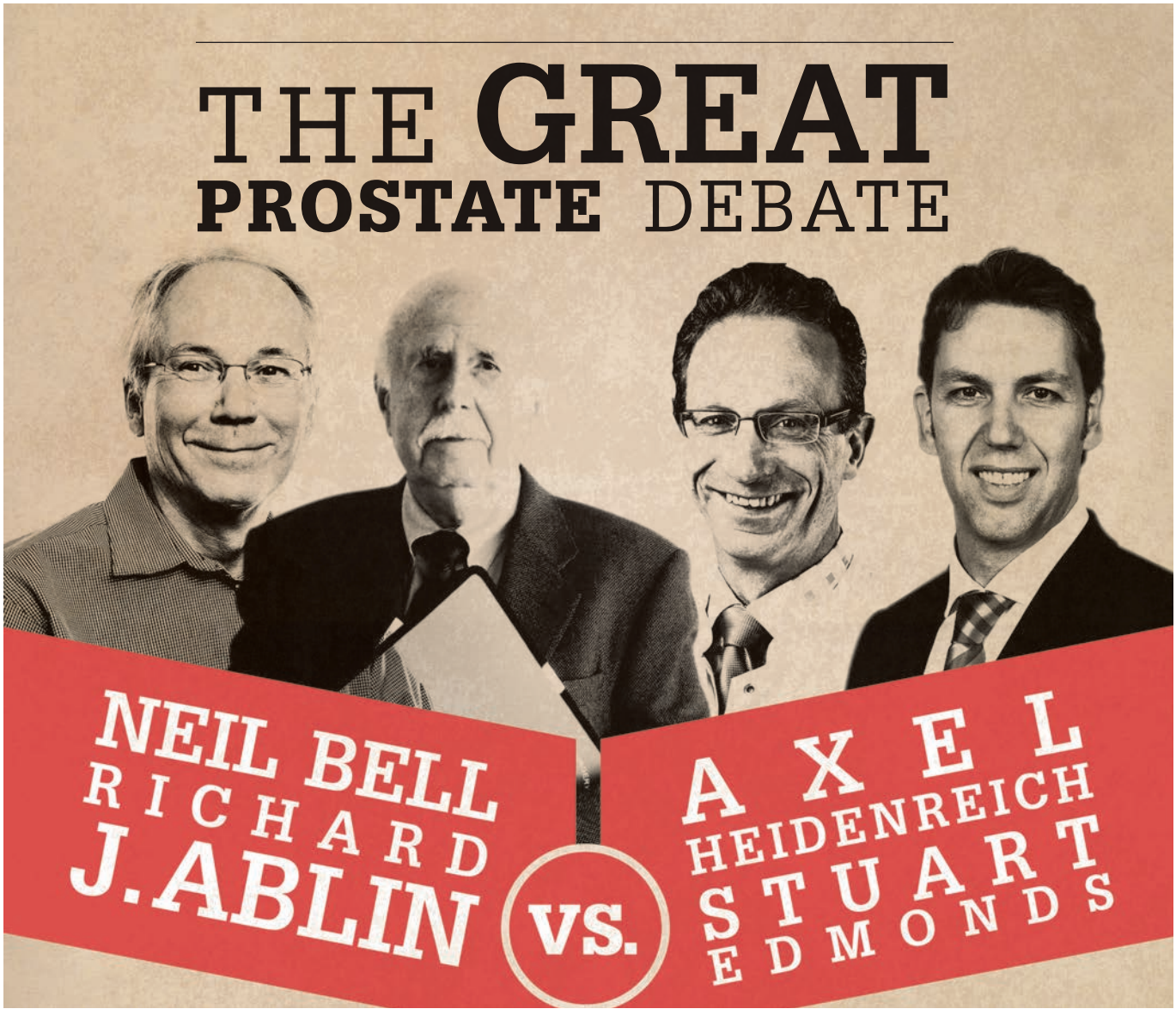
the
Pathologist



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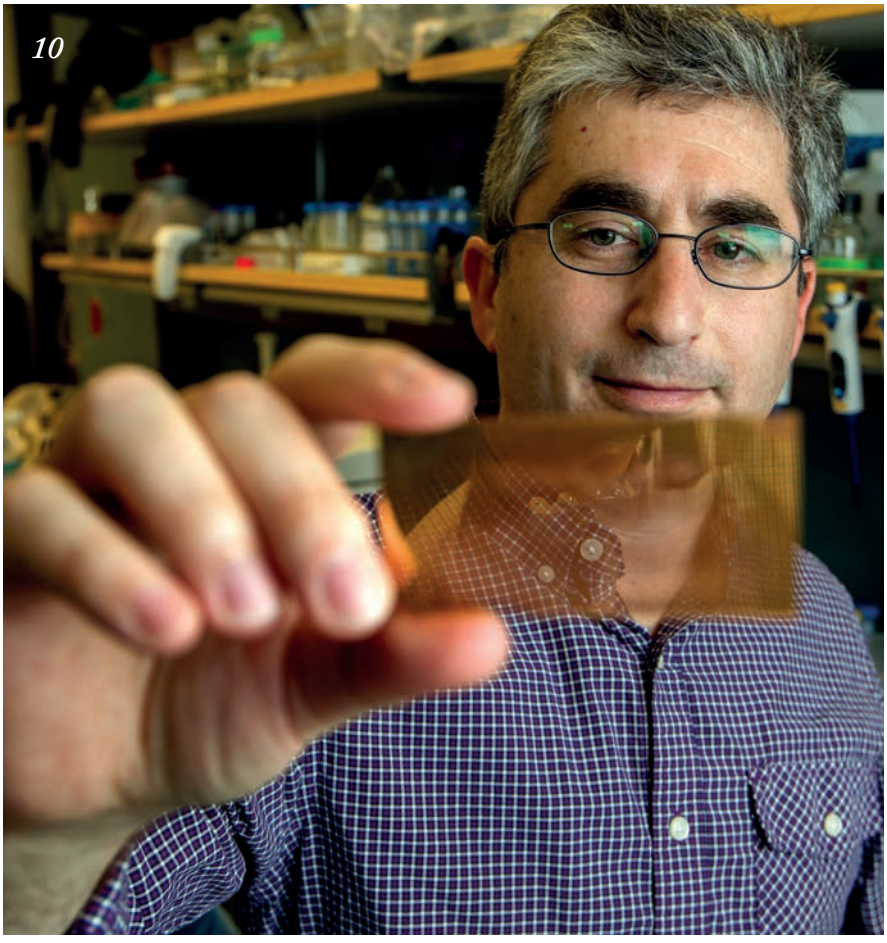

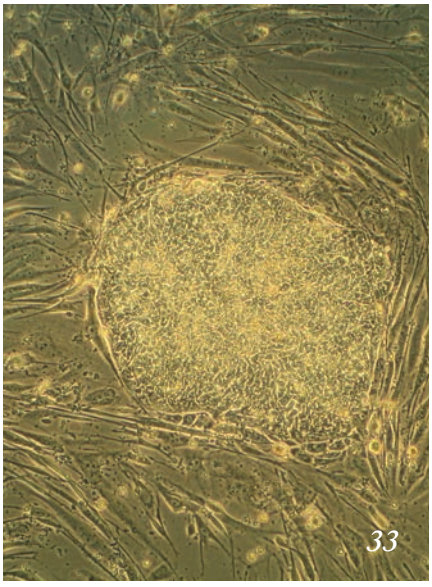

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Fighting Pigeonholes

Last month, we threw down the gauntlet and urged you to face reality: pathologists don't always get the respect they deserve.

"The Last Respite of the Socially Inept?" – last month's cover feature on negative pathology stereotypes – prompted a swift response from many of you. Certainly, we hoped to stir up the hornet's nest a little. After all, the public face of pathology is an issue that concerns us all. Negative stereotyping is damaging the field of pathology, frightening away the brightest medical students and painting an inaccurate picture of life in the lab. The big question is: how can we change that negative image?

Well, it's not easy. And sure enough, you let us know how frustrating it can be; "like hitting your head against a brick wall" was one of the phrases used. We all know what a stereotype is: "a widely held but fixed and oversimplified image or idea of a particular type of person or thing" (Oxford Dictionary) – and the real crux of the problem lies in the word "fixed".

Changing people's opinions isn't easy, especially when those views are deeply ingrained and constantly reinforced by the media (that is to say, fixed). Ironically, even those pathologists who go out of their way to show that they're just as friendly, capable and hardworking as any other specialty face challenges; people just can't seem to understand why a doctor with such outstanding qualities would "be forced" to become a pathologist – much less want to become one…

And yet, there's immense value in what you do. After all, pathology is an essential, integral and constantly evolving aspect of patient care – but can you do more to ensure that your work is not overlooked or taken for granted?

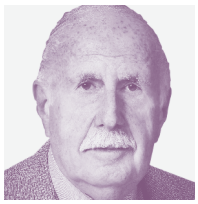
The lab isn't the only place where you can make a difference. An enthusiastic and approachable mentor is more likely to encourage a student to consider pathology than an intelligent but dispassionate one who simply quotes textbooks from memory. And in the wider world, showing people that there's life beyond the television portrayal of the forensic pathologist can surprise them and challenge their preconceptions.

The more visible you are – and the more you show both medical students and members of the public that the stereotypes are tired and outdated – the better the outlook for the field. If we all make a conscious effort to shift the stereotype, more promising young students will be attracted to a career in pathology, adding further momentum to the cause.

In terms of shining a spotlight on pathology and pathologists more generally – well, we will do our best.

Have you experienced negative stereotyping? Do you have a suggestion for combating it?
We would love to hear your opinions and experiences. Email fedra.pavlou@texerepublishing.com.

Rich Whitworth
Associate Editorial Director, Texere Publishing



Richard J. Ablin

Richard is a professor of pathology at the University of Arizona College of Medicine, Arizona, US, and president of the Robert Benjamin Ablin Foundation for Cancer Research, founded in memory of his father, who was diagnosed with metastatic prostate cancer in 1978. He received his PhD in microbiology and continued his training as a postdoctoral fellow at the University at Buffalo, The State University of New York. After discovering prostate-specific antigen in 1970, he became a vocal opponent of screening asymptomatic men using PSA. He serves on the editorial board of several journals and is co-editor of the book series Cancer Metastasis – Biology and Treatment.

Richard airs his strong views on PSA screening on page 16.

Julia Baker

Julia is a senior veterinary pathologist at Charles River Frederick, Maryland, US, and has been working in the fields of toxicologic and diagnostic pathology for over 25 years. She is a member of the Executive Board of the International Society of Ocular Toxicology, and also serves on the Global Executive Steering Committee of the International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice (INHAND) project, chairing the Special Senses organ working group. Away from the microscope, Julia works as a convergent media artist, using digital brushes to blend her own photography with layers of pure light, texture, and paint effects.

Julia explains how pathologists can help save costs on preclinical pathology for cell-based therapeutics on page 33.

Andrew Don-Wauchope & Janet Simons

Andrew is a medical biochemist with the Hamilton Regional Laboratory Medicine Program where he works in both the core and chemistry-immunology laboratories. He is an associate professor in pathology and molecular medicine at McMaster University and his research interests include the evaluation of laboratory tests for clinical use, which involves systematic reviews and the evaluation and development of guidelines.

