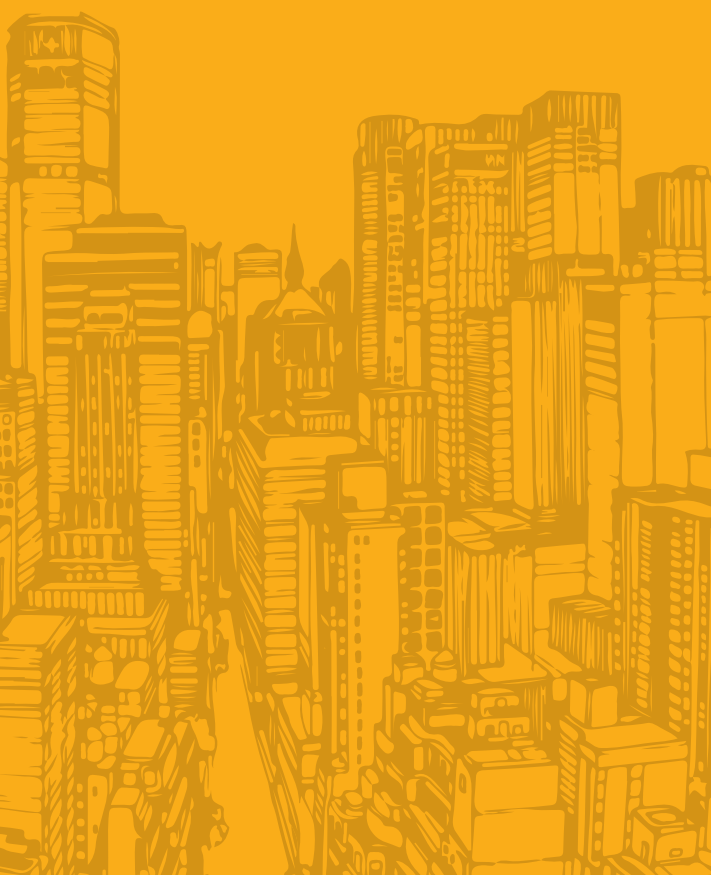
*Established:* 2012.

*Located:* Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania, USA.

*Website:*
www1.pennmedicine.org/personalized-diagnostics/

A joint initiative between Penn Medicine's Department of Pathology and Laboratory Medicine and the Abramson Cancer Center, the Center for Personalized Diagnostics' current goal is to analyze the genetic characteristics of individual cancers. With that information, patients can make important decisions faster and receive more targeted treatment. The center aims for turnaround times under two weeks, including review by subspecialized pathologists, and also contributes to translational and clinical research.

The CPD currently employs four technologists, three faculty, two genetic counsellors, two bioinformaticians, one clinical fellow and one research and development scientist – and the numbers are growing rapidly. The center also hosts a clinical fellowship.



have kept most of our testing in-house, spurred a number of research efforts, and allowed us to provide more clinically applicable tests. Clinicians have definitely had a say in every part of the process, and that turns out to be very empowering.

Today, our major focus is still on somatic cancer diagnostics, but we are beginning to think about branching out into other areas. In our first year, we returned about 1,000 results. We've grown considerably, and are now offering more tests, including both substantially larger panels and small panels designed to allow testing of very small specimens, including cytopathology specimens. We'll soon hit our 5,000th patient sample – and this year, we will more than double our initial annual volume. It's been incredibly gratifying to see our entire team pull together and accomplish something I'm sure many people thought we couldn't. Penn is a very collaborative institution, and that has really worked in our favor, letting us offer cancer patients new treatment options and broaden the realm of possibility for treating difficult diseases.

### Advice to others

Precision medicine is a big topic. I'm sure that the only reason many members of the public have heard about precision medicine is because of the announcement made at the President's 2015 State of the Union address, which was focused mainly on building a large research cohort. Many of our peer institutions are developing research-focused precision medicine projects. In our newly launched Penn Center for Precision Medicine, we chose instead to focus on developing ways to move ideas, processes, or technologies derived from precision medicine into patient care, and to measure the outcomes of these interventions (see http://tp.txp.to/0316/penn-cent for more information).

Based on my interactions with people in the community, I think we have a long way to go in educating people about the benefits of current precision medicine approaches to cancer, which include genomic testing (see our short video about cancer testing at http://tp.txp.to/inside-cpd-video). That's the message I would like to emphasize to the public. I recently met a woman who had been diagnosed with metastatic lung cancer several years previously. She was advised to say goodbye to her young children. And yet, for several years now, she has been on a series of targeted therapies that have kept her disease stable. She has been able to watch her children grow up. Precision medicine may be in its infancy, but even today, it has tremendous power to help individual patients.

*David Roth is Director of the Penn Center for Precision Medicine and Simon Flexner Professor and Chair of Pathology and Laboratory Medicine at the University of Pennsylvania, Philadelphia, USA.*

# FIELD OF GENES

## The Penn State Hershey Institute for Personalized Medicine benefits from a unique "build it as you need it" growth pattern

*By James Broach*

I didn't start my career intending to focus on personalized medicine. For many years I was a yeast geneticist, first at Cold Spring Harbor Laboratory, then at the State University of New York at Stony Brook, and finally at Princeton. I studied numerous topics – how cells respond to their environment, how their genes are regulated – but all within the purview of basic research in model organisms. That changed about four years ago, when the vice dean for research at Penn State Hershey asked if I wanted to come out and start an institute for personalized medicine. It was too good to pass up – a wonderful challenge and an opportunity to take everything I had learned about genomics in model organisms and apply it to people.

### Starting from scratch

I had to build from the ground up when establishing the Institute for Personalized Medicine (IPM). In Pennsylvania, we have access to money from the "tobacco settlement." The Tobacco Master Settlement Agreement provides the Milton S. Hershey Medical Center with a significant amount of funding each year that can be spent at the discretion of the vice dean for research. Fortunately, because he's the one who suggested the program, he was very supportive of our financial needs. We also had a grant from the National Institutes of Health (NIH) to renovate the physical space we needed to house the institute – so having the initial resources available early on helped us get the IPM off the ground.

Early on, I recruited Glenn Gerhard. He's a clinical geneticist with a strong research program in the application of genomics to medical issues, and he also had experience in setting up and running a biobank. So he knew all the ins and outs of obtaining patient consent, establishing databases to handle samples, working with the institutional review board for approvals, and generally understanding the ebb and flow of the biobanking process. He was a great addition to the program, and I credit him with much of our early success.

My job at that point was to recruit genetically interesting patient populations from the various clinical departments – those who looked like they might be predisposed to a particular disease or who could be stratified by response to a particular treatment. I used that knowledge first to identify the questions we wanted to ask, and then to select the patients whose data might help us answer those questions.

This is very different to the way most other biobanks and institutes for personalized medicine operate. Many use what I call a "field of dreams" approach – they say, "if we build this institute and get 100,000 samples, then somebody smart will come along and figure out what to do with it." That requires a lot of upfront infrastructure and funding without any assurance of downstream income to offset it. Ours, by contrast, was a focused, "build it as you need it" approach, so that we could closely link funding to individual projects.

> **"Our partnerships with clinicians have kept most of our testing in-house, spurred a number of research efforts, and allowed us to provide more clinically applicable tests."**
>
> **David Roth**

### Advancing ALS

We currently have about 25 different collaborations, with another 15 or so in preparation. We started with amyotrophic lateral sclerosis (ALS, or Lou Gehrig's disease) and have expanded outward – first into other neurological diseases, and now into cancer, cardiovascular, pulmonary, urinary and more. Ever since people realized we were a resource that would enable them to pursue their more ambitious research questions, I haven't had any trouble finding collaborators.

ALS is a syndrome, not a disease – it's diagnosed based on patient presentation, rather than underlying etiology. That may be why it's so difficult to treat; trying to find a single drug to address a range of diseases that we classify as "ALS" would be like trying to find one drug to cure all of cancer. Our hope was that, by applying genomic tools, we might be able to identify the etiologies of the disease – and then come up with new treatment approaches.

As a geneticist, I recognized early on that the known ALS-associated genes were all dominant alleles. From genetics in model

organisms, we know that if you select for mutations that generate a given phenotype, only 10 percent are dominant alleles – so we were just hitting the tip of the ALS gene iceberg. To overcome that, we focused heavily on examining the genomes of patients previously considered "sporadic" and their families, in order to see whether their disease was actually the result of recessive mutations. And we've been successful in that. In fact, we've increased the number of potential genes that are causative in ALS by a factor of 10.

## Handling the hype

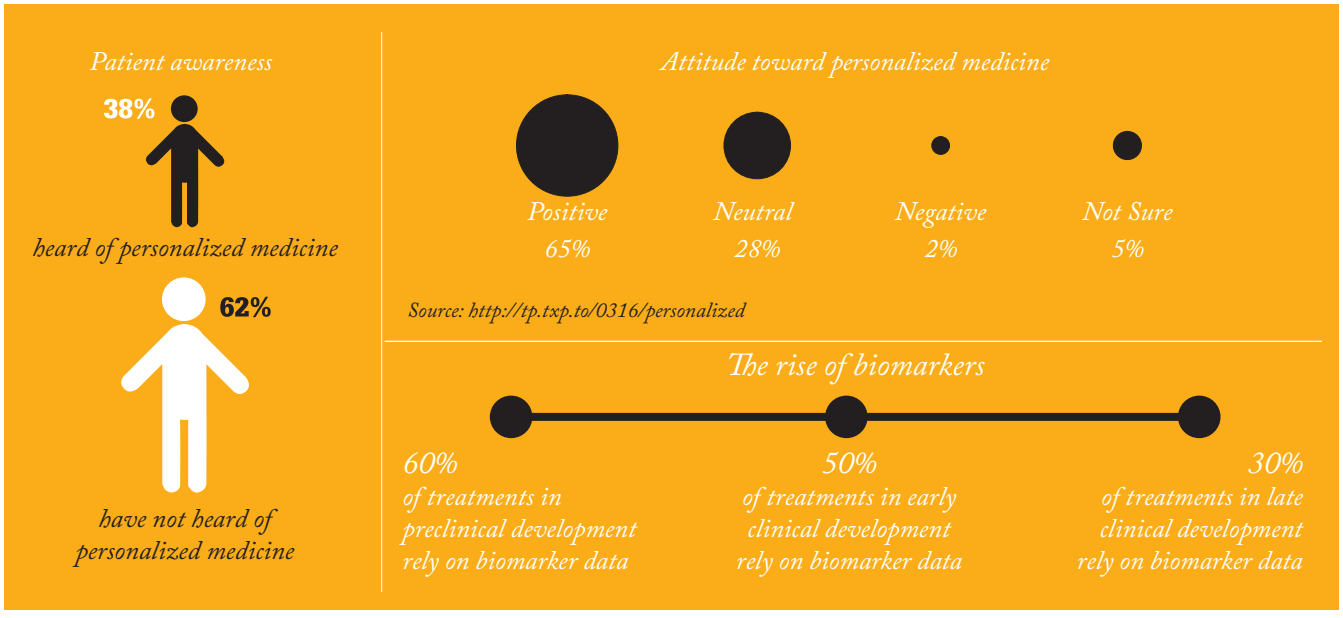
Despite our early successes, running a center like the IPM isn't easy. Funding is always an issue, for instance – there are always more collaborations than we have money to pursue. There's also the issue of translating basic science into clinical applications. Our work to date has been strictly research, but precision medicine's real impact is on patient treatment – so we have to get the genomic information back to the clinic. That's not happening as quickly as we'd like; it's taken a while to get the IPM's clinically certified laboratory established, and in many cases where we've established potential tests, clinicians aren't always ready to integrate genomic information into patient care.