A third year resident in medical biochemistry at McMaster University in Hamilton, Ontario, Janet Simons received her BSc in Biochemistry from the University of Waterloo before joining McMaster for her MD training. She is interested in helping clinicians make more efficient use of laboratory resources and increasing the number of evidence based recommendations for laboratory testing in clinical practice guidelines.

On page 28, Andrew and Janet discuss the role of the pathologist in the creation and assessment of clinical practice guidelines.

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Upfront

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

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

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Photo credit: Norbert von der Groeben/Stanford School of Medicine

Chipping Away at Diabetes

Sensitive and specific point-of-care testing with plasmonic gold chips

Historically, the two major types of diabetes were easy to distinguish: type 1 (T1D, the autoimmune form) appeared in childhood and type 2 (T2D, the metabolic form) was considered the adult-onset form of the disease. The distinction has faded in recent years – significantly more adults are developing T1D, while the rapid rise in childhood obesity is accompanied by a corresponding increase in childhood T2D (1). But though the diseases present with similar symptoms, they need very different treatment approaches – so how can we quickly distinguish between the two types?

A group led by Brian Feldman at Stanford University in Califiornia, USA, have devised a potential solution: a plasmonic gold chip that allows near-infrared fluorescence-enhanced (NIR-FE) detection of antibodies that attack the islet cells of the pancreas. The chip features multiplexed islet cell antigen microarrays with nanostructured gold islands; the combination of the gold substrate and nanogaps supports techniques like electric field enhancement and surface plasmon resonance that yield 100-fold improvements in NIR-FE detection.

The chip increases the efficiency of diagnosis as well as the sensitivity. While current tests for diabetes require several milliliters of blood drawn in a laboratory, a test using lower sample volumes would allow diagnosis at the point of care, saving time and reducing patient stress and inconvenience. The plasmonic chip can reliably detect islet antigen-specific autoantibodies even in ultralow blood volumes like those obtained from a finger prick. Using only two microliters of blood with no processing required, the chip was able to diagnose T1D with the same sensitivity (100 percent) and specificity (85 percent) as standard radioassays (2).

With such results, it may come as a surprise that this is the first time protein microarrays on plasmonic gold chips have been used for human disease diagnosis. And the future looks bright; the microarrays can perform isotype-specific analysis of the autoantibodies and may one day even be used to anticipate the onset of diabetes. One thing is certain: as the incidence of both types of diabetes continues to rise, the need for rapid and efficient diagnostic technologies will only become greater. *MS*

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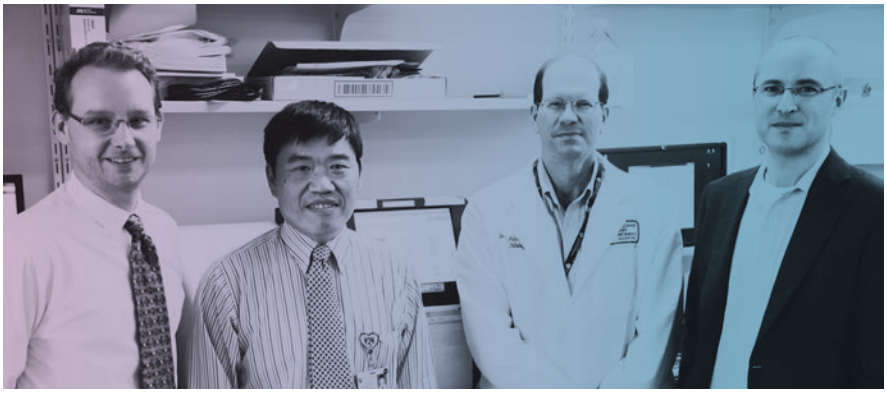
Faster Blood Cancer Screening

Could Rapid Heme Panel become a mainstay in blood cancer diagnosis and management?

A team of pathologists at Dana-Farber/Brigham and Women's Cancer Centre have developed a quick, high-tech genetic test that can provide deep sequencing information on 95 cancer genes in just a few days. We spoke to Jon Aster, a professor of pathology at Harvard Medical School and one of the developers of Rapid Heme Panel (RHP), to find out more.



Rapid Heme Panel team members included Michael Kluk, Frank Kuo, Jon Aster, and Coleman Lindsley (left to right).

Why develop a new test?

Before RHP, we had to send out tests for important genes in acute myeloid leukemia (AML), namely, *NPM1*, *FLT3* and *CEBPA*. The tests had to be performed individually and results could take up to a fortnight. We were also doing bead-selection-based targeted exome sequencing in patients with hematologic malignancies, but these were treated as research and were not recorded in the medical record. We saw a need for a test with a faster turnaround (approximately three to seven days) that would provide sequence variant information to clinicians treating patients with suspected or diagnosed AML, myelodysplastic syndromes (MDS), and myeloproliferative neoplasms (MPN).

How does RHP testing work?

A template is created by primer extension, and the Illumina Mi-Seq platform is used to sequence amplified patient DNA. Once we have the genetic data, an informatics pipeline is used to filter out non-pathogenic single nucleotide polymorphisms. Finally, a report is created that lists pathogenic variants, their allele frequencies and their significance based on the most recent scientific evidence.

Were there any challenges?

RHP relies on analyzing 1,330 amplified DNA sequences that cover 95 genes known to be pathogenic drivers in blood cancers. As you can imagine, developing a test that involves simultaneous amplification of so many sequences is not trivial. It required several rounds of primer design and a lot of testing with cell line and sample controls. In fact, it took about a year to develop and validate. The greatest challenge we are facing now is capacity: we can do up to 42 tests per week with our current equipment – and at present we are at almost 100 percent capacity.

Could you highlight the advantages?

The main advantage is that the test can be performed in five days or less and requires only one tube of blood. It provides information to help pathologists establish a diagnosis, and enable clinicians to select the appropriate therapy in a range of situations; for patients with relapsed refractory disease, RHP includes analysis of genes that may help select suitable candidates for clinical trials. Patients with de novo acute leukemia can be risk stratified based on genetic analysis, and patients who present with abnormal blood counts (who could have myeloid neoplasms or possibly benign hematologic conditions) can be more easily diagnosed. We have now used this test on several hundred samples, and RHP adds value to results beyond our previous capabilities.

What is the impact on pathologists?

Because RHP provides excellent sequencing depth (generally >1000x) it is possible to detect minor clones and to follow clonal evolution during treatment, which may have prognostic importance. In principle, it could also be used to detect and follow chimerism in a post-transplant setting, and it may also be possible to extend its applications to other hematologic malignancies (for example, myeloma and lymphoma).

RHP can also help pathologists establish earlier and more definitive diagnoses, allowing clinicians to better select the best therapy for patients – that actually enhances the value of what the pathologist provides.

Finally, RHP is relatively inexpensive; the price of the deep sequencing information is about a third of the cost of the three tests it is replacing. The sequencing and informatics pipeline we have created only requires the pathologist to consider and interpret a few variations per sample (typically one or two, or at most six or seven), meaning the time required to evaluate and sign out a case is typically only a few minutes – enabling rapid molecular diagnosis.

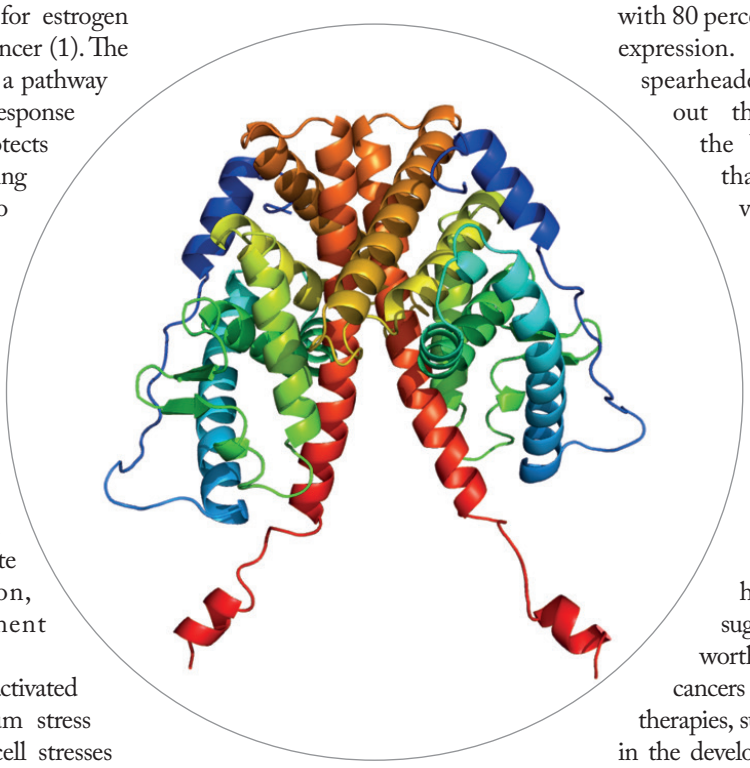
Signaling Cellular Stress

Atypical estrogen induction of protein pathways leads to treatment resistance and poorer outcomes in some breast cancers

Researchers from the University of Illinois at Urbana-Champaign, USA, have discovered a new function for estrogen in the pathology of breast cancer (1). The hormone atypically activates a pathway called the unfolded protein response (UPR). UPR normally protects cells against stress by activating downstream pathways to reduce protein production and by increasing the protein folding capacity of the endoplasmic reticulum. UPR is inactive in healthy and unstressed cells, but overactive in numerous types of cancer, including breast tumors. Chronic activation of the pathway appears to facilitate the survival, proliferation, angiogenesis and treatment resistance of tumors.

In normal cells, the UPR is activated by the endoplasmic reticulum stress sensor, which responds to cell stresses such as hypoxia or an accumulation of unfolded protein (2). Acting via estrogen receptor α (ERα), estrogen induces rapid pre-activation of the UPR before normal stress signals are present. "This is a new role for estrogen in the pathology of cancer," said David Shapiro, the biochemistry professor who led the study. "Others have shown that stress activates this pathway, helping to protect some tumors. What is new is our finding that estrogen can pre-activate this pathway to protect tumors."

When estrogen binds to ERα, it initiates a series of molecular changes in the cell. One of these involves opening calcium channels in the endoplasmic reticulum, allowing calcium ions to flood the cell. "That's a signal to activate the UPR pathway – the stress pathway," Shapiro said. "It's also a signal that many researchers think has something to do with cell proliferation. The calcium itself may be a proliferation signal." The pathway induces additional chaperone production, which Shapiro refers to as an "assembly line" for protein packaging, and also mediates cell death. In a normal cell, excessive stress will induce apoptosis, but in a cancer cell with mild, ongoing UPR activation, the opposite occurs – the apoptosis pathway becomes more difficult to induce, allowing malignant cells to survive under conditions that would otherwise have caused their death.



By looking back at genetic data from breast tumor samples, the researchers were able to correlate UPR activation with disease outcome. They found that patients expressing the UPR signature had a higher rate of recurrence, reduced time to relapse and reduced overall survival. Ten years after diagnosis, only 15 percent of women with high UPR expression were disease-free, compared with 80 percent of women with minimal expression. "[Neal] Andruska, who spearheaded the research and carried out the computer analysis of the breast cancer data, found that UPR activation is a very powerful prognostic marker of the course of a woman's disease," said Shapiro. "Our marker helps identify breast cancers that are likely to be highly aggressive and therefore require intensive therapy."

The function of estrogen in anticipatory activation of the UPR pathway was hitherto unknown, but suggests a new therapeutic target worthy of further investigation. For cancers that are resistant to current therapies, such knowledge can be useful in the development of new and effective treatments. *MS*

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Body Mass Cancer

Could high BMI be responsible for nearly half a million cancer cases each year?

We know that obesity increases the risk of a range of diseases – but exactly what impact does high body mass index (BMI) have on cancer incidence? A research team led by Melina Arnold of the International Agency for Research on Cancer (IARC) attempted to answer that question with a global population-based study. The headline: 481,000 new cases (or 3.6 percent of cancer worldwide) in adults in 2012 could be attributed to high BMI. Perhaps more telling is the fact that a quarter of those obesity-related cancers could have been avoided had the mean BMI value from 1982 been maintained over the past 20 years... But we continue to get fatter.

The authors looked at the incidence of cancers associated with a high BMI (including rectal, colon and oesophageal cancer) in 12 geographical regions. Perhaps unsurprisingly, the incidence of obesity-related cancer was much higher in developed countries (see Figure 1), with around 64 percent occurring in North America and Europe. Notably, women are at higher risk than men, with endometrial and breast cancer accounting for a large portion of female cancer cases.

But are all of these cases attributable to weight? The authors allowed a 10-year lag-time, linking BMI information from 2002 to cancer cases in adults over 30 in 2012. However, the effect of bodyweight on cancer risk is poorly understood, so the accuracy of the results given the chosen 10-year timeframe is uncertain – especially as a variety of cancers were included in the study. Geography (and hence ethnicity) was also not fully accounted for.

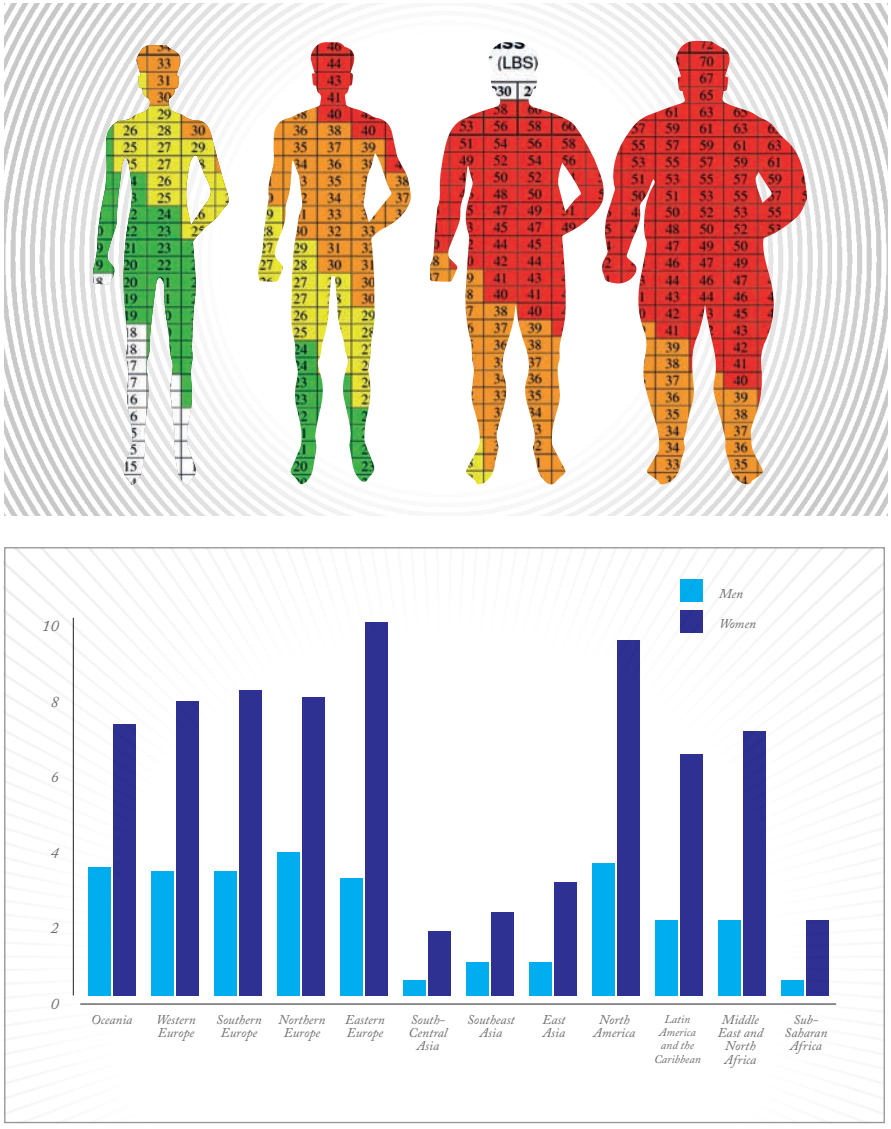

Figure 1. Proportion of all cancer cases attributable to high BMI by region. For further information, see reference 1.

Despite any limitations of the study, it does seem very clear that large increases in BMI are affecting public health. "Our findings add support for a global effort to address the rising trends in obesity," says Melina Arnold, "The global prevalence of obesity in adults has doubled since 1980. If this trend continues it will certainly boost the future burden of cancer, particularly in South America and North Africa, where the largest increases in the rate of obesity have been seen over the last 30 years." *RM*

Reference

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Antimicrobial Arms Race

Can microbial "dark matter" give us a new weapon against drug-resistant pathogens

As the battle between humans and drug-resistant pathogens grows increasingly fraught, a team from Northeastern University in Boston (Massachusetts, USA) has used an innovative device to isolate microbial "dark matter" – bacteria that cannot typically be cultured in the laboratory. By analyzing the ability of 10,000 potential candidates to halt methicillin-resistant *Staphylococcus aureus* (MRSA), the team discovered 25 that showed promise (1).

To find these unculturable bacteria, the researchers used a device called an iChip, which dilutes soil samples so that individual bacterial cells can be sorted into separate chambers. After sorting, the iChip is buried again so that nutrients and growth factors from the natural soil environment can diffuse through the chambers and allow the bacteria to grow. In the lab, only about one percent of soil bacteria can be cultured, but the iChip permits the growth of about 50 percent of those bacteria.

The research team then screened 10,000 of the resulting isolates for antimicrobial activity against MRSA – and found, among others, a new species of bacterium provisionally named *Eleftheria terrae*. Genome sequencing revealed that the bacterium was a new genus of Aquabacteria, a group of microbes not otherwise known to produce antibiotics. From *E. terrae,* researchers were able to isolate and examine a novel compound, which they named teixobactin. Teixobactin shows excellent potency against Gram-positive pathogens – including drug-resistant strains. It acts by inhibiting peptidoglycan biosynthesis in the cell wall and demonstrated exceptional activity against *Clostridium difficile, Bacillus anthracis* and MRSA.

Even when plating at low doses, the researchers were unable to obtain teixobactin-resistant mutants of either MRSA or multi-drug-resistant *Mycobacterium tuberculosis*. After testing on plated bacteria, the effectiveness of the compound against MRSA and *Streptococcus pneumoniae* was assayed in mice, where it showed good potency and efficacy with low toxicity. If teixobactin behaves similarly in human studies – and without toxic effects – it could become our next weapon against drug-resistant pathogens.

Unfortunately, because of its action against the cell wall, Gram-negative pathogens will remain safe... for now. However, with proven systems like the iChip coming into play, we may force new dark matter into the light in our fight against Gram-negative infections. *MS*

Reference

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Imaging Alzheimer's

MRI probes causative proteins a decade before disease symptoms

Alzheimer's disease currently affects an estimated one in nine people over the age of 65, a number that shoots up to one in three at 85 (1). Despite its prevalence, there is currently no method for early detection. Now, a team of researchers and engineers at Northwestern University, Illinois, USA, have developed a non-invasive magnetic resonance imaging (MRI) approach to detect Alzheimer's before patients become symptomatic (2).

"We have a new brain imaging method that can detect the toxin that leads to Alzheimer's disease," said William Klein, co-leader of the research team and the original discoverer of the amyloid beta protein that causes Alzheimer's symptoms in the brain. "Using MRI, we can see the toxins attached to neurons in the brain."

The group's technology features an MRI probe that couples a magnetic nanostructure with an antibody against amyloid beta proteins, which then show up as dark areas in MRI scans of the brain (see Figure 1). Tested intranasally on mouse models with and without Alzheimer's disease (as well as on preserved human brain tissue), the scans showed clear differentiation between control and affected brains.

Conventional technologies used to observe Alzheimer's disease pathology, such as positron emission tomography, detect plaques – abnormal clusters of protein fragments – that only occur in the latter stages of the disease. The new approach detects the amyloid beta oligomers themselves, which can appear as long as a decade before plaques begin to form. Oligomers attack the synapses of neurons, damaging them and ultimately causing neuron death. As time progresses, the oligomers accumulate, forming the amyloid plaques that current probes target. Vinayak Dravid, research team co-leader and Abraham Harris Professor of Materials Science and Engineering, says, "Noninvasive imaging by MRI of amyloid beta oligomers is a giant step forward towards diagnosis of this debilitating disease in its earliest form."

The new probe isn't just useful for

disease detection – it also conclusively establishes the molecular basis for Alzheimer's disease. There are currently no effective drugs for the disease, but now that a biomarker has been identified, it may allow researchers to design better treatments. Furthermore, Dravid suggests that the new MRI method could also be used to assess how well a new drug is working: "If a drug is effective, you would expect the amyloid beta signal to go down."

But that's not all. Preliminary evidence suggests that the MRI probe may also have a therapeutic effect because it directly binds to amyloid beta proteins in the brain. The research team observed that the behavior of mice with Alzheimer's disease improved even after receiving a single dose of the probe. If the new technology offers both early detection and potential treatment, then it could be the ultimate triumph. *MS*

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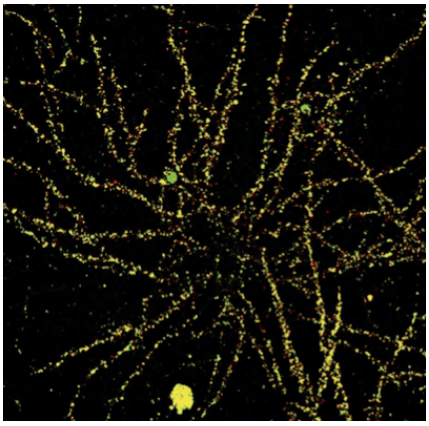
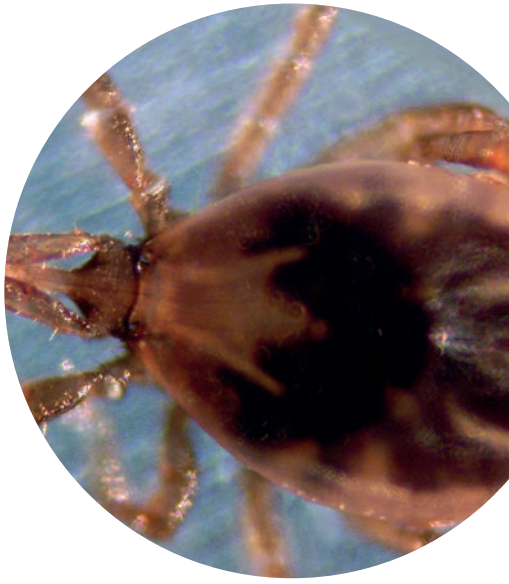


Figure 1. The antibody-conjugated MNS (red) binds to amyloid beta oligomers (green) on neurons in the hippocampus (2).