There's been a lot of hype around precision medicine, and there's an expectation that it's going to revolutionize medical treatment. But that's a slow process, and precision medicine is still a long way from being commonplace and having the massive impact that we anticipate. It's becoming state-of-the-art care for cancer – you shouldn't get cancer treatment without at least having your tumor sequenced over the potential drivers, because therapy really does depend on the nature of the tumor. But in other areas, progress is slower. Ultimately, the more we understand about polygenic diseases, the more we'll be able to use that information to be able to tailor treatment to the patient.

That's where patient self-advocacy can play a role, too. I think that there's going to be increased recognition that genomic information can be informative about patients' disease predisposition and treatment options. Connecting genomic information to medical records so that it's clinically useful is one step in the right direction, but the next is to create and implement a mechanism for getting that information back to the patient as quickly as possible. It's their data – their genome – that we've sequenced, and patients have a growing interest in obtaining as much information as possible so that they can advocate for their own medical care. I think it's important for them to have that kind of information. Here at Hershey, we have open portals so that patients can look at their own medical records. I think that kind of openness needs to extend to genomic information as well, particularly when it's linked to medical care. We're moving into a new era of healthcare; instead of an all-knowing physician and a patient who listens, we now involve a team of people – and the patient is just as important as any other member of the team.

## Dealing with data

One concern precision medicine raises is the need for protocols regarding data collection and processing. We're in an exponential phase of data accumulation, but we don't know how to take full advantage of it. Part of the problem is that we can generate data for a particular purpose – for instance, treatment or



billing – but when we try to mine that data for research, it's not useful. Collecting data that can be valuable from multiple perspectives is a big challenge, and disparate data often contain key information. Twitter is better at predicting flu outbreaks than epidemiological data. So there are ways to take advantage of the information out there, but there's also a lot of noise.

> **"The people who can cross the boundaries between life sciences and computing are the ones who will lead the personalized medicine programs of the future."**
> **James Broach**

Separating signals from noise is one of the exciting directions I think informatics will take in the next couple of years.

There's a lot of data out there, if only we can learn to use it properly. For example, the IPM has an alliance with the New York Genome Center for studying ALS. We share detailed phenotypic and genomic data – and we do it in an established common language, so that researchers from any site can understand the information provided by any other. There is a strong push to establish common data languages, so that it becomes easier to aggregate information on patient populations.

I think it's important for medical researchers to be well versed in both computer analysis and biology. At the IPM, we spend a lot of time training the next generation of physicians and basic researchers to have a foot in both doors, because it's so important for laboratory medicine professionals to understand what information is already out there and how best to take advantage of it. The people who can cross the boundaries between life sciences and computing are the ones who will lead the personalized medicine programs of the future.

*James Broach is Director of the Institute for Personalized Medicine and Chair of the Department of Biochemistry and Molecular Biology at Penn State College of Medicine, Hershey, USA.*

## Penn State Hershey Institute for Personalized Medicine

*Established:* 2013.
*Located:* Penn State College of Medicine, Hershey, Pennsylvania, USA.
*Website:* www2.med.psu.edu/ipm

The Institute for Personalized Medicine features a biorepository that stores blood and tissue samples along with a database of patient information, and a genome sciences core that handles nucleic acid analyses from quality assessment to next-generation sequencing. The IPM's ongoing projects include investigations into ALS, autism, diverticulitis, epilepsy, aneurysms, Parkinson's disease, osteoporosis and cancer – and researchers anticipate projects examining psychiatric disorders, bone healing and age-related macular degeneration.
The institute includes one pathologist, three CLIA lab staff members, four consenters, seven genomics core members, 10 bioinformaticians, and approximately 12 students and 25 clinicians conducting IPM-based research projects.

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Waseem Asghar

# Meet the Winner

## Waseem Asghar

Waseem Asghar, Assistant Professor at the Departments of Computer Engineering & Electrical Engineering, Computer Science, and Biological Sciences, Florida Atlantic University, USA, has been chosen as the winner of the 2016 Humanity in Science Award for "development of a new paper and flexible material-based diagnostic biosensing platform that could be used to remotely detect and determine treatment options for HIV, *E. coli*, *Staphylococcus aureus* and other pathogens."

Waseem will be presented with a humble prize of $25,000 during an all-expenses-paid trip to Analytica 2016 in Munich, and his work will feature in an upcoming issue of The Analytical Scientist.

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Analytical science has been at the heart of many scientific breakthroughs that have helped to improve people's lives worldwide. And yet analytical scientists rarely receive fanfare for their humble but life-changing work. The Humanity in Science Award was launched to recognize and reward analytical scientists who are changing lives for the better.

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# The Problem With Panels

**Newborn screening programs that seek out common mutations often fail to diagnose cystic fibrosis in non-white populations. Why – and what can we do?**

*By Iris Schrijver*

Cystic fibrosis (CF) is a serious genetic condition that affects about one in every 3,500 people. It's inherited in an autosomal recessive manner, meaning that both copies of the causative *CFTR* gene require a mutation to cause disease. Those with such mutations have defects of the exocrine epithelial cells in multiple tissues and experience symptoms including failure to thrive, chest infections, difficulty breathing, pancreatic insufficiency, infertility and more. The best way to minimize the impact of these symptoms is to diagnose CF early and begin treating it from a young age – but in certain cases, that's easier said than done.

In some countries, including the United States, newborn screening programs can diagnose CF by detecting common mutations in the *CFTR* gene. But the "common" disease-causing mutations aren't always the same (1) – and I noticed that, in this area, non-white patients are underserved. The molecular tests offered are often not representative of the sequence changes that are encountered in non-white individuals. Like many countries, the United States is a melting pot of ethnic diversity, so it's important to have a good grasp of the diversity of mutations that arise – not just in white populations, but in other ethnicities as well.

*At a Glance*

- *Cystic fibrosis is a life-threatening disorder whose impact can be minimized by early diagnosis and treatment*
- *Newborn screening programs have dramatically reduced patients' age at diagnosis, but rely on detecting common mutations that occur far less frequently in non-white populations*
- *When these patients aren't spotted early, they are diagnosed only after clinical symptoms occur and their chances of survival and thriving are reduced*
- *In order to better serve our ethnically diverse population, clinicians should consider more comprehensive screening methods like next-generation sequencing*

*"We're looking in the wrong place; the locations we target based on research in white populations aren't the ones most commonly mutated in other ethnicities."*

## Where screening falls short

CF newborn screening has now been implemented in every single state in the USA, but identification of CF variants in non-white people remains suboptimal. If we know the sequence variants in each population, we can improve genotype-phenotype correlation, and that can affect a range of things including results reporting, counseling for families, accurate prognoses, and therapy decisions. Better yet, more familiarity with the mutation spectrum will allow us to optimize newborn screening programs based on the ethnic composition of the population. That can make a huge difference to patients, so my objective was to take the first step by establishing which mutations are present in non-white CF patients.

We've known for some time that many of those mutations were not being identified in routine screening. The panels as they exist today, both for carrier screening and for newborns, are based on early knowledge of the mutation spectrum in white and Ashkenazi Jewish patients. CF is most common in "white" – northern European Caucasian – individuals, with a frequency of about one in 2,500. It occurs less often in other populations (one in 11,000 Native Americans; one in 15,000 black people; and one in 35,000 Asian people) – but when it does, we often fail to find the relevant mutations during screening. That's because we're looking in the wrong place; the locations we target based on research in white populations aren't the ones most commonly mutated in other ethnicities. To find them, we need to adopt a more comprehensive screening approach so that we can facilitate equity in diagnosis and improve our patients' quality of life.

## Delays and deterioration

Despite these genetic differences, disease presentation is the same regardless of a patient's ethnicity. With newborn screening, CF patients in the United States are diagnosed at a median age of two to four weeks – and they can "hit the ground running" in terms of disease management. But in non-white patients, identification occurs significantly later.

One of the hallmarks of CF is failure to thrive, so late-diagnosed patients gain less weight, experience less overall growth, and develop pulmonary issues. It's a life-threatening disorder.

Non-white populations face another disadvantage: CF just isn't at the forefront of the differential diagnosis. It's much more likely that a physician would consider infectious diseases before a relatively rare genetic disease like CF. When I was collaborating with researchers in Thailand, I learned that some mothers will bring their child to the attention of a physician by saying, "I don't know what's wrong with my child, but she tastes salty." That's due to the abnormal salt and chloride metabolism typical of CF, but mothers sometimes notice that before physicians even think of the disease. So it's important to improve our genetic tests, both to avoid delays in diagnosis and to ensure that we can unambiguously detect CF in our patients.

## We can do better

Our work has really highlighted the limitations of panel testing in a diverse population. The next step is for newborn screening programs to consider more inclusive test approaches that improve diagnosis – and therefore enhance prognosis – for CF patients of non-white and mixed ethnicities. I think that, especially in ethnically diverse areas, it comes down to appropriate testing in each setting and for each patient. Every newborn screening program in the United States currently performs its own cost-benefit analysis. With screening, of course, you build in the assumption that you're going to fail to detect some individuals in order to be cost-effective, but that should be minimized. I think that if you look carefully at available algorithms and regional demographics, you can achieve desirable sensitivities for all populations and avoid disproportionate disease burdens in minority ethnicities.