Ticking Multiple Boxes

Preclinical data for a new multivalent Lyme disease vaccine looks promising



Lyme borreliosis is the most common vector-borne infection in the northern hemisphere – and it is rapidly becoming more prevalent. Recent studies reported 300,000 Lyme disease cases in the US (1) and over 65,000 in Europe (2), despite known underreporting. And though a vaccine was developed for use in humans, it was voluntarily withdrawn from the market because of poor performance (3). There is currently no vaccine available for protection against *Borrelia* – the causative bacteria.

Previous generations of Lyme borreliosis vaccines have shown that the disease can be prevented by immune targeting of outer surface protein A (OspA), a dominant antigen of the *Borrelia* spirochete. When present in the gut of a tick, the spirochete abundantly expresses the OspA lipoprotein, which appears to act as an antibody shield for the tick while it feeds. Studies have already shown that antibodies targeting the C terminal domain of OspA are crucial for protection against Lyme borreliosis. Vaccines based only on that portion of the protein confer partial protection.

To address the ongoing need for Lyme borreliosis prevention, a research group from Valneva SE recently designed a multivalent vaccine based on the OspA protein (4). Because there are four *Borrelia* species in Europe expressing a total of six different OspA serotypes, the vaccine contains three separate proteins, each of which contains the C terminal halves of two OspA serotypes linked to form heterodimers. The researchers also introduced disulfide bonds to stabilize the protein fragments and a lipidation signal to increase immunogenicity.

The vaccine was tested in mice challenged with either infected ticks or in vitro grown spirochetes. Four to six weeks later, the researchers tested their blood using an ELISA assay against *Borrelia garinii* and extracted DNA from ear or urinary bladder tissue to attempt PCR or qPCR detection of spirochete DNA. In all cases, the vaccine introduced a significant degree of protection. Renowned vaccinologist Stanley A. Plotkin said, "These preclinical data are an encouraging step towards a vaccine that is badly needed" – and if the new vaccine performs as well in clinical trials as it has to date, the future of Lyme disease prevention looks promising. *MS*

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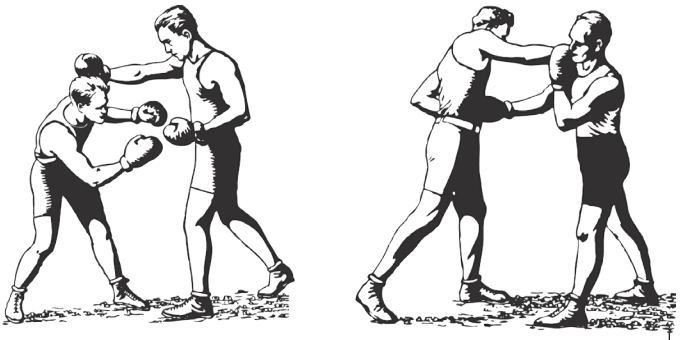
THE GREAT PROSTATE DEBATE

NEIL BELL RICHARD J. ABLIN VS. AXEL HEIDENREICH STUART EDMONDS

ALSO FEATURING **LEONARD BOKHORST**

Prostate-specific antigen (PSA) assay: essential part of prostate cancer diagnosis or public menace? We fire up the discussion with five expert views on the controversial PSA test.

By Roisin McGuigan



The prostate-specific antigen (PSA) assay has been around for over 30 years, but ever since its approval by the FDA in 1986 for the surveillance of prostate cancer (PrCa), it has created confusion, disagreement and division amongst the healthcare community in terms of its potential to screen for the disease.

Recently, the Canadian Task Force on Preventative Health Care updated their guidelines to advise against PSA screening. This wasn't without controversy – many still feel that although PSA test results must be interpreted with caution, it's still the best option available to identify patients who require biopsy for a more definitive PrCa diagnosis. But what does the evidence say?

Recent data released by the European Randomised Study of Screening for Prostate Cancer (ERSPC) (1) – the largest ever PrCa screening study (13 years and more than 180,000 male participants from eight countries) – led its investigators to come down on the anti-screening side of the debate. Nevertheless, the study did demonstrate a 21 percent reduction in PrCa mortality in men who undergo screening compared with those who don't. That figure rises to 27 percent when selection bias due to non-participation is accounted for. However, the investigators believe that the potential downsides of screening still outweigh the benefits, estimating that roughly 40 percent of men were over-diagnosed by screening.

On the other side of the debate, many remain in favor of PSA screening in certain situations, including the American and Canadian Urological Associations and the charity group Prostate Cancer Canada. The counterargument is that PSA testing is effective if used carefully, and that there is no alternative for early PrCa diagnosis.

So, the big question: is PSA screening an essential diagnostic tool or a dangerously misused assay?

Reference

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LIFESAVER OR HEALTHCARE NIGHTMARE?

The man who discovered prostate-specific antigen speaks out on the test's misuse and the potential harm caused.

How did you discover prostate-specific antigen (PSA) and have you been surprised by its impact?

In the late 1960s, I was looking for a cancer-specific marker for prostate cancer (PrCa), but what I found instead was PSA – an antigen specific to the prostate, not cancer. Did I anticipate the huge impact it would have on the medical community? In a word, no. I never thought it would be used for screening; the only use I could see, because of its specificity for the prostate, was to monitor men who had already been diagnosed and treated. The re-appearance of PSA could suggest positive surgical margins following prostatectomy, a recurrence of the disease following other treatment, and/or the presence of micrometastasis not identified at initial diagnosis. So at that time, I continued my search for an antigen specific to PrCa.

How was the PSA test developed?

Approximately ten years after my discovery, Beckman Coulter (formerly Hybritech) developed the first validated blood test for PSA levels. At first, it was only used as I had envisioned – for monitoring of patients after treatment for PrCa, and it was approved by the FDA for this purpose in 1986. But more and more people started using the test off label to screen for PrCa. It was like a tsunami. In 1994, the FDA approved the test in concert with a digital rectal examination (DRE) for screening, i.e., the diagnosis of PrCa in asymptomatic men. On the

basis of the submitted data, I believe the FDA made a terrible mistake – one that has resulted in the over-diagnosis and over-treatment of millions of men with their attendant morbidities.

Why are you such a vocal opponent of PSA screening?
There are four main arguments (or cruxes) against the test:

- *It's not cancer-specific*
  PSA is specific to the prostate, not to PrCa. Changes in PSA level could be caused by benign prostate hyperplasia (BPH) or prostatitis, not just PrCa.
- *There is no diagnostic level*
  The level of PSA defined as 'elevated' is arbitrary. One man could have a level of 11 ng/ml and be cancer free, while another could have a level of 0.5 ng/ml and have PrCa. Consequently, the false positive rate when using PSA can be as high as 80 percent; to use a test this inaccurate on asymptomatic men is tantamount to criminal.
- *It cannot distinguish between indolent (non-aggressive) and aggressive PrCa*
  The two types of PrCa can be likened to a "turtle" and a "rabbit": symbolizing indolent and aggressive PrCa, respectively. If, by analogy, you have them both in a box without a lid, the "turtle" would crawl around the box and go nowhere, whereas the "rabbit" may at any time jump out, meaning that the cancer will spread. Some cancers are turtles allowing a patient to live for years without treatment. The level of PSA cannot tell you which type of cancer you're dealing with.
- *Prostate cancer is an age-related disease*
  By the time men reach 70 years of age, as many as 80 percent will have PrCa. In many cases the cancer will be a "turtle". Therefore, a PSA-prompted biopsy may or may not (related to the age of the individual) find cancer – but if it does, is it a "turtle or a "rabbit" – and that's something the PSA test cannot tell you this, so millions of men are being over-diagnosed and over-treated.

What are the consequences of over-treatment and over-diagnosis?
The results of the inappropriate treatment of an indolent PrCa can be devastating for patients. Incontinence and impotence are two common side-effects. And let's not forget the potential psychological aspect. Loss of bladder control can be life altering and debilitating; erectile dysfunction can have a profound effect on wellbeing and may negatively affect relationships. Supporters of screening argue that despite these risks it is still better to be alive – but some men's lifespans after treatment may be identical to those who weren't screened. These men are receiving unnecessary treatment that is leaving them maimed for the rest of their lives.

"I first started searching for a PrCa marker over 45 years ago and, to this day, I'm still looking."

What about large, long-term studies, such as the European Randomized Study of Screening for Prostate Cancer (ERSPC), that indicate a reduction in mortality through screening?
I believe that particular study is flawed – just how deeply flawed I can't say, as the investigators won't release all of their data. Firstly, the ERSPC claimed to show a 21 percent decrease in mortality from PrCa in men who were screened versus those who weren't (1). However, many of the men who were not screened and who developed PrCa received hormonal monotherapy, which (according to some recent studies) may actually cause progression of localized disease (2). If non-screened men had then received a treatment that accelerated their cancer, it could have seriously compromised the results of the study. Another issue is that large amounts of the data were taken from the Göteborg, Sweden study (3), which showed very large reductions in PrCa mortality; including this patient data may have skewed the overall results in favor of screening. There is a strong possibility that the overall reduction in mortality is in fact a much lower number.

Why has screening been so widely adopted in some countries given its low accuracy?
In the US at least, I think there are two main reasons for this: the sway of celebrity and the almighty dollar.

By way of example in terms of the quest for the almighty dollar, in 1989, five years before the FDA approved the PSA test as a screening tool, Schering-Plough found a way to "supercharge" the market for their PSA test. They paid an advertising firm US$1.2 million to promote screening, so from the very beginning this test has been well marketed by people who stand to profit from it. In the US alone, we spend three billion dollars a year on screening asymptomatic men. In the last 20 years (since FDA approval in 1994) that's 60 billion dollars. Having the test itself isn't too costly for the patient, but it creates a domino effect, by prompting an ultrasound, a biopsy, and so on. When you tell a man he has cancer (perhaps the most feared word in any language), his first inclination is to do whatever it takes to get rid of it. Although there are many outstanding urologists who have the patient's interests at heart, they can sometimes be overshadowed by opportunists

who see this process merely as a means of making money.

The influence of our celebrity culture is another problem. For example, in recent years, the famous baseball player Reggie Jackson has been coming on the radio to tell the public that instead of buying shirts or ties for Father's Day, they should offer something meaningful: a PSA test. A point I often make is that depending on your father's age, giving him a PSA test is often synonymous with actually giving him PrCa – he may well have an indolent form (a "turtle"). In reaction, he follows up with a biopsy and suddenly an essentially healthy man believes he is at risk of dying from PrCa. I have another example: when Rudy Giuliani – New York City mayor on 9/11 – was diagnosed with PrCa (partly through PSA level assessment), he opted for radiation therapy. When the public see people they admire getting treatment and the type, it can influence their decisions. "If it's good enough for Rudy, it's good enough for me."

What other options are there for detecting and managing PrCa?

Right now, if you are an asymptomatic man with a normal DRE and no elevated risk of PrCa, I would say the best thing you can do is nothing. Patients who are at a higher risk (the two well-known examples being men with a family history of PrCa and black men) may wish to consider monitoring PSA levels over time, to spot any substantial elevation. But all this can tell you is that something may be wrong with their prostate – for example, prostatitis or BPH – and not necessarily that they have cancer.

When PrCa has already been diagnosed, watchful waiting (better known as active surveillance) is an option for men who are unsure whether to proceed with treatment, potentially risking their quality of life to treat an indolent cancer that may not need to be treated. First initiated by British urologist, Chris Parker, this involves establishing the histological characteristics of the tumor (Gleason score), PSA level and results of a DRE. The patient can then be reassessed periodically to monitor any clinical changes that may warrant further action.

As for direct replacements for PSA screening, there are several currently being developed (see What's the Alternative), but I think there is still some way to go before we have a reliable, validated test. It is noteworthy, however, that two of these prospective tests – the Prostate Health Index (PHI) and Progensa Prostate Cancer Antigen 3 (PCA3) – have been reviewed by the National Institute for Health and Care (NICE, the UK's healthcare watchdog), which has indicated that neither of the tests improve diagnosis sufficiently to be recommended for clinical practice (4).

MILESTONES IN PSA TESTING

1970 Richard J. Ablin and his team discover PSA

1980 PSA is measured quantitatively in the serum for the first time (1)

1986 FDA approves PSA for monitoring patients with PrCa following treatment

1994 FDA approves PSA in conjunction with a DRE for screening asymptomatic men for PrCa

2011 US Preventive Services Task Force recommends against PSA screening

2014 Canadian Task Force on Preventive Health Care recommends against PSA screening

At the 13 year follow up, the European Randomised Study of Screening for Prostate Cancer (ERSPC) investigators report that although screening appears to reduce mortality in a small percentage of patients, there is not sufficient evidence to justify it for widespread (population) use (2)

What do you think the future holds for PrCa diagnosis?

I first started searching for a PrCa marker over 45 years ago and to this day, I'm still looking. By training I'm an immunologist, specializing in immunopathology, and a lot of my work has focused on trying to understand why a tumor progresses to metastatic disease. I'm currently collaborating with a group at the University of Cardiff, Wales, to find a reliable PrCa marker, and while my colleagues and I don't have anything definitive enough to discuss yet, we remain hopeful.

WHAT'S THE ALTERNATIVE?

Biomarker	*Overview*	*Status*
proPSA	The [-2] isoform of proPSA (an inactive precursor of PSA) has been shown to increase in PrCa and is associated with more aggressive cancers (1)	FDA approved
Prostate Health Index	Blood test based on PSA which combines total PSA, free (unbound) PSA and the isoform p2PSA to increase accuracy (2)	FDA approved
Prostate Cancer Antigen 3 (PCA3)	A non-coding RNA expressed by the gene PCA3, shown to be overexpressed in PrCa. A commercial kit (Progensa) has been developed by the company Gen-Probe (3)	FDA approved
Oncotype Dx Prostate Cancer Assay	Gene expression assay used on needle biopsy tissue to predict aggressive cancers (4)	Clinical Laboratory Improvement Amendments (CLIAs)-based laboratory-developed test
Polaris	Used on biopsy tissue; measures the expression of 46 genes in order to provide information on cell cycle progression and therefore aggressiveness of disease (5), developed by Myriad Genetics	CLIAs-based laboratory-developed test
Prostarix	Post-digital rectal examination urine test which measures the concentration of a panel of metabolites, to identify metabolic abnormalities associated with PrCa (6) developed by Metabolon	CLIAs-based laboratory-developed test
TMPRSS2:ERG	A fusion gene present in between 40 and 80 percent of PrCas (7)	CLIAs-based laboratory-developed test
Mi-Prostate Score	A multiplex analysis of TMPRSS2:ERG gene fusion, PCA3, and serum PSA, commercially developed by University of Michigan MLabs (8)	CLIAs-based laboratory-developed test
ConfirmMDx	Epigenetic assay, utilizes gene methylation patterns to aid in identifying PrCa following negative biopsy (MDxHealth) (9)	CLIAs-based laboratory-developed test
Prostate Core Mitotic Test	Mitochondrial DNA deletion assay for use on biopsy tissue (10, 11)	CLIAs-based laboratory-developed test
4k Score	Four prostate-specific kallikrein assays to increase accurate prediction of aggressive cancer, developed by OPKO Inc. (12)	Potential biomarker
ProMark	Multiplex immunofluorescence in situ imaging of biopsy tissue to measure protein biomarker levels, to distinguish latent and aggressive disease, developed by Metamark Genetics (13)	Potential biomarker

"As for the PSA test, I believe it will continue to lose favor as more organizations start to appreciate the drawbacks of screening and change their stance on it."

Ideally, the replacement for PSA screening would: i) be more reliable – we would have assurance that a normal test indicates absence of cancer, and ii) reduce mortality, without significant morbidity following treatment for an abnormal test.

As for the PSA test, I believe it will continue to lose favor as more organizations start to appreciate the drawbacks of screening and change their stance on it. That said, I suspect its use will continue for some time. There will always be people who believe something (however ineffective) is better than nothing – or who have a financial interest in PSA. Until we do have an alternative, it is of critical importance that doctors, patients and the public in general understand the severe limitations of this test – and the potentially harmful consequences.

Richard J. Ablin is a professor of pathology at the University of Arizona College of Medicine, The Arizona Cancer Center and The BIO5 Institute, Tucson, AZ, and the author of "The Great Prostate Hoax: How Big Medicine Hijacked the PSA Test and Caused a Public Health Disaster".

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PSA SCREENING SAVES LIVES

Early detection, aided by screening, can help more men survive prostate cancer

STUART EDMONDS

Recent guidelines released by the Canadian Task Force on Preventive Health Care, which recommend against prostate-specific antigen (PSA) screening, do a disservice to both the general population and to health care providers. When developing their recommendations, the Task Force did not take into account the views of people living and working with the disease on a day-to-day basis, and I do not believe these recommendations reflect the reality of prostate cancer (PrCa) screening and diagnosis. Prostate Cancer Canada (PCC) is concerned that this new recommendation will negatively impact the doctor-patient relationship, and will result in men failing to be informed about a test that could directly impact their chances of surviving PrCa. Further, men simply don't need another reason to put off taking the test, or seeing their doctor in general.

We know that early detection saves lives. The alternative to testing is a loss of the excellent survival rate early detection gives us. We also know that men diagnosed at a later stage are more likely to die of PrCa as late detection of this disease may potentially limit treatment options and diminish survival. While the PSA test isn't perfect, elevated levels are currently our best indicator that something may be wrong with the prostate. This is especially necessary for a disease like PrCa, in which symptoms may only present themselves once the disease has progressed significantly.

PCC still recommends that men be screened using the PSA test because we believe the benefits of screening outweigh the negatives. The PSA test is a necessary entry point into an important diagnostic pathway. This simple blood test, combined with other risk factors, is an important resource for doctors to detect PrCa early and then monitor and treat as is appropriate for that individual.

"The PSA test is a necessary entry point into an important diagnostic pathway. This simple blood test, combined with other risk factors, is an important resource for doctors to detect PrCa early and then monitor and treat as is appropriate for that individual."

The negatives of over-treatment (a problem often cited by critics of PSA screening) can be mitigated using active surveillance. Active Surveillance is an evidence-based approach that involves closely monitoring low-risk PrCa and aims to improve quality of life by reducing or delaying radical treatment until absolutely necessary. In Canada, active surveillance has become a standard option for the management of PrCa and is often the treatment of choice for men with low risk disease. Eliminating screening using the PSA test would make this method impossible.

Men should have an informed discussion with their health care provider to understand the pros and cons of screening, and they should know that a positive PSA test does not imply a need for immediate treatment. PCC believes in "smart screening", a personalized approach where men are encouraged to be tested to establish a baseline PSA in their 40s, with follow up based on individuals' risk profile.

Not everyone who is diagnosed with PrCa will require treatment, but anyone who does need treatment must first be diagnosed. PSA screening, when used appropriately, remains an essential tool to detect prostate cancer in its earliest stages when it is curable. Until a proven alternative becomes available, we will continue to advise doctors and patients of the continuing relevance of the PSA test as a screening tool.

Stuart Edmonds is the Vice President of Research, Health Promotion and Survivorship at Prostate Cancer Canada, a national foundation dedicated to the elimination of the disease through research, education, support and awareness.

RISKS ECLIPSE BENEFITS

The Canadian Task Force on Preventative Health Care recommends against screening. But changing the opinions of clinicians and patients is no easy task.

When it comes to prostate-specific antigen (PSA) testing, Canada has a lot in common with other countries – it is a widely used screening method for prostate cancer (PrCa), and most men in the right age range are likely to have had their PSA levels measured at least once. Most urologist associations and regional guidelines encourage screening, and many doctors and patients believe it is the best way to detect PrCa early. As part of the Canadian Task Force on Preventative Health Care (CTFPHC), my colleagues and I reviewed the effectiveness of PSA screening, and we have now recommended against it. However, opinions of the test vary greatly and it still plays a large role in PrCa detection, despite the lack of supporting evidence.

Based on what I believe was an extremely rigorous review of the current literature (including the European Randomized Study of Screening for Prostate Cancer (1) and the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial) using GRADE methodology (a systematic review system which is becoming an international standard (2)) we concluded that there is no strong proof that screening will significantly reduce mortality – we found only a 0.1 percent absolute risk reduction in PrCa mortality). It appears that the potential problems (for example, overtreatment) eclipse the small potential benefit (see infographic) (3).

We therefore recommend against screening men in all age groups, with a strong recommendation against screening for men under 55 and over 70, and a weak recommendation for those 55–69 years old, which essentially means that men in this age range may wish to consider their own preferences with regards to screening. The recommendations against screening also apply to men at higher risk (for example, those with a family history of PrCa). However, we do suggest that clinicians may wish to discuss the pros and cons of testing with high-risk patients.

Despite finding little evidence to support the use of PSA testing, the task force recommendations don't reflect current practices in Canada, and as an independent organization funded by the government, they are not necessarily approved or followed. Many urologists we have spoken to – as well as the patient advocacy group Prostate Cancer Canada – do not agree with our findings, and have concerns about our study. Screening is a subject which still causes a lot of division within the healthcare community, both in Canada and around the world.