*"NGS may not only be more comprehensive, but also more cost-effective than our current methods."*

Doctors performing screening need to be aware that common panels don't include some of the variants present in non-white populations. With the attention currently focused on personalized medicine, we can advocate for a more inclusive approach that could help propel equity in mutation detection. I personally believe that we should consider a more comprehensive approach like next-generation sequencing (NGS) – which doesn't have to be much more expensive than our current tests, because the cost of sequencing is rapidly decreasing. The trick is to get people to consider these newer approaches, rather than simply opting for standard panels because they come to mind first, or because they're assumed to be more economical. That doesn't have to be the case – NGS may not only be more comprehensive, but also more cost-effective than our current methods (2). But regardless of how we approach the challenge, now that we better understand the diversity of disease mutations, I would like to see appropriate testing applied to every patient.

*Iris Schrijver is a Professor of Pathology and, by courtesy, Pediatrics (Genetics) at the Stanford University Medical Center, Stanford, CA, USA.*

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# Getting Personal

## Patient-derived xenografts are a valuable tool for the oncological drug development of the future

*By Anne-Lise Peille*

Despite the great progress we've made in understanding cancer biology, the unfortunate reality is that most newly developed anticancer agents still fail clinical trials. Why? Either because of poor drug efficacy, or because of their substantial side effects (1). When this happens, it's often not just because of an incomplete comprehension of human cancer biology – other factors also play a role, including the use of unsuitable preclinical models for treatment evaluation and a lack of appropriate biomarkers for patient selection. So how can we overcome these barriers? The answer lies in patient-derived xenografts (PDXs), which are fast becoming the new gold standard models for oncological drug development.

For decades now, conventional cell lines have been the standard tool for in vitro drug testing; without a doubt, their ease of use has improved our understanding of tumor biology. Unfortunately, cell lines aren't particularly good at predicting drug responses in the clinic (2,3) – and it's not hard to see why. Tumor cell lines are maintained in 2D culture, without a tumor microenvironment. These artificial growing conditions cause significant biological changes and lead to clonal selection, which prevents conventional cell lines from reflecting the overall profile of the original tumors or the complexity of tumor subpopulations. Even implanting the cells into mouse models for in vivo drug testing doesn't improve their ability to predict the efficacy of anticancer drugs (4).

PDXs circumvent these limitations. They're based on implanting human tumor cells into immunocompromised mice to develop models that are exclusively passaged from mouse to mouse with no in vitro culture. To obtain PDX models, dissociated cells or tumor fragments are usually implanted subcutaneously. Although human stroma is replaced by its mouse counterpart during the first passages, this direct implantation maintains realistic growth conditions to preserve tumor architecture, intratumoral heterogeneity and the molecular profile of the original patient tumor (2).

Once numerous studies showed that PDXs ably mimicked patient sensitivity to chemotherapies and targeted therapies (3), their use in drug development grew rapidly and large collections were set up (1,5) (Figure 1). In addition to their role in drug development, PDXs offer the opportunity to perform integrative analyses of their histological, molecular and sensitivity profiles, meaning that researchers can use them to identify predictive biomarkers at a preclinical stage. That's useful for many reasons – not just preclinical drug testing, but also selection and stratification of patients for future clinical trials (Figure 2).

*"Pathologists have been cast in the unusual role of analyzing PDXs as they would patient tumors."*

### A crucial role for pathology

So how do pathologists fit into this scheme? One of pathologists' major responsibilities in cancer research is the management of biobanks – vital for the appropriate storage of clinical samples, the traceability of such samples, and the collection of relevant patient information. Pathologists analyze the morphological and histological features of collected tumor samples, as well as examine clinically approved predictive biomarkers to elucidate molecular characteristics. This contributes to a better comprehension of tumor heterogeneity and a clearer classification of the tumors, both of which can influence patients' therapeutic options. By interacting closely with research scientists, clinical pathologists also contribute to discovering predictive or prognostic molecular signatures and to developing companion diagnostic tools crucial to boosting the success rates of drugs in clinical trials (6).

### *At a Glance*

- *Patient-derived xenografts (PDXs) are widely used in oncology research for tumor biology investigation, drug screening and preclinical biomarker identification*
- *Pathologists have been cast in the unusual role of analyzing PDXs as they would patient tumors in order to guarantee their relevance*
- *PDX engraftment techniques have improved, but several challenges remain, including the development of such models in an immunocompetent system*
- *As PDXs grow in popularity, we're developing new tricks and techniques to increase their usefulness for patient stratification and treatment design*

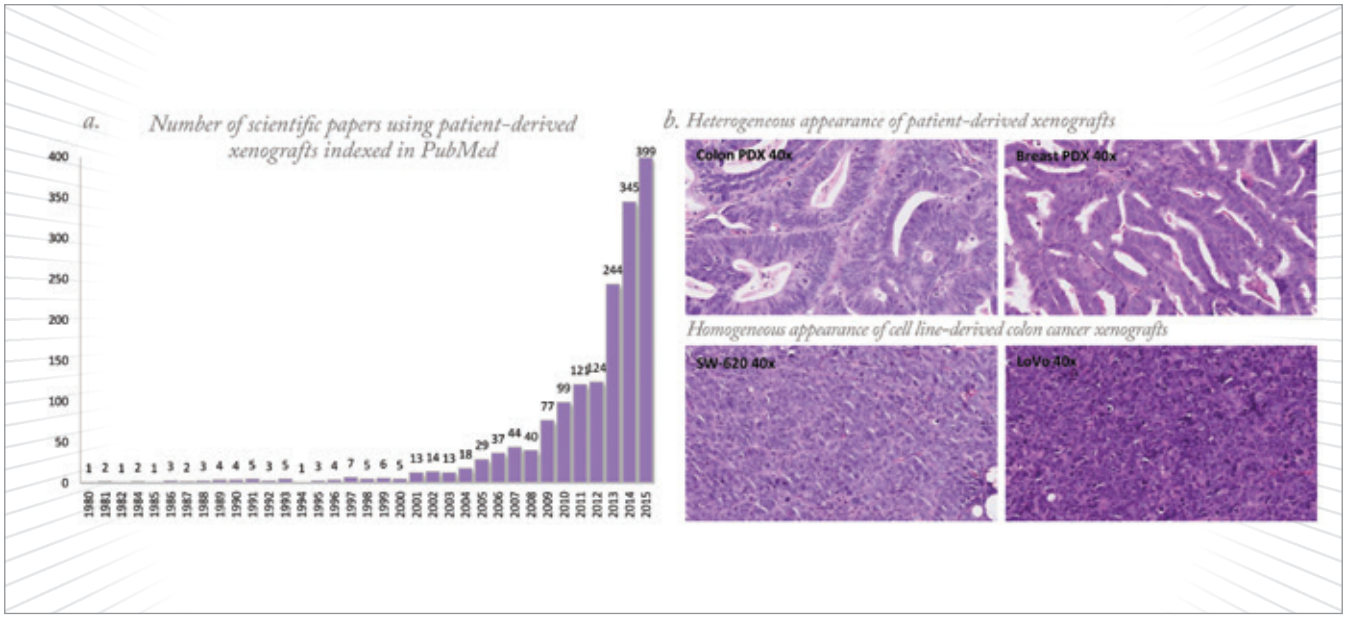


Figure 1. a. The number of scientific papers on the use of patient-derived xenografts in oncology research, indexed in PubMed, per year. b. PDX establishment allows the preservation of the tumor architecture, in contrast to xenografts developed from commercial cell lines.

To be clinically relevant, a PDX model should accurately represent intra- and intertumoral complexity. To that end, pathologists have been cast in the unusual role of analyzing PDXs as they would patient tumors, in order to guarantee their pertinence in oncology research. Together with surgeons and clinicians, pathologists improve the PDX engraftment rate by guaranteeing sufficient tissue withdrawal and decreasing the time between resection and xenotransplantation. After initial engraftment in the mouse, pathologists track the major characteristics (vascularization, stromal content, necrosis, and relevant clinical biomarkers) of the PDX during establishment and over multiple passages to ensure that it remains representative of the patient tumors. They also study the potential changes in murine organs during treatment in order to evaluate the compounds' safety and efficacy. Clearly, this extensive characterization by pathologists is key to choosing the most appropriate models for testing compounds, interpreting responses obtained in vivo, and identifying biomarkers and potential indications for subsequent clinical trials.

## Revving up research

While PDX models are already having a significant impact on the research and development of novel therapies, they're also now widely used for tumor biology investigation, drug screening and, most recently, preclinical biomarker identification. Patient tumors represent a limited source of material for cellular and molecular analyses, whereas PDXs provide an unlimited source of FFPE, DNA, RNA and protein samples. More material means more potential analysis, which in turn means more potential to improve patient care! And as new targeted therapies and high-throughput technologies emerge, the way we diagnose cancer is changing. Therapies are guided not only by tumor histotypes, but by the expression levels or mutation status of particular genes – molecular classifications that represent significant advances in tumor categorization. These recent technical advances and the ability to study both the mouse stroma and the human tumor cells have enabled scientists to describe new molecular classifications (7) and to study tumor heterogeneity in more detail (8). Ultimately, we hope that knowledge will facilitate the discovery of new treatment options for tricky patients.

Several biomarkers are already approved for testing in specific cancer histotypes and for certain targeted therapies. Most prominently, the *ERBB2* (*HER2*) amplification in breast and gastric cancer serves as an indication for trastuzumab treatment – and other cancer subtypes that overexpress *ERBB2* may benefit from the same therapy. But not all *ERBB2*-amplified cancers respond well to trastuzumab, highlighting the need to develop new compounds and identify better predictive biomarkers (9). That's where PDXs become valuable – and indeed, recent investigations conducted on *ERBB2*-amplified PDXs have led to more efficient anti-*ERBB2* compounds (10)!

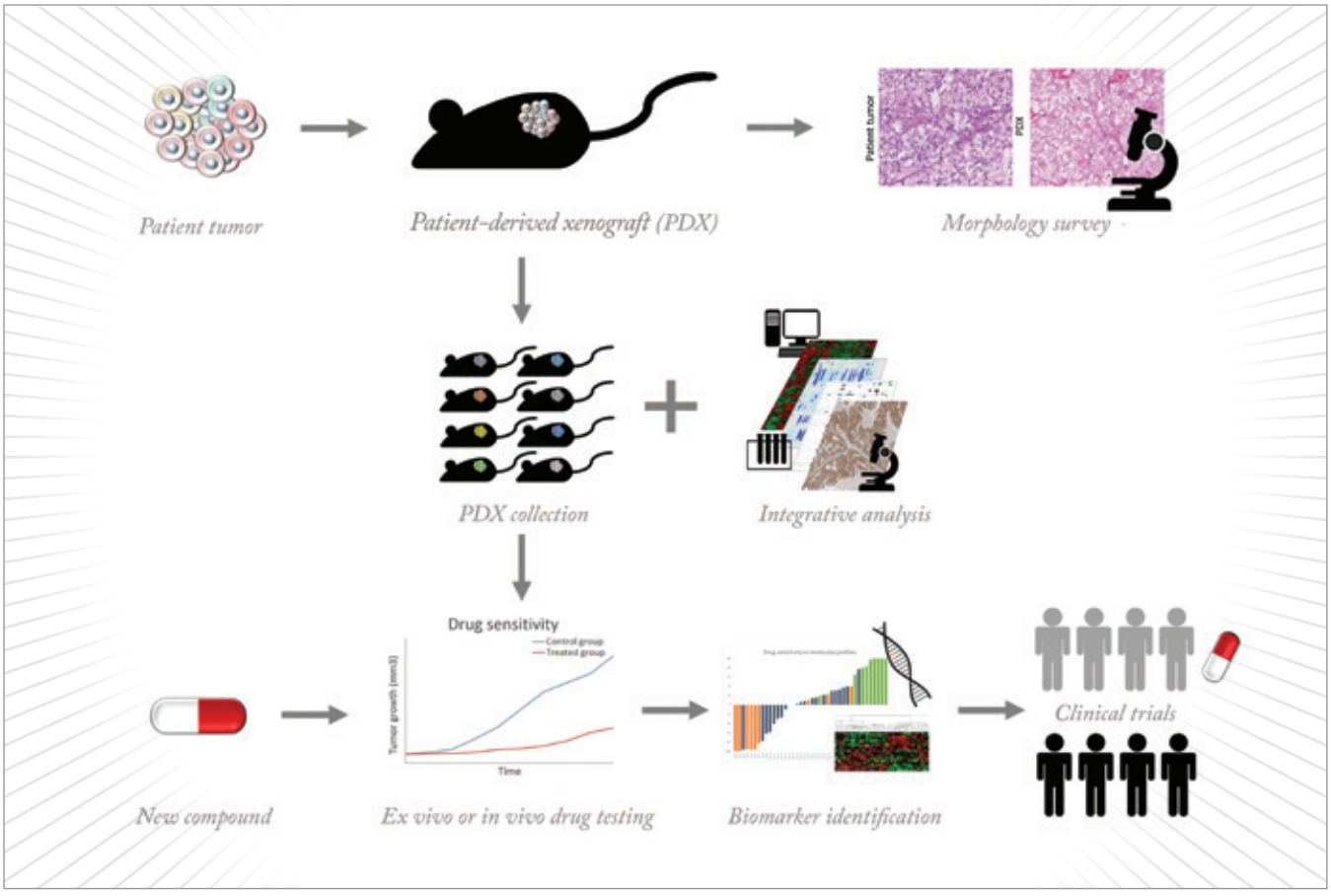


Figure 2: Example of a preclinical study using PDX models. First, fragments of tumors are implanted in mice to establish PDX models. Then, PDX models, characterized for histology and molecular features by pathologists, biologists and bioinformaticians, are used for ex vivo or in vivo drug testing. Finally, sensitivity data are compared to histology and molecular profiles to identify predictive biomarkers that can be validated during clinical trials.

## Looking at limitations

For all their benefits, PDX models are not without challenges. Most are established by subcutaneous implantation of tumor cells on the flanks of immunocompromised mice. Although the site allows rapid transplantation during passages and easy tumor growth monitoring during drug testing, it's an important change in the anatomic microenvironment and can influence the engraftment rate of some histotypes. The replacement of human stromal cells by their mouse counterparts during the first passages of the PDX can also affect tumor growth conditions. Finally, it's essential for the mouse to have a degree of immunosuppression in order to establish the model system (11) – but the lack of an intact immune system makes it impossible to test immunotherapies (Figure 2).

Depending on the tumor histotypes and degree of cell aggressiveness, the engraftment time varies between two and 12 months (12,13). Although PDXs from gastric, pancreas and colon cancers – not to mention metastases and aggressive tumors in general – are relatively easy to establish, hormone-dependent cancers like breast cancer are much more challenging, and therefore underrepresented in PDX collections (14,15). Biases like these force researchers to test large PDX collections and include rare subtypes to be sure they're effectively recapitulating cancer variety. Such extensive in vivo drug testing can be expensive and time consuming – but fortunately, it's not the only way to use these models. Single-mouse trials have recently proven their clinical relevance (1), and ex vivo drug screening of PDX cell suspensions cultured in 3D is another efficient and cost-effective strategy.

## Model perfect?

As PDX models become more popular, collections have grown and engraftment techniques have improved. To make full use of these tools for cancer research, though, we need to resolve several issues:

the impossibility of testing immune-based therapies; the transfer from drug-oriented to patient-oriented studies with the development of personalized "avatar" mouse models; and the microenvironment approximation of heterotopic PDX.

Major progress has already been made in immuno-oncology. Evaluating immune-based therapies requires relevant models growing on immunocompetent mice, and researchers are currently attempting to develop PDX in humanized mice by transferring human hematopoietic cells into immunodeficient mice (16,17). Furthermore, mouse avatars have emerged as an interesting translational platform for individualized medicine. The avatar concept is based on generating PDX from a particular patient and testing several drugs to identify the most effective treatment for that patient. It has demonstrated its promise in several pilot studies (18,19) – but, like any other method, avatar models have limitations, including engraftment times and rates that can be incompatible with clinical settings.

The absence of an adequate microenvironment in heterotopic PDX is another hurdle we will need to overcome to get the most out of these models. To circumvent it, PDX can be developed from orthotopically implanted tumors (which are placed in the organ of origin to mimic the original anatomical microenvironment as closely as possible). It usually increases the engraftment rate (3) and better reflects the patient's drug sensitivities (20). However, it's also labor-intensive, involves complex surgery, and requires efficient imaging techniques to monitor tumor evolution during drug sensitivity evaluation. We need more in-depth studies to evaluate, on a large scale, the feasibility of using orthotopic, humanized or avatar models for drug testing and personalized therapy.

All of these considerations lead us to consider PDX as an attractive preclinical model for studying tumor biology, new therapeutic options and predictive biomarkers. It's my hope that these models will help us improve stratification into clinical trials and develop personalized treatment approaches for our patients.

*Anne-Lise Peille is Laboratory Head of Biobanking and Molecular Analytics at Oncotest in Freiburg, Germany, a Charles River company dedicated to preclinical oncology using PDXs.*

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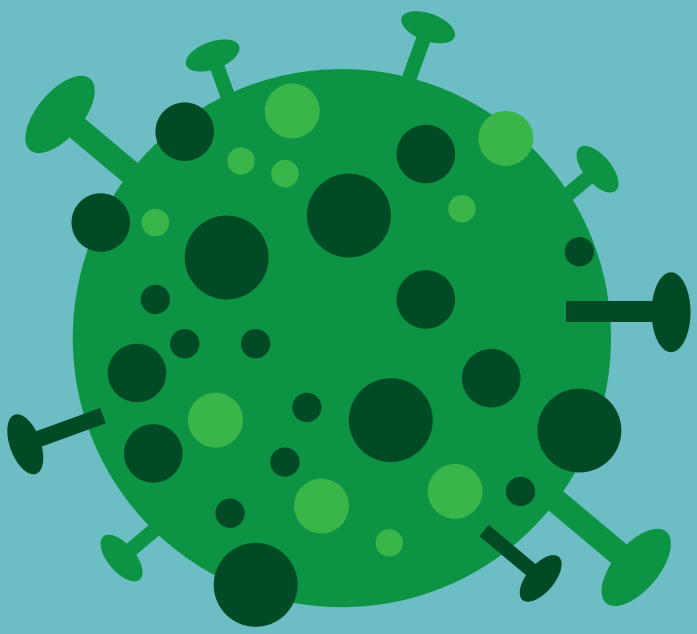


# Viruses Face the Ultimate Test

**Shotgun sequencing is a powerful tool in microbial genomics, but viral genomes have proven tough to crack. Enter ViroCap and its ability to enhance shotgun sequencing to detect the vast majority of viruses infecting humans and animals in a single test. For viruses, is there anywhere left to hide?**

*By William Aryitey*

Ever since Watson and Crick's discovery of the double helix, we have longed to unlock the secrets of every base pair. The completion of the Human Genome Project, while an amazing achievement, was just the start – we immediately set to work on mapping as many variations, mutations and interactions that influence our genes in health and disease as we could. And why stop at the human genome? After all, the very first genomes to be sequenced were those of microbes, and there are obvious benefits to knowing the genetic makeup of the thousands of tiny organisms that live within and alongside us, especially those that cause disease. The development of new, faster genome sequencing technologies has made it feasible to sequence any microbial DNA found in a sample – the basis of the relatively new science of metagenomics.

## At a Glance

- *Viruses in the blood are difficult to detect, due to issues with sensitivity, specificity, and breadth of testing*
- *Metagenomic shotgun sequencing (MSS) can capture viruses without preconceptions – but it's limited by viral genomes' small sizes and extensive variability*
- *ViroCap, an MSS enhancement tool, allows the detection of virtually any viral genome previously sequenced, and even unknown ones that share sequences with known viruses*
- *The technique has obvious applications for the laboratory and the clinic – and its developers have made it freely available to other researchers*

## (Shot)gunning for viral genomes

It was the identification of a gene – 16S ribosomal RNA – found in all bacteria back in 1971 that made sequencing of bacterial pathogens relatively simple, explains Greg Storch, Professor of Pediatrics at Washington University School of Medicine. Greg invented ViroCap together with Kristine and Todd Wylie, and says, "There is no single gene that we can amplify across all viruses, and that's why we have to take the much broader approach of metagenomic shotgun sequencing (MSS)."

MSS, in which all the DNA and RNA in a sample are (i) fragmented at random, (ii) sequenced, and (iii) reconstructed into a consensus sequence, has been a useful tool for studies of the microbiome. Kristine Wylie, Assistant Professor in the Department of Pediatrics at Washington University School of Medicine, has previously used MSS both to examine the normal human virome as part of the Human Microbiome Project, and to detect the presence of viruses in children with unexplained fevers. "We loved the approach of sequencing because we were able to look at all viruses without having any preconceptions of what we might be looking for, or what we might find," she says. However, MSS is limited by the small size and extensive variability of many viral genomes, which leads to poor sensitivity. Kristine and her collaborators thought they could do better.