"The controversy surrounding PSA means that we are not likely to see widespread changes in the near future."

The controversy surrounding PSA means that we are not likely to see widespread changes in the near future; but despite the resistance we are encountering, time will tell. Three years ago, the US preventative task force published a similar guideline, which generated a considerable amount of dispute in the literature – but since then, there has been a decrease in PSA screening.

I believe there is likely to be a similar reaction to our recommendations. Attitudes will begin to change – slowly. Many people still associate screening programs with a reduction in PrCa mortality, but our results show this reduction is very small. Moreover, countries without such screening programs, such as the UK, have also seen PrCa mortality drop, which indicates that other factors, such as improved therapies, are influencing survival.

Screening can often do more harm than good, and patients and clinicians alike must weigh the benefits of screening against the potential health risks; this is a medical debate I expect to continue for some time to come.

Neil Bell is a member of the Canadian Task Force on Preventative Health Care, a family physician and a Professor of the Department of Family Medicine, University of Alberta.

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IF 1,000 MEN* ARE SCREENED USING THE PSA ASSAY...

1000
MEN SCREENED

102
MEN WILL DIAGNOSED WITH POSITIVE CANCER

33

Out of the 102 men diagnosed with prostate cancer (PrCa), 33 would not have experienced symptoms. Most will choose to go ahead with treatment due to uncertainty over the severity of their cancer, and may experience side-effects.

5

Five men will die of PrCa despite taking part in PSA screening.

1

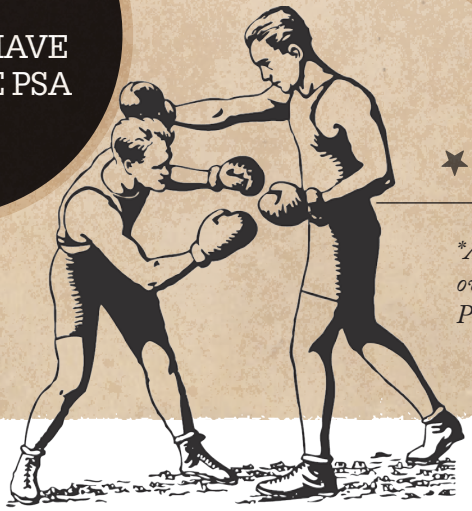
One man who would have died from PrCa will survive due to PSA screening.

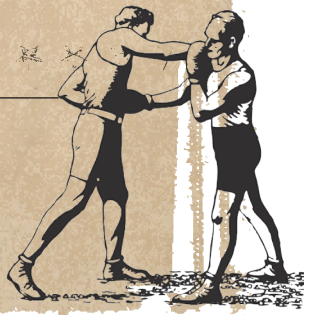
178
MEN WILL HAVE A POSITIVE PSA TEST, BUT PRCA WILL NOT BE DIAGNOSED DURING FOLLOW-UP SCREENING

4

Four of these 178 men will experience biopsy complications, including infection and severe bleeding.

720
MEN WILL HAVE A NEGATIVE PSA TEST





**Aged 55 – 69 years and screened over a 13 year period. Abnormal PSA threshold of 3.0 ng/ml*

Information provided by the Canadian Task Force on Preventive Health Care, based on data taken from FH Schroder et al., "Screening and prostate cancer mortality: results of the European randomized study of screening for prostate cancer (ERSPC) at 13 years follow-up", Lancet, 384, 2027–2035 (2014). PMID: 25108889.

INTELLIGENT AND INDIVIDUALIZED

PSA is still a valuable tool – it is our approach that needs to change.

AXEL HEIDENREICH

As a urologist and former chairman of the Prostate Cancer Guideline Working Group of the European Association of Urology (EAU), I've been involved in creating and updating the European guidelines for the use of PSA for the testing and screening of prostate cancer (PrCa). I believe the key to success with PSA is to use it in the right way – PSA monitoring and subsequent treatment must be individualized, risk-based and, above all, intelligent.

In Germany, we don't perform mass PSA screening due to the lack of convincing data and, simply because the tests are not reimbursed by insurance companies. Having said that, many urologists in private practice here believe that early detection of PrCa can be achieved with annual PSA assessments – a practice that's not based on scientific evidence. The most recent German guidelines for the diagnosis and treatment of PrCa are to inform men at the age of 45 years (40 years if there is a family history) with a life expectancy of at least 10 years that PSA is an option, although it does have potential consequences. If the patient chooses to undergo PSA testing, the timing of follow-up examinations will depend on the serum PSA concentration and may vary between one year (PSA > 2.5 ng/ml) and four years (PSA < 1.0 ng/ml).

The EAU guidelines differ from the German ones; they are formed by an interdisciplinary group consisting of urologists, medical oncologists, radiation oncologists, pathologists and an advisory panel of radiologists and physicians from nuclear medicine. They are frequently updated and take into consideration all new published trials on diagnosis and treatment. The EAU does not recommend PSA mass screening, but does recommend baseline PSA serum concentration assessment in men under 50 years. Depending on this initial measurement, follow-up should be every 1–2 years (PSA >1.0 ng/ml) or 4–6 years (< 1.0 ng/ml) (1).

"Although PSA is useful, there is always room for improvement. Risk calculators have been validated but are rarely used."

Despite the EAU's guidelines being based on the most up-to-date evidence available, a recent survey which analyzed PSA and diagnostic work-up practices in a cohort of ~600 men, revealed compliance levels of only 35–45 percent. Such statistics show that we have a lot of work to do when it comes to raising awareness and educating healthcare providers – be it through symposia, meetings or publications such as this. When used correctly, I believe that PSA and free total PSA ratio are currently the best routine options for daily practice.

Although PSA is useful, there is always room for improvement. Risk calculators have been validated but are rarely used. For example, the Prostate Cancer Prevention Trial (PCPT) and the European Randomised Study of Screening for Prostate Cancer (ERSPC) calculators combine PSA level with other parameters, such as age, family history, and digital rectal examination results, to give a modified risk score. Such resources are currently underutilized, and I think they could significantly decrease the number of unnecessary prostate biopsies currently performed based on PSA assessment alone.

In the future, we may see entirely different approaches, such as new serum or urinary biomarkers, which may further improve diagnosis. One example is TMPRSS2-ERG, a fusion gene present in 40–80 percent of PrCa. However, it is still unclear how findings like this could be incorporated into early detection methods. Until new approaches are validated and proven, I expect PSA to remain the standard.

Axel Heidenreich is the director and chairman of the Department of Urology at the University of Aachen and the Certified EURO Prostate Cancer Centre, Germany. He has also served as chairman of the Prostate Cancer Guideline Working Group of the European Association of Urology until 2013.

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CUTTING PROSTATE CANCER DEATHS

Regular PSA tests could reduce prostate cancer mortality by as much as 51 percent – but methods must improve before mass screening can be used safely.

A study of the Rotterdam arm of the European Randomised Study of Screening for Prostate Cancer (ERSPC) found that screening can significantly reduce deaths from prostate cancer (PrCa) – if the screening protocol is strictly followed.

"In the Netherlands, there is no official screening program – if a man wants a PSA test, he needs to consult his doctor. However, Data from the Dutch central agency for statistics shows that in 2011, 24 percent of men over 40 had taken a test in the last five years for screening purposes, demonstrating the test's popularity" explains Leonard Bokhorst, amedical researcher at the Erasmus Medical Center, the Netherlands.

Leonard and his colleagues conducted an analysis of the Dutch section of the ERSPC, with some surprising results. They studied a total of 34,833 men in the Dutch arm, who received either regular PSA screening, or no screening at all. "We found that the effects of screening can be diluted by nonattendance (seen in around 5 percent of participants) and contamination – that is to say, men also seeking tests from their own doctors, which approximately 20 percent of participants did," he explains, "correcting for these factors meant that the risk of death from prostate cancer for those who took part in screening was reduced by up to 51 percent, as compared to no screening at all(1)."

This finding resulted from using the following screening protocol:

- screening started in the age range of 55–69 years
- a four year testing interval was used until the age of 75
- a biopsy was taken if PSA level is abnormal (in this case, a level of higher than 3.0 ng/ml)

"Without following a strict screening protocol, the reduction in mortality may be lower, and the potential for undesirable side effects, such as over-diagnosis, may be greater," says Bokhorst.

So based on these results, did Bokhorst and his colleagues conclude that PSA screening is effective? "PSA is currently the only proven tool for reducing PrCa death through early detection, but the risk of negative side effects means that screening for PrCa with PSA alone is currently not recommended. The key to better PrCa screening is to improve the balance of harm versus benefit. Supplementary tools and information, such as including risk calculators, are extremely important; doctors should start making use of these complementary methods to better predict PrCa risk. The use of MRI also looks like a promising technique to detect men at increased risk of especially aggressive PrCa. Such approaches can reduce the amount of unnecessary biopsies and reduce over-diagnosis of low risk PrCa."

"The key to better PrCa screening is to improve the balance of harm versus benefit. Supplementary tools and information are extremely important."

If screening methods are improved, suggests Bokhorst, they may become a viable option for the detection of PrCa – but only in well-informed patients who are aware of the negative side effects. Until then, mass screening programs are simply not a sensible option.

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Join the debate

It appears that the jury is still out on PSA testing – what do you think? Send your comments to the editor (fedra.pavlou@texerepublishing.com) or get in touch with us online at www.thepathologist.com or on Twitter @pathologistmag.