"The initial idea came from work we had previously done with whole-exome sequencing," explains Todd Wylie, Instructor in Pediatrics at Washington University School of Medicine. Exome sequencing uses targeted capture to sequence only expressed genes, which is quicker and cheaper than whole-genome sequencing because expressed genes account for just one percent of the human genome. "We thought that using the same idea, we could build a panel that could target other species, such as bacteria or viruses, to enhance the sensitivity of MSS," says Todd. Focusing their efforts on viruses, the team created "a comprehensive viral targeted sequence capture panel that could be used to assess all viruses known to infect vertebrate cells and detect divergent viruses" – or, more concisely, ViroCap (1).

## If the ViroCap fits...

ViroCap is an MSS enhancement tool that provides the sensitivity required to accurately detect virtually all previously sequenced genomes of viruses infecting vertebrates, plus viruses that have yet to be sequenced but share some of their genetic code with a virus on the panel. The test targets 34 viral families, with 190 annotated genera and 337 different species, shown in Figure 1. A comparison of ViroCap viral sequence reads versus standard MSS can be seen in Figure 2. The results are impressive, and the new tool has understandably generated a lot of excitement.

"In relation to detecting viruses that are actually present in the sample, it appears to be pretty accurate, because we're able to obtain a very large proportion – up to 100 percent – of the sequence of viruses that are in clinical samples. Once we have that much sequence, there's very little doubt about

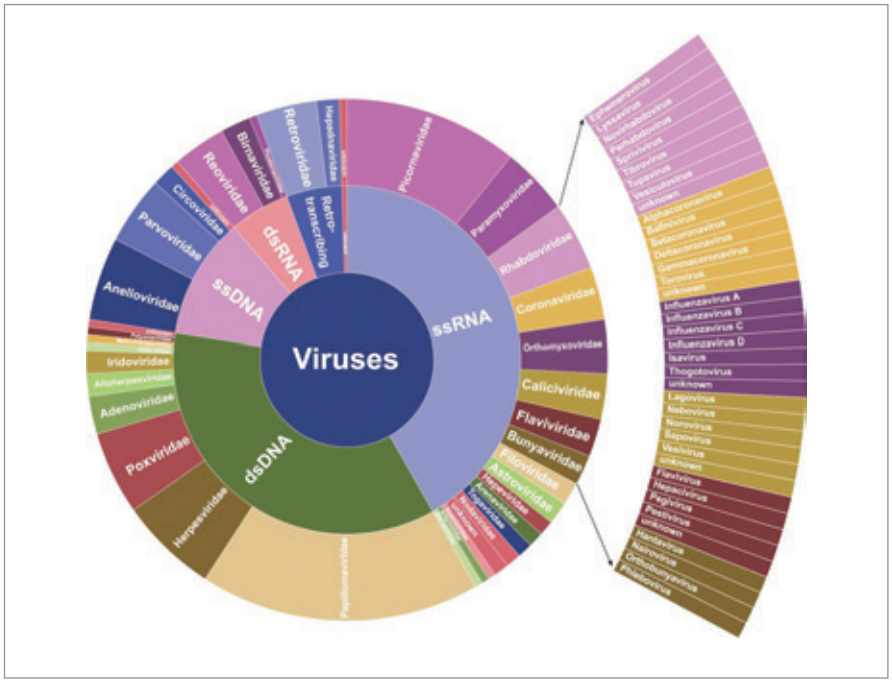



Figure 1. Taxonomic distribution of target genomes included in ViroCap (1).

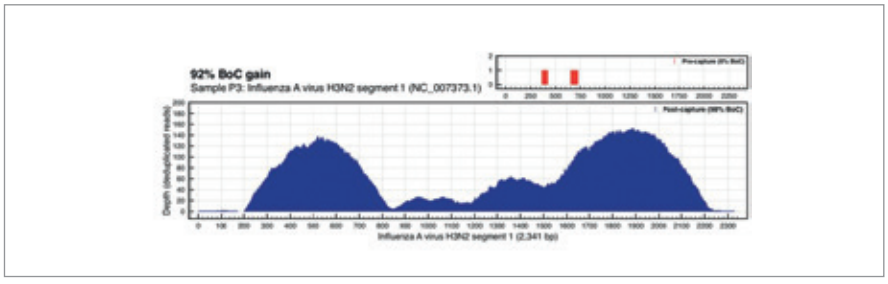



Figure 2. Targeted sequence capture enrichment, influenza A virus (1).

the identity of the virus", says Greg. Even a group of viruses with a very high degree of variability – the anelloviruses – were identifiable by ViroCap.

Several microarray-based techniques are available for viral characterization and discovery; however, they only target relatively small, discrete regions of the viral genome, while ViroCap targets the complete genome. As a result, microarrays can only indicate that the virus is present, whereas ViroCap provides sequences – and sometimes the complete sequence. By providing instant information on taxonomy, strain typing, virulence characteristics, and antiviral drug resistance genotype, ViroCap has the potential to be a game-changing technology for both research and clinical applications.

## Translation will take time

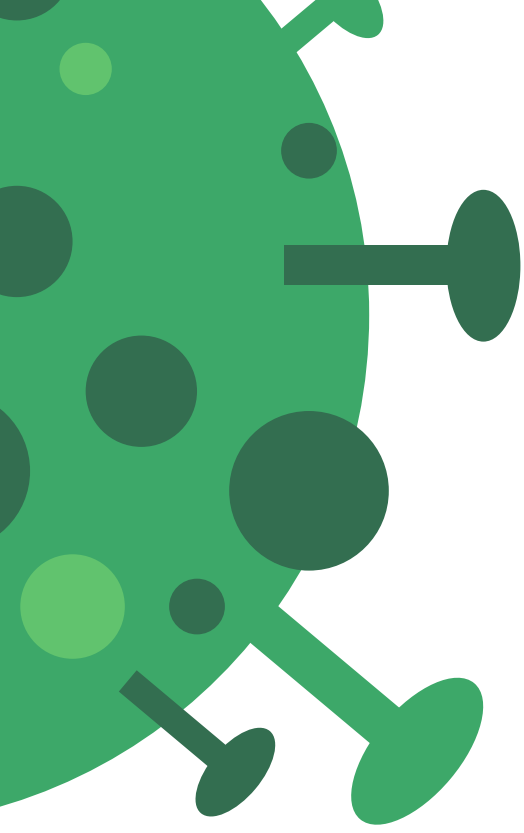
A test able to detect and identify almost any viral pathogen has obvious applications for clinical diagnostics. However, it's likely to take years before it reaches doctors, according to Todd: "To get ViroCap into the clinic, as with most new inventions, we will need to increase the speed and decrease costs."

Greg agrees, "It's relatively slow and expensive in its current form so you can think of it as more a proof-of-concept than anything else for clinical laboratory testing. But we anticipate that rapid advances in technology and refinements to the technique will lead to progress in both of those dimensions. We do think that this will come into clinical testing over the next few years."

*"There is no single gene that we can amplify across all viruses, and that's why we have to take the much broader approach of metagenomic shotgun sequencing."*

Initial results hint at the potential diagnostic power of the technique – in a study of children with unexplained fevers, ViroCap increased the breadth of coverage from 2 to 83 percent compared with MSS and identified several viruses not picked up by MSS at all.

Those looking to harness ViroCap to analyze clinical samples will need to bear in mind that the results won't provide information on clinical significance. "ViroCap is not judgmental. It reveals the presence of viruses in a clinical sample, but additional information would be required to determine whether the virus or viruses are significant," says Greg. "You definitely need a human interrogator to interpret the information."



The novelty of the approach could also bring up regulatory challenges. "It's not clear what the path would be to FDA approval of a technology like this where the capability of detection is very open. It's an area that's under very active discussion, both in microbial genomics and human genomics. The big question is how the FDA will validate tests based on next generation sequencing," says Greg.

## Research ready

For now, the team are focusing on how ViroCap can be used in microbiology and infectious disease research. "We are very interested in applying this technology to a whole variety of questions, both clinically relevant and more fundamental research questions," says Greg. "In particular, we want to apply it to diseases of unknown etiology, where there's some reason to be suspicious that viruses are important. One of the big areas is neurologic disease, such as encephalitis or meningitis, and another area is fever of unknown origin, both in adults and children, where current diagnostic testing does not always reveal an answer."

The response to the technology has been overwhelmingly positive say the researchers. "It's generated a lot of interest and excitement in our field, and in the scientific community and the public at large," says Todd.

Kristine says that scientists doing similar work immediately saw the potential for the technique. "There are a lot of scientists trying to use sequencing to look at viruses, and we've all experienced the same problems with sensitivity. So people are very excited for their own research, because this solved a problem that they have all been running up against."

*"When Washington University was involved in the Human Genome Project they made the sequences from the day available every night. We are continuing that tradition."*

## Collaboration goes viral

The team has already been approached by researchers working in a wide variety of research areas, according to Greg: "ViroCap has very broad-based applications because it has the potential to enhance the detection of all viruses that infect vertebrates. So we've heard from a very diverse selection of people in a variety of settings."

The team is making the technology available to researchers immediately. The reason is simple, says Greg, "We are scientists, and we want to promote the scientific process. ViroCap is very big, and we think that researchers around the globe will find applications for it, and so we want the scientific process to move forward."

The researchers are proud of Washington University's long history of data sharing. "When Washington University was involved in the Human Genome Project they made the sequences from that day available every night. We are continuing that tradition," Greg says. Researchers who would like to make use of the technology are encouraged to contact the researchers directly. Todd Wylie can be reached at *wylie_t@kids.wustl.edu*.

Looking forward, the team plan to keep developing their informatics pipeline while working on decreasing the time and cost of ViroCap. They also hope to overcome the limitations that may be preventing ViroCap from reaching its full potential, including filtering out bacterial vector sequences and building up the viral library to give the largest possible range of detectable sequences. The team even suggests that ViroCap could be modified to become a tool for broader pathogenic identification, including the detection of other human pathogens, such as bacteria, fungi and protozoa – a truly universal test.

"There is no end to the potential applications," concludes Greg.

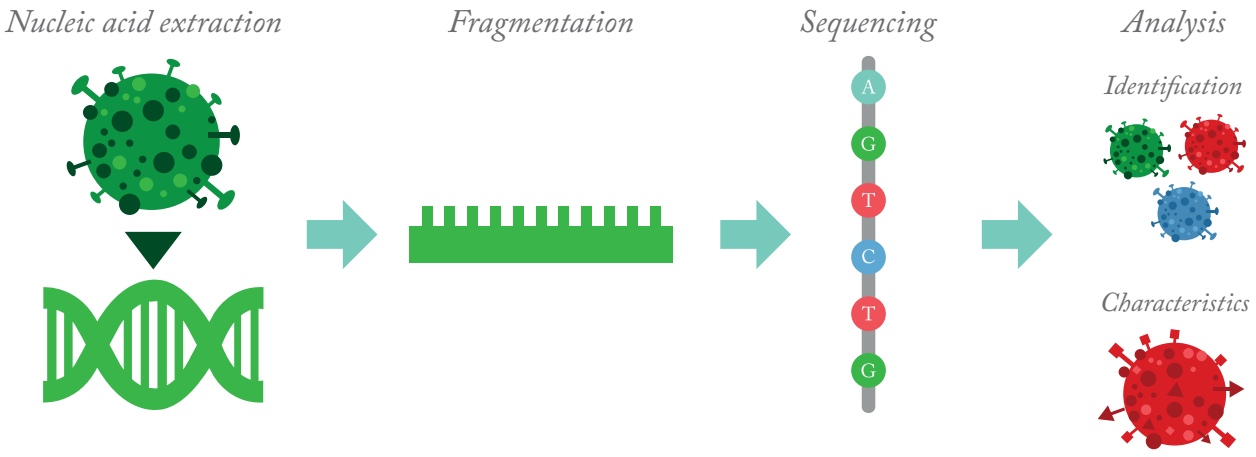

*Reference*

1. *TN Wylie et al., "Enhanced virome sequencing using targeted sequence capture", Genome Res, 25, 1910-1920 (2015). PMID: 26395152.*

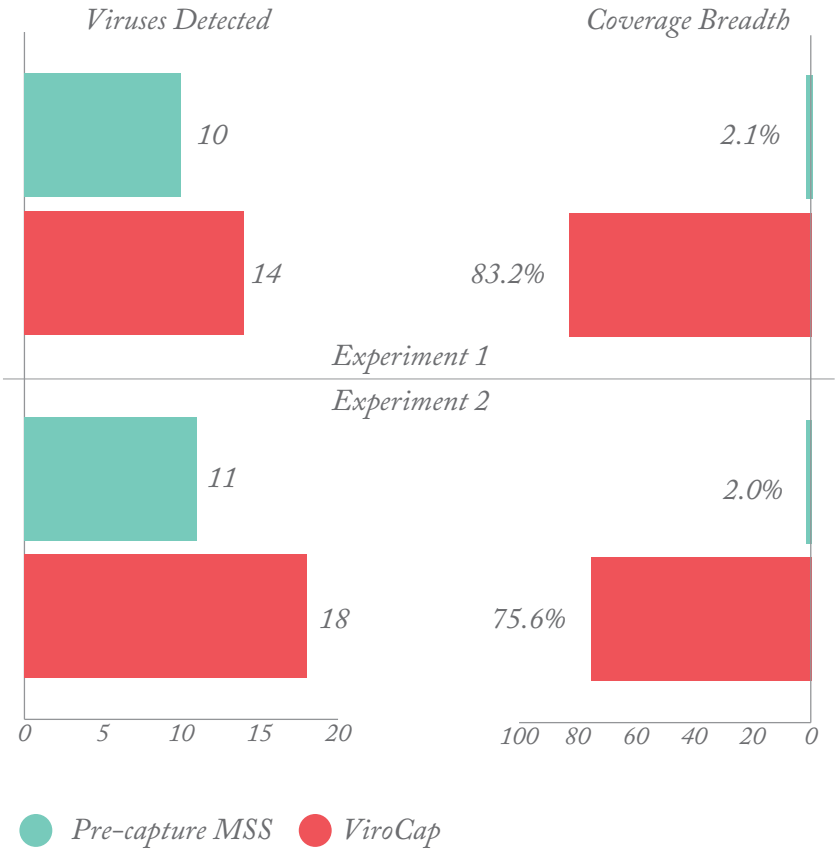


*First published in The Translational Scientist (www.thetranslationalscientist.com), a sister publication of The Pathologist.*

## How does metagenomic shotgun sequencing work?




## Comparing performance




## By the numbers

**337**
*Species of virus detected by ViroCap*

**16**
*Samples tested with ViroCap in the recent publication*

**52**
*Percent increase in viruses detected*

**~62**
*Percent genomic sequence identity required for complete coverage*

**~20**
*The number of viruses detected by the most expansive PCR assays*

**Unlimited**
*The number of viruses detected by ViroCap*

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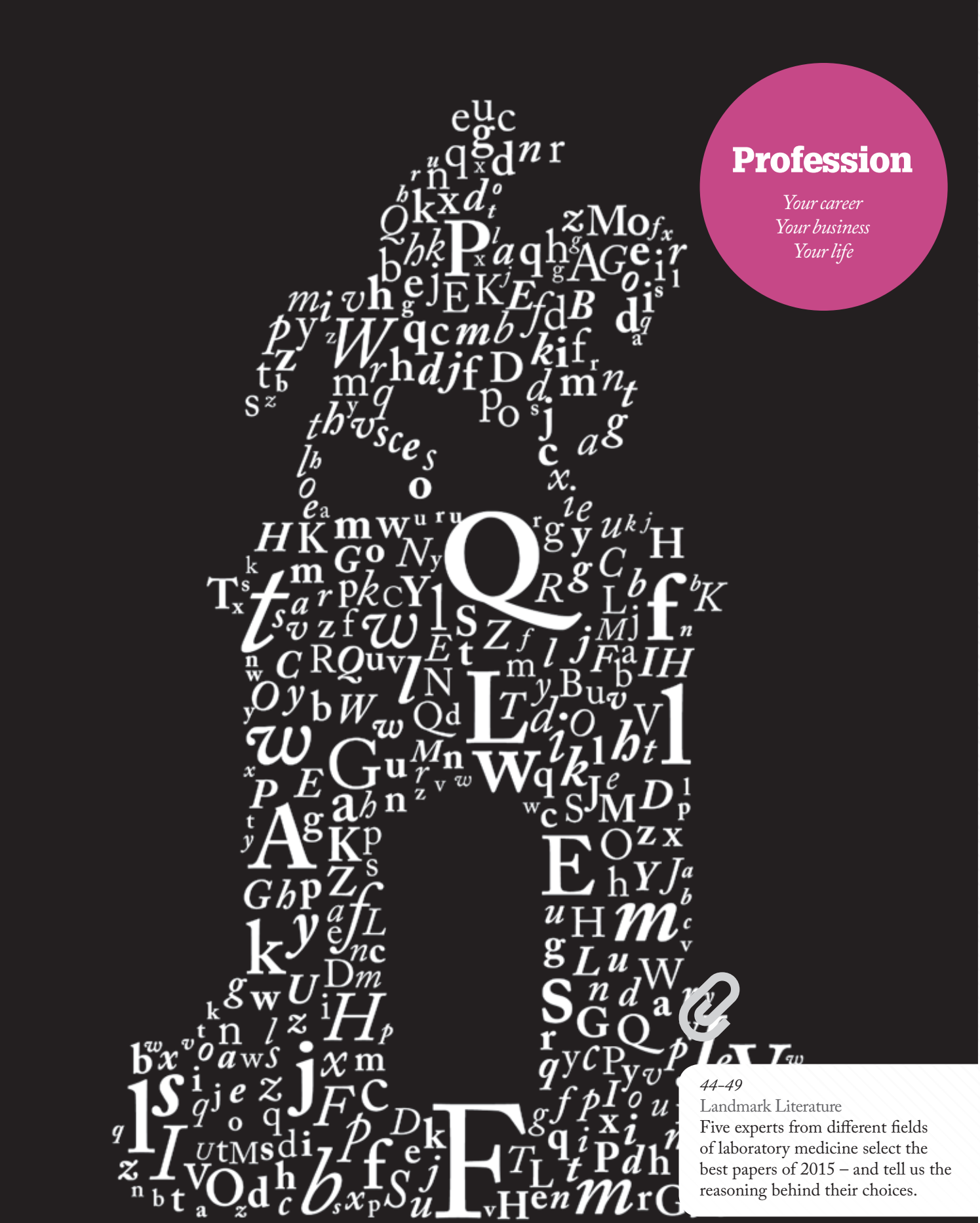

## Profession

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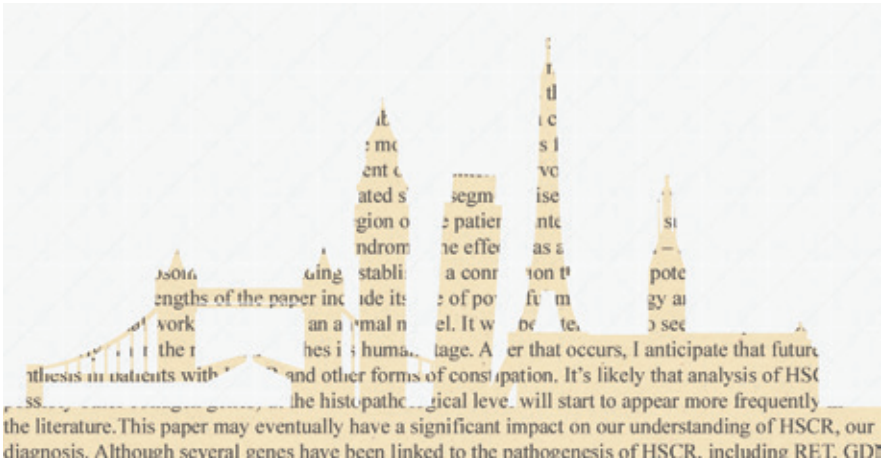
# Landmark Literature

**We asked an impossible question at the end of 2015: which piece of literature stood out from the crowd and showed the greatest potential for pushing the field of laboratory medicine forward?**

*In this series of articles, five experts boldly share their answers.*




# Variations on a Drop

**James' landmark paper: MM Bond, RR Richards-Kortum, "Drop-to-drop variation in the cellular components of fingerprick blood", Am J Clin Pathol, 144, 885–894 (2015). PMID: 26572995.**

*By James Nichols*

This manuscript describes the variability of complete blood count (CBC) results in a fingerstick capillary sample. It was an initial pilot study, but the results have ramifications for the reliability of point-of-care testing devices that use capillary blood samples. Although the paper only looked at CBC and hemoglobin, anything that provides a quantitative value – glucose meters, coagulation devices, serology, disease markers and more – can be tested by fingerstick, so all of these tests may be subject to the variability the authors observed.