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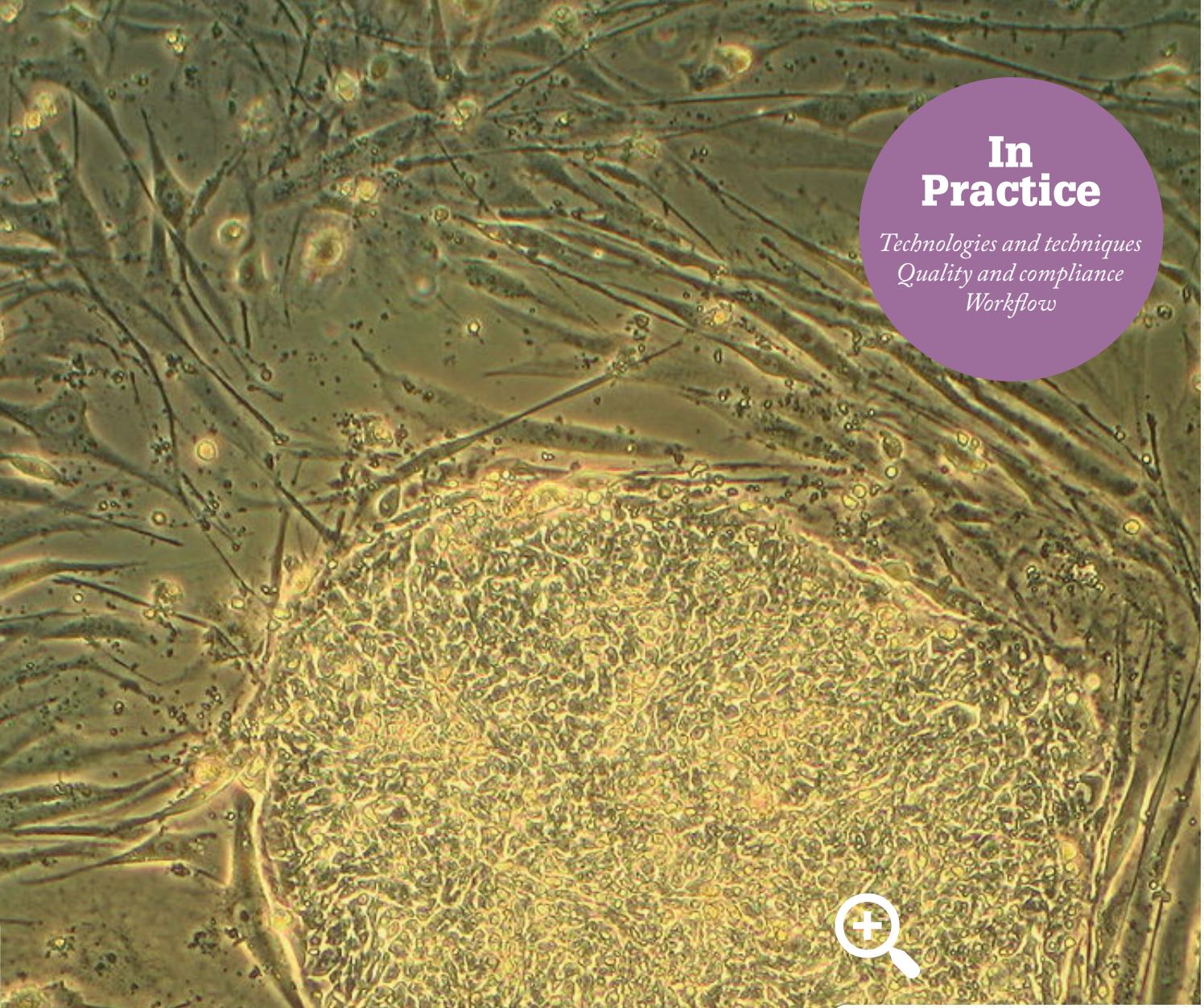

In Practice

Technologies and techniques
Quality and compliance
Workflow

Reshaping Recommendations

Making clinical practice guidelines work for pathologists

By Janet E. Simons and Andrew C. Don-Wauchope

Most clinical and laboratory medical professionals are aware that there's considerable interdependence in their areas of expertise – for instance, in disease diagnosis, screening, prognosis and treatment. Clinicians use clinical practice guidelines (CPGs) to provide the best possible care to their patients, but it isn't only clinical practitioners who encounter these guidelines in their work; pathologists, too, must consider the recommendations made. Though some are authored by specialty pathology societies, there are many others that contain recommendations that directly impact pathologists. Many CPGs that include recommendations for lab-based testing are written by clinicians with minimal or no input from pathologists.

But CPGs are not the final word in medicine. The evidence that supports laboratory tests is often different to the evidence supporting other areas of medical practice – and this is one of the main challenges CPGs encounter in making recommendations that relate to pathology (1). To make these guidelines work for everyone, it's important for pathologists not only to have insight into how CPGs are developed and how they should be evaluated by the people who are actually using them, but also to get involved in pulling them together.

Getting started with standards

Because of the huge volume of CPGs out there and the wide variety of ways to report and assess evidence, many pathologists trying to unravel the details have found themselves frustrated. One way of evaluating CPGs is to measure them against the standards (see Supplementary Table 1 online) published by the Institute of Medicine (IOM) (2). Though many national organizations and specialist societies have developed their own handbooks for writing CPGs (3, 4), some of those handbooks have been criticized for not meeting IOM standards (5, 6). As the IOM's suggestions become more widely adopted and are incorporated into more agencies' procedures, it is hoped that CPGs will become more consistent and easier to apply.

The first step to good guideline development is posing a relevant clinical question. It's a delicate balance – the question needs to be specific enough to allow a focused review of the evidence, but also broad enough to allow application in a variety of clinical settings. A good example is the United Kingdom's National Institute for Health and Care Excellence (NICE) diagnostic guideline for genetic testing in adults with locally advanced or metastatic non-small cell lung cancer (NSCLC). It sets out to "identify which test and test strategies for EGFR-TK mutation testing in adults with previously untreated, locally advanced or metastatic NSCLC are clinically and cost effective for informing first-line treatment decisions…" (7). It's a useful guideline because it evaluates all of the possible testing strategies and only then makes recommendations for use in the defined populations.

Evaluating evidence

Clinical evaluations are classified as case reports, case series, retrospective or prospective observational cohorts, case-control studies, cross-sectional studies, and non-randomized or randomized clinical trials. Any of these can be used to support medical recommendations, but there is a perceived hierarchy in levels of evidence, as shown in Figure 1, which describes the hierarchy of levels developed by the Oxford Centre for Evidence-Based Medicine (CEBM) and the 6S model.

The lowest level of evidence is mechanistic reasoning, which rarely applies to laboratory medicine. For diagnostic tests, there are typically very few randomized controlled trials, so clinical researchers most often rely on cross-sectional studies to provide evidence. The 6S model for applying evidence to clinical practice extends the levels of evidence beyond systematic review to include even higher levels: synopses of synthesis, such as the Database of Abstracts of Reviews of Effects (DARE) ; summaries, such as evidence-based practice guidelines; and systems, such as computerized decision support systems (8). The 6S model requires CPGs to be evidence-based, rather than opinion-based, and considers the role of well-conducted systematic review essential to guideline development. The IOM standards, too, require systematic review – but it can be challenging, though not impossible, to accomplish, especially in the context of pathology.

Many of the organizations commissioning CPGs have developed their own procedures to help with interpreting the evidence used to make recommendations. These procedures are usually based on similar principles,

At a Glance

- *Many clinical practice guidelines (CPGs) are written with minimal input from pathologists*
- *Adhering to these guidelines can be difficult because the level of evidence supporting laboratory tests often differs from evidence in other areas of medical practice*
- *CPGs must not only be carefully written, but also rigorously evaluated to ensure that they're meaningful*
- *In order to best make use of CPGs, pathologists should involve themselves in guideline creation and assessment*

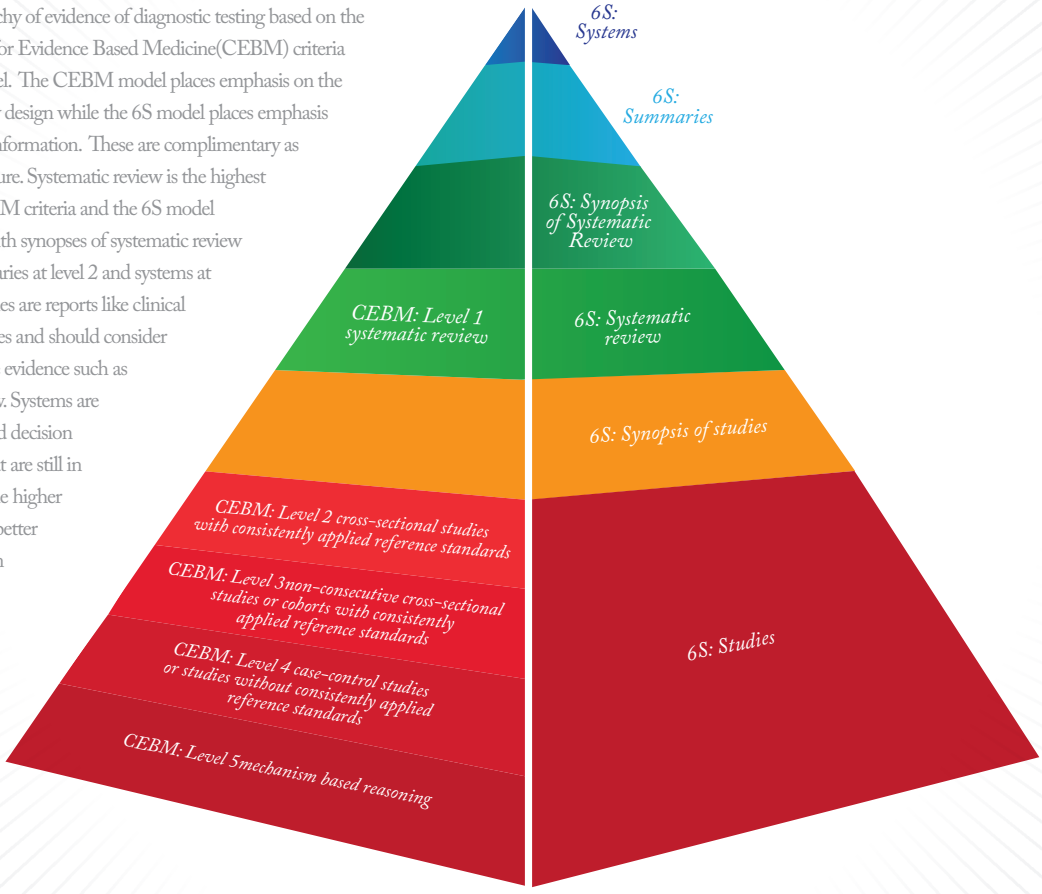

Figure 1. Hierarchy of evidence of diagnostic testing based on the Oxford Centre for Evidence Based Medicine(CEBM) criteria and the 6S model. The CEBM model places emphasis on the underlying study design while the 6S model places emphasis on synthesised information. These are complimentary as shown in the figure. Systematic review is the highest level in the CEBM criteria and the 6S model continues this with synopses of systematic review at level 3, summaries at level 2 and systems at level 1. Summaries are reports like clinical practice guidelines and should consider the best available evidence such as systematic review. Systems are the computerized decision support tools that are still in development. The higher up the scale the better the confidence in the evidence.

"The first step to good guideline development is posing a relevant clinical question."

but often they aren't consistent between organizations. The Grading of Recommendations Assessment, Development and Evaluation (GRADE) project strives to standardize the way CPG recommendations are evaluated. Initiated in 2007, GRADE is seeing increasing adoption, but it still isn't the only system in use, particularly as there remains some debate about its appropriateness for diagnostic tests (1, 9). For instance, one common recommendation is the use of the B-type natriuretic peptide (BNP) test to exclude heart failure in a primary care setting; it's ranked differently in different CPGs (see Table 1).