It's been known for some time that glucose meters often give different results with capillary samples than with venous samples. But even between capillary samples, there's variation. Some of this is due to operator technique; people who squeeze the finger to get enough blood for the device end up contaminating the sample with interstitial fluid and other non-blood substances. It's even more of a problem when you are sampling for coagulation tests or other devices. But I think this paper is interesting because it looks at standard blood samples by fingerstick – no difficult draws or unusual sampling methods – and determines that there is variability even within a single drop of blood.

As a pilot analysis, it's quite in-depth. The authors looked at multiple samples from 11 different donors. In practice, though, I wouldn't recommend the use of multiple fingerstick collection from the same patient – it increases the time and expense of testing. It's useful for method validations – examining the variability we see with a particular sample type and looking at reproducibility with different patients and operators. Part of the advantage of point-of-care testing is its speed, and the ability to take action on the spot. If you have to run three tests from three different fingersticks on same patient, wait for the results, and then average them, you delay the intervention.

*"This paper is interesting because it looks at standard blood samples by fingerstick – no difficult draws or unusual sampling methods – and determines that there is variability even within a single drop of blood."*

A possible limitation of the paper is that the authors needed to dilute the samples, because they weren't able to collect sufficient amounts of blood for cell counts on a larger automated hematology analyzer. That dilution step could add variability to the test results. To mitigate its effects, the authors also used a HemoCue device to analyze hemoglobin. This analyzer only requires 5 µL of sample, so there's no need for dilution, and it showed similar variability to the larger hematology analyzer in different portions of the fingerstick blood samples.

I don't think there's any way of overcoming the issue of variability while maintaining small sample sizes – but I also don't think we should stop taking small samples. It doesn't preclude the use of capillary sample tests; it's just something we should be aware of as we perform the tests, like any other limitation or source of variation. We'll consider it as we educate our operators, too; we have nearly 6,000 operators doing glucose testing, and we'll want them to be aware of their sampling technique and try to minimize variation.

It will be interesting to see if similar variability is seen in portions of a blood drop for other common point-of-care tests, like glucose, hemoglobin A1c, coagulation or chemistries (like sodium, potassium, creatinine and even cardiac markers). As technologies become more sensitive – using smaller and smaller sample sizes to detect increasingly minor changes – I expect that variability will be a continuing problem. It's a good opportunity for engineers and designers to collaborate with the end-users of the instruments to figure out ways to minimize its effect. In the future, we might even find that certain technologies are more prone to these effects than others, on the basis of sample volume or sampling technique. That may help us to steer more towards those technologies that are less dependent on variability within the drop.

*James Nichols is Director of Clinical Chemistry and Professor in the Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, USA*

# A Paper to Circulate

**Ian's landmark paper: KL Spindler et al., "Circulating free DNA as biomarker and source for mutation detection in metastatic colorectal cancer", PLoS One, 10, e0108247 (2015). PMID: 25875772.**

*By Ian Cree*

This paper is one of a number published over the last year that examine the potential for liquid biopsy in colorectal cancer. That's a clear indication of a subject worthy of study – but there are several reasons I like this piece in particular. For one, it's open-access, which makes it available to a much larger readership than one published in a traditional, subscribers-only journal. For another, it's an example of careful research – the authors assembled a good population, used control groups wisely, conducted a well-defined study, and reported on the limitations as well as the results of their research.

Circulating free DNA (cfDNA) has been put forward as a potential tool for early diagnosis, monitoring of disease burden, and as an alternative to tissue biopsy for determining the *RAS* mutation status of a patient. In the paper I've chosen, the authors use a series of Phase II clinical trials to obtain plasma from 229 well-documented metastatic colorectal cancer patients and 100 controls, using the REMARK guidance (REporting recommendations for tumor MARKer prognostic studies, available at http://tp.txp.to/0316/bjc). DNA was quantified using qPCR for the peptidylprolyl isomerase A (cyclophilin A) gene, and a laboratory-developed amplification-refractory mutation system assay for *RAS* gene alterations was used to determine the presence of *KRAS* mutations.

The paper has some limitations. For instance, it was not clear what product sizes were identified by the authors' PCR methods, which is important as fragmentation of DNA in plasma will affect the sensitivity of the assay. The colorectal cancer cases were mainly advanced, but no analysis based on tumor load by radiology or another tumor marker like carcinoembryonic antigen (CEA) was given. Nevertheless, the authors should be congratulated on performing a meticulous study with reasonable patient numbers and a control group. They are quite right in saying that "differences in investigational methodologies make direct comparisons (between studies) difficult" – although personally I think "impossible" might be a better term!

*"The authors should be congratulated on performing a meticulous study with reasonable patient numbers and a control group."*

That issue does, however, call attention to a gap in our current work. There is a strong need for the community involved in this type of research to use well-defined comparators in their papers. A tumor marker like CEA is the obvious solution for colorectal cancer, though this is more difficult for many other tumor types. The authors call for "combined efforts to compare, validate and prospectively investigate the role of cfDNA quantification in cancer, with the overall perspective of translating results into clinical care." Based on the results of several such studies, this is clearly the next step – we need large, multicenter studies to provide the analytical and clinical validation necessary for this technology to enter routine clinical practice.

*Ian Cree is Molecular Pathologist at University Hospitals Coventry and Warwickshire, Visiting Professor at Coventry University, and Honorary Professor of Pathology at the Institute of Ophthalmology, University College London, UK*

# Hyperspectral Disease Diagnosis

**Peter's landmark paper: JT Kwak et al., "Improving prediction of prostate cancer recurrence using chemical imaging", Scientific Reports 5, Article number: 8758 (2015). PMID: 25737022.**

*By Peter Griffiths*

The diagnosis of cancers at an early stage is critical for the long-term survival of patients. For solid cancers, such as lung, breast and prostate cancer, this is currently accomplished by staining tissue samples with hematoxylin and eosin (H&E) dyes followed by histopathological examination; time to results is typically days rather than hours. Furthermore, diagnoses performed in this way are quite subjective. Indeed, if four histopathologists examine a stained tissue sample, there could be four different diagnoses! Clearly, a technique that is faster, more accurate and less subjective than H&E staining is needed.

My paper of choice details a very careful collaborative study of the prediction of prostate cancer recurrence.

For at least two decades, vibrational spectroscopists have attempted to demonstrate the feasibility of using infrared spectroscopy in medical diagnosis. In the early studies, a FT-IR microspectrometer equipped with a single-element detector was used in the mapping mode, where spectra were measured sequentially, with the sample being moved in steps of a few micrometers.

Although the results showed promise, the time required to acquire enough spectra to fully classify tissue samples was too long. Furthermore, an insufficient number of samples were usually tested, so that any results were rarely statistically significant. As a result, optimism for such measurements was not justified.

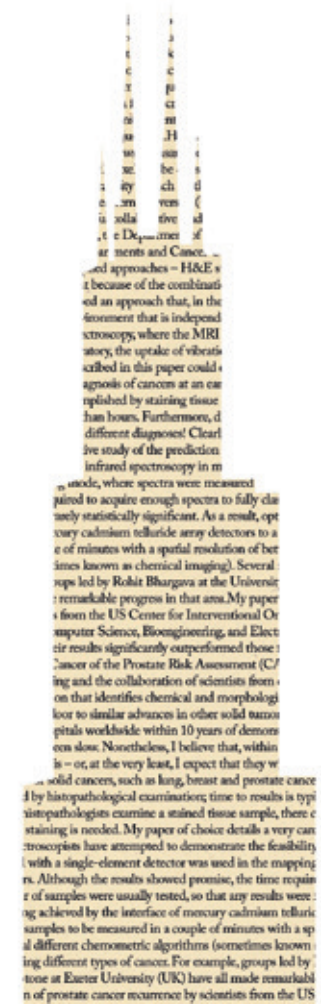
Hyperspectral imaging achieved by the interface of mercury cadmium telluride array detectors to a standard, continuously scanning FT-IR spectrometer allows thousands of spectra of tissue samples to be measured in a couple of minutes with a spatial resolution of between 1 and 10 μm. The spectrum measured at each pixel can be classified by several different chemometric algorithms (sometimes known as chemical imaging). Several research groups have demonstrated the applicability of such methods in predicting different types of cancer. For example, groups led by Rohit Bhargava at the University of Illinois (USA), Max Diem at Northeastern University (USA) and Nick Stone at Exeter University (UK) have all made remarkable progress in that area.

My paper of choice details the results of a very careful collaborative study of the prediction of prostate cancer recurrence by scientists from the US Center for Interventional Oncology at the National Institutes of Health, the Department of Pathology at the University of Illinois at Chicago, and the Computer Science, Bioengineering, and Electrical and Computer Engineering, departments and Cancer Center of the University of Illinois, Urbana-Champaign. Their results significantly outperformed those found using the most commonly applied approaches – H&E staining with classification using the Kattan nomogram or the Cancer of the Prostate Risk Assessment (CAPRA-S) score.

The paper stands out because of the combination of very high-quality spectroscopy and data processing and the collaboration of scientists from different disciplines. The paper described an approach that, in the words of its abstract, "provides a histologic basis to a prediction that identifies chemical and morphological features in the tumor microenvironment that is independent of conventional clinical information, opening the door to similar advances in other solid tumors."

In contrast to magnetic resonance spectroscopy, where the MRI technique was rapidly commercialized and adopted in hospitals worldwide within 10 years of demonstrating its feasibility in the laboratory, the uptake of vibrational spectroscopic techniques for medical diagnosis has been slow. Nonetheless, I believe that, within the next decade, the techniques described in this paper could displace current staining techniques for histopathological analysis – or, at the very least, I expect that they will be used alongside them.

*Peter Griffiths is Professor of Chemistry Emeritus, University of Idaho, Owner, Griffiths Consulting LLC, Moscow, USA*

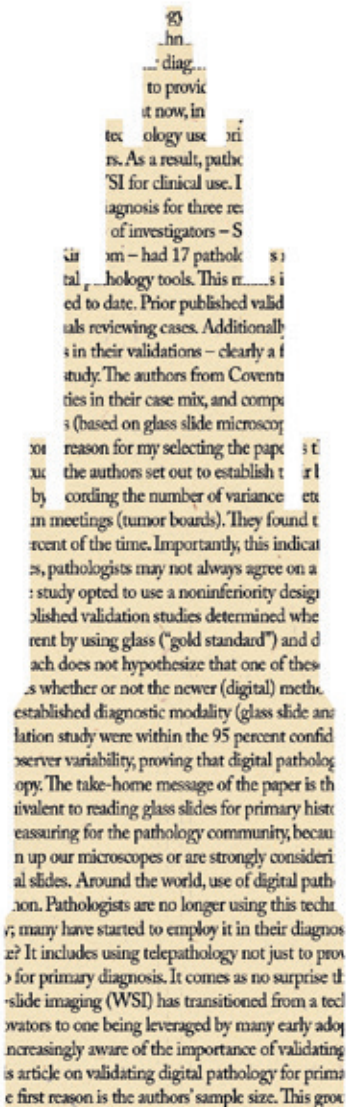


# Diagnosis: Digital

**Liron's landmark paper: DR Snead et al., "Validation of digital pathology imaging for primary histopathological diagnosis", Histopathology, [Epub ahead of print] (2015). PMID: 26409165.**

*By Liron Pantanowitz*

Around the world, use of digital pathology is becoming increasingly common. Pathologists are no longer



using this technology for education and research only; many have started to employ it in their diagnostic work. What does this look like? It includes using telepathology not just to provide second opinions, but also for primary diagnosis. It comes as no surprise that now, in many countries, whole-slide imaging (WSI) has transitioned from a technology used primarily by innovators to one being leveraged by many early adopters. As a result, pathologists are increasingly aware of the importance of validating WSI for clinical use.

I selected this article on validating digital pathology for primary diagnosis for three reasons. The first reason is the authors' sample size. This group of investigators – Snead and colleagues, from Coventry in the United Kingdom – had 17 pathologists report on 3,017 cases (10,138 slides) using digital pathology tools. This makes it one of the largest WSI validation studies published to date. Prior published validation studies have included, on average, eight individuals reviewing cases. Additionally, most prior studies used between 60 and 600 cases in their validations – clearly a far less comprehensive overview than this latest study. The authors from Coventry also included a broad distribution of subspecialties in their case mix, and compared the diagnoses in their original pathology reports (based on glass slide microscopy) to those rendered using digital slides.

The second reason for my selecting the paper is that, before embarking on their validation study, the authors set out to establish their baseline discrepancy rate for pathologists by recording the number of variances detected at their multidisciplinary team meetings (tumor boards). They found that they were concordant 98.78 percent of the time. Importantly, this indicates that even when examining glass slides, pathologists may not always agree on a diagnosis.

The third reason is because the study opted to use a noninferiority design for their validation. Many previously published validation studies determined whether or not diagnostic outcomes were different by using glass ("gold standard") and digital modalities. The noninferiority approach does not hypothesize that one of these methods is superior, but rather establishes whether or not the newer (digital) method is at least as effective as another, better-established diagnostic modality (glass slide analysis). The results of the Coventry validation study were within the 95 percent confidence interval for intra- and inter-observer variability, proving that digital pathology is non-inferior to glass slide microscopy.

> *"This makes it one of the largest WSI validation studies published to date."*

The take-home message of the paper is that digital pathology techniques are equivalent to reading glass slides for primary histopathological diagnosis. This is reassuring for the pathology community, because many of us have either already given up our microscopes or are strongly considering giving them up in exchange for digital slides.

*Liron Pantanowitz is Professor of Pathology and Biomedical Informatics and Director of Pathology Informatics, University of Pittsburgh Medical Center, Pittsburgh, USA*

# Collagen and the Colon

**Miguel's landmark paper: R Soret et al., "A collagen VI-dependent pathogenic mechanism for Hirschsprung's disease", J Clin Invest, 125, 4483–4496 (2015). PMID: 26571399.**

*By Miguel Reyes-Múgica*

Rodolphe Soret and his colleagues in Canada and France recently published what I consider a landmark paper in pediatric pathology. It provides new insights into the origins of a highly prevalent childhood disease. Hirschsprung disease (HSCR) is a congenital form of megacolon that occurs when the ganglion cells of the digestive tract fail to develop, impairing or eliminating function. The disease occurs in approximately one of every 5,000 live births, making it a very common disorder in the pediatric population – and therefore one worthy of extensive investigation. The paper I chose is extremely important because it describes a new mechanism to explain the pathogenesis of HSCR.

The researchers began by generating a mouse model of HSCR, named Holstein. The model was created to carry a mutation that results in increased secretion of collagen VI, which the authors determined renders the extracellular matrix unwelcoming to neural crest cell migration in the embryonic bowel. Why is this mutation relevant? The absence of ganglion cells in HSCR arises from a lack of neural crest migration into the large intestine during prenatal development. In the mouse model, this defect is further complicated through interaction with other extracellular matrix proteins like fibronectin, interfering with the development of the enteric nervous system. The authors also examined cross-sections of muscle strips from a human HSCR cohort of 16 children (12 with isolated short-segment disease and four with combined HSCR and Down syndrome). They observed that the myenteric ganglia from a ganglionated region of the patients' intestines were surrounded by abundant collagen VI microfibrils, lending strength to this conclusion. In the children with Down syndrome, the effect was accentuated – and since the human collagen VI genes (*COL6A1* and *COL6A2*) are located on chromosome 21, this finding establishes a connection that could potentially explain the frequent association of HSCR with Down syndrome.

The strengths of the paper include its use of powerful methodology and well-designed experiments. The potential weakness, however, is that the experimental work was done in an animal model. It will be interesting to see the reproducibility of these initial findings across other species, particularly when the research reaches its human stage. After that occurs, I anticipate that future research will focus on the genes involved in collagen synthesis in patients with HSCR and other forms of constipation. It's likely that analysis of HSCR specimens for variations in collagen VI (and possibly other collagen genes) at the histopathological level will start to appear more frequently in the literature.

This paper may eventually have a significant impact on our understanding of HSCR, our ways of classifying it, and our methods of diagnosis. Although several genes have been linked to the pathogenesis of HSCR, including *RET*, *GDNF*, *NRTN*, *EDNRB*, *EDN3*, *ECE1* and others, there's still a case of "missing heritability." We still don't have a plausible explanation for the many forms of HSCR that are not related to these mutations – and, in time, it may emerge that collagen gene upregulation holds the key.

*Miguel Reyes-Múgica is Marjory K. Harmer Endowed Chair in Pediatric Pathology, Chief of Pathology and Head of Laboratories, Children's Hospital of Pittsburgh, University of Pittsburgh School of Medicine, Pittsburgh, USA*

# Westgard Rules!

**Sitting Down With… James Westgard,** Emeritus Professor in Pathology and Laboratory Medicine at the University of Wisconsin Medical School and President of Westgard QC, Inc., USA.

You are one of the world's most recognized experts in laboratory QA/QC; what inspired you to follow this career path?

I majored in chemistry in college, in part because my father had studied it. I was then inspired to pursue a graduate degree by a couple of professors who were outstanding teachers. Teachers have been critical in my personal development and I don't think they are properly appreciated at all levels of education. The actual decision to work in laboratory medicine was somewhat fortuitous! I finished my graduate studies mid-year while my wife was under contract to teach elementary school for the whole year. I had a couple of colleagues who worked in the medical school and wanted some help to implement new automated analytic systems. Having been trained in analytical chemistry and performed graduate studies on automated continuous flow systems, it was a good fit. The position at the University of Wisconsin turned out to be the job of a lifetime!

And your interest was piqued during your first project evaluating the performance of a new multi-test analyzer?

That's right. In studying the scientific literature on evaluation of methods, I found that the statistical results and decisions on acceptability of performance didn't make sense; decisions often depended on correlation statistics, rather than the size of errors occurring and their effect on the use and interpretation of test results. That led us to propose new criteria for judging the acceptability of performance, including the introduction of the concept of Total Analytical Error. That experience helped me understand the importance of employing proper tools and techniques to measure and manage quality.

Westgard Rules are internationally recognized. Why do you feel that you have been so successful?

Westgard Rules have a theoretic rationale, but they were also evaluated for practicality by the medical technologists in our clinical chemistry laboratory, who found them to be a logical and fit with their thoughts about inspecting QC results. It was just that no one had formalized those thoughts as "rules" and justified and approved their use in the laboratory. We did a lot of education and training over the years, documented the practice in the scientific literature and laboratory textbooks, expanded the applications to tests outside of clinical chemistry, and then further to laboratories outside the US.

Analytical error is still unacceptably high. Why, and what needs to change?

Laboratories need to pay attention to the quality of all phases of the Total Testing Process. There has been a tremendous amount of work on preanalytic errors in the last 20 years and those error rates have been significantly reduced. Analytical errors actually have more serious impact on patient care and I worry now that laboratories continue to assume analytical errors are not as important as preanalytic errors. Our studies to characterize the quality of processes using sigma metrics are now showing that preanalytic processes are often as good or better than analytic processes. On a scale of 3 to 6, where 3-sigma represents the minimum acceptable quality for production and 6-sigma world class quality, we observe that preanalytic processes have often improved to better than 4-sigma, whereas there are some critical analytic tests, such as HbA1c, that often operate at 3-sigma or less.

What advice would you give to laboratory professionals who feel that things could be done better?

Quality of service and quality of work life should go hand-in-hand. Everyone has a responsibility to contribute to providing the services they would want to receive for themselves, for their family, for everyone. This often means additional training, but anyone who is committed to quality can find ways to make improvements, starting with their own work processes.

If you could start your career over again, would you do anything differently?

I would probably include some formal study of statistics. On the other hand, I have sometimes found that my lack of it has been an advantage, because I start by focusing on the problem and then work with data to understand what statistical tools are useful. Statisticians often have their favorite tools and techniques and attempt to define the apparent problem so that it fits the statistics, rather than solve the real problem. I've been fortunate to work on some interesting problems and develop practical solutions that others have also found useful.

What is your most satisfying achievement?

I would definitely say my success as a teacher. I see myself first as a teacher, second as a researcher, and third as a laboratory analyst or service provider. I'm also incredibly proud of my son Sten, who developed and maintains the www.westgard.com website, publishes our books and training materials, and continues our teaching and training around the world. Together we have been able to help laboratories develop a better understanding of quality and provide tools and techniques to improve quality management.

Is there anything that you would still like to achieve before you can say… "yes, I'm happy now"?

I am actually happy and have been for many years. But, it would be nice to see the term Total Analytic Error and its definition appear in the official international vocabulary of metrology (VIM). That would be heaven!

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