The GRADE system aims to evaluate risk of bias, inconsistency, indirectness, imprecision, and publication bias. The best means of achieving these goals is a systematic review that addresses the clinical question posed by the guideline; if good review technique is followed, the review team should be able to evaluate most of the GRADE components and develop an overall impression of the strength of the evidence – which can be reported as high, moderate, low, or very low (see Table 2). For example, a CPG relating to high blood triglyceride levels reads, "The Task Force recommends basing the diagnosis of hypertriglyceridemia on fasting triglyceride levels and not on non-fasting triglyceride levels (1|+++o)" (10). Tools like data collection tables can be used to estimate precision and consistency, whereas others like the Quality Assessment of Diagnostic Accuracy

Guideline	*Year*	*Recommendation*	*Grade of evidence*
American College of Cardiology Foundation; American Heart Association	2013	In ambulatory patients with dyspnea, BNP is useful in diagnosis of heart failure, especially in the setting of clinical uncertainty.	Class I, Grade A
		BNP is useful to support clinical judgment in the early diagnosis of acutely decompensated heart failure.	Class I, Grade A
National Heart Foundation of Australia and Cardiac Society of Australia and New Zealand	2011	BNP may improve diagnostic accuracy in patients presenting with new-onset dyspnea.	Grade B
European Society of Cardiology	2012	Consider using NPs to rule out heart failure.	Class IIa, Level C
Canadian Cardiovascular Society	2012	Use NPs in settings of intermediate pre-test probability for acute heart failure to rule in or rule out.	Strong Recommendation, Moderate Quality Evidence (1|+++o)
		Use NPs to rule in or rule out a diagnosis of chronic heart failure.	Strong Recommendation, High Quality Evidence (1|++++)

Table 1. Heart failure clinical practice guidelines (CPGs): recommendations for B-type natriuretic peptides in the diagnosis of heart failure. Evidence can be reported as high (++++), moderate (+++o), low (++oo), or very low (+ooo).

"Hopefully, a better understanding of CPG development and appraisal will encourage more pathologists to contribute to the process."

Studies (QUADAS-2) can be used to evaluate risk of bias and directness (11).

With regard to the BNP test, we've recently applied the Agency for Healthcare Research and Quality (AHRQ) grading process to the questions set for systematic reviews of BNP in heart failure (12, 13). We used the AHRQ process for both diagnostic and prognostic questions, and were able to achieve most of the principles set out by the GRADE project. We found that, for diagnostic tests, the types of evidence available usually result in deficiencies in bias and directness, which can become even more challenging when factoring in screening, prognosis, and treatment monitoring.

Considering CPG quality

The Appraisal of Guidelines for Research and Evaluation II (AGREE II) instrument assesses the quality of CPGs to help with developing new guidelines as well as with reporting their recommendations (14). Already validated for laboratory tests (5), AGREE II takes into account the things that need to be considered when preparing or presenting CPGs – especially the domain covering the rigor of development, which has historically been the most challenging to address. The AGREE II domains (available online at www.agreetrust.org along with the tool itself) include:

Question	*Diagnostic measure*	*Risk of bias*	*Inconsistency*	*Indirectness*	*Imprecision*	*Publication bias*	*Strength of evidence*
Use of BNPs for the diagnosis of heart failure in the emergency department	Sensitivity	Low	Consistent	Direct	Imprecise	N/A	For both BNP and NT-proBNP: High or ++++
	Specificity	Low	Inconsistent	Direct	Imprecise	N/A	For BNP: High or ++++ For NT-proBNP: Moderate or +++o
Diagnostic performance of BNPs for the diagnosis of heart failure in primary care	Sensitivity	Low	Consistent	Direct	Imprecise	No evidence	High or ++++
	Specificity	Low	Inconsistent	Direct	Imprecise	No evidence	Moderate or +++o

Table 2. Grading of evidence for the diagnostic use of B-type natriuretic peptides in heart failure.

- Domain 1: Scope and Purpose
- Domain 2: Stakeholder Involvement
- Domain 3: Rigor of Development
- Domain 4: Clarity of Presentation
- Domain 5: Applicability
- Domain 6: Editorial Independence

The AGREE II tool works well for CPGs, but to specifically look at considerations for medical tests in CPGs, a European working group has suggested a comprehensive checklist of items to consider (15). This includes important factors in the post-analytical phase, such as reference intervals, cutoff values and turnaround time. Additional web-based resources for creating and evaluating guidelines are available (see Table 3).

Only after a full evaluation can we begin implementing CPGs in our practices. Complete evaluations should include an examination of the clinical question posed by the guideline, the population encompassed by the question, the intervention being applied, and the expected outcome. If all of these factors match a specific practice, then the guideline can be considered and adapted for that practice's use – but even with all of these checks, there's still no way of knowing how stringent the CPGs development was. That's where the appraisal tools come in – to provide an idea of the rigor behind the development of a given guideline. To be sure that a guideline is appropriate for the laboratory as well as the clinic, we need to ensure that it's been evaluated, that the evaluation was rigorous enough, and that it included enough specifics of laboratory medicine to be meaningful.

Hopefully, a better understanding of CPG development and appraisal will encourage more pathologists to contribute to the process. Lab medicine is an important component of CPGs, and the methods and evidence used in the laboratory don't always look like those found in other areas of specialization. If we want to ensure that these guidelines are just as useful for pathologists as for clinical practitioners, we need to get involved in the process of creating and assessing them, so that they are as helpful as possible for the laboratory professionals who use them.

Topic	*Description*	*URL*
Some sources of guidelines	National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines	http://bit.ly/1C6L30B
	The College of American Pathologists Practice Guidelines	http://bit.ly/1C6L30B
	Cancer Care Ontario practice guidelines	http://bit.ly/1w9u7Sk
	National Institute for Health and Care Excellence	http://bit.ly/1tZ4lG0
	The National Guideline Clearing House	http://www.guideline.gov
Examples of tools	Oxford Centre for Evidence-Based Medicine	http://bit.ly/14odImc
	6S Hierarchy of Evidence-Based Resources	http://bit.ly/1B9CVy6
	AGREE II	http://www.agreetrust.org
Examples of source of evidence summaries	Database of Abstracts of Reviews of Effects (DARE)	http://bit.ly/1sp8GBq
	MacPLUS Federated Search	http://bit.ly/1sp8GBq

Table 3. A selection of web-based resources (not comprehensive) for writing and assessing CPGs.

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Supplementary table is available online.

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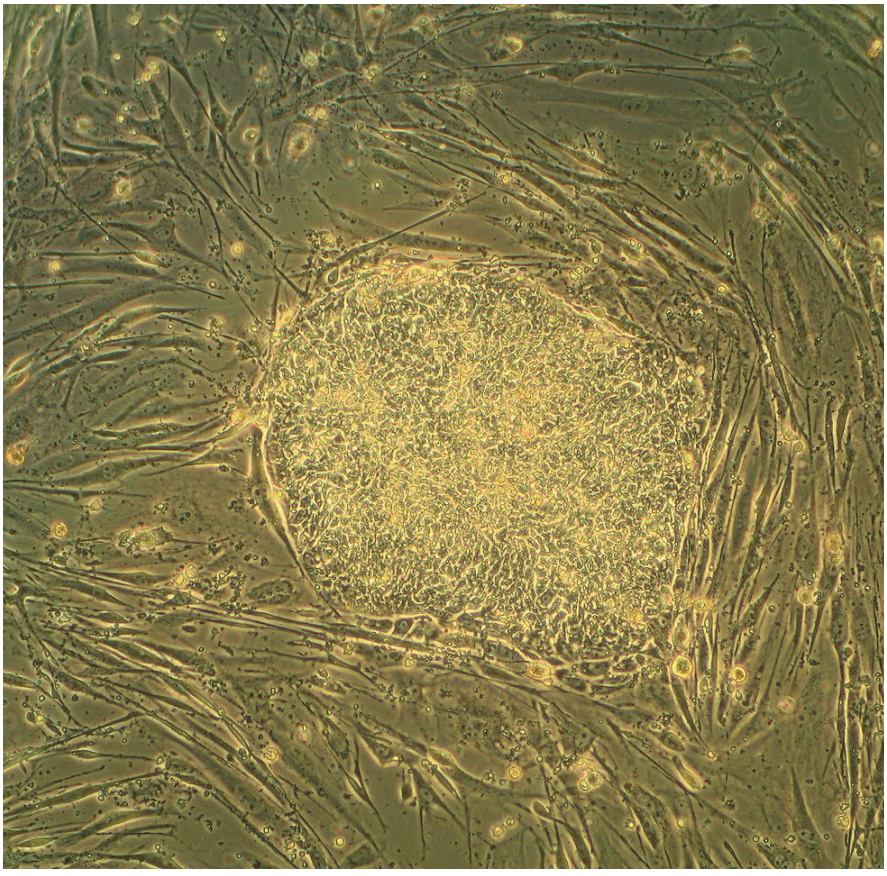
The Devil is in the Detail

How to optimize the preclinical pathology applied to cellular therapeutics

By Julia Baker



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The development of stem cell-derived cellular therapeutics has long been dogged by controversy. Cell-based medicines have been hailed by some as the panacea of all ills, while others have seemingly declared them the root of all evil. These therapeutic agents fall under the umbrella of regenerative medicine because they are intended to restore or replace the structure and function of damaged organs, thereby curing previously untreatable injury or disease. In recent years methods have been developed that allow the harvest of stem cells from embryos without causing embryonic death, and for the induction of pluripotency in adult cell lines. These developments have rendered such therapies more "palatable" to the public, and today, both the scientific literature and popular press feature stories of success – and also of failure – with growing regularity.

Having acted as a study pathologist on several preclinical programs designed to evaluate the safety of cell-derived therapeutic agents, and having witnessed their potential efficacy, I can certainly share some of the excitement. However, my experience has also made me keenly aware of the massive cost (both financial and in terms of time) that can be incurred in the absence of a carefully crafted pathology protocol.

The regulatory pathway for conventional drugs and small molecules is clearly defined, with specified species and time requirements. In contrast, the requirements for preclinical safety and efficacy studies for cellular therapeutic agents tend to be much more fluid, with each study being considered on a case-by-case basis by the US Food and Drug Administration's (FDA) Center for Biologics Evaluation and Research (CBER). Rather than having access to a predefined list of species choice and study type, companies can, and should, not only take advantage of pre-Investigational New Drug (IND) meetings, but also pre-pre-IND discussions with the agency to develop a customized protocol that provides scientifically sound data without breaking the bank.

A unique challenge facing this type of study is the fact that we are administering one living system (the cell

At a Glance

- *Recent developments have rendered the rapidly developing field of cell-based therapies more palatable to the public*
- *The regulatory requirements for preclinical safety and efficacy studies for cellular therapeutic agents tend to be much more fluid than for conventional drugs and small molecules*
- *Pathologists need to be able to demonstrate the presence of the cells in the tissues of the host animal, show the continued viability of those cells, and confirm that the cells are differentiating into the intended cell type*
- *Careful selection of biomarkers and animal models, together with tissue triage and DNA technologies can help you design a high-quality study at lower cost*

line) into another (the animal model). The viability of the administered cells may be threatened by immune responses in the host, or by the dosing apparatus. The cells themselves are very sensitive to their microenvironment, and may assume a very different morphology from that in culture when they are placed in their intended location, or transported to other parts of the body. It's therefore vital that pathologists demonstrate the presence of the cells in the tissues of the host animal during the study, show the continued viability of those cells, and confirm that the cells are differentiating into the intended cell type.

Know your targets

For each of these tasks, it is critical that we develop reliable biomarkers to confidently identify the administered cells, even if they take on a different morphology in differing microenvironments. Biomarkers may take the form of immunohistochemical (IHC) stains, antigenic determinants for use in quantitative polymerase chain reaction (qPCR) or in situ hybridization (ISH), or targeted growth factors produced as a result of endogenous stem and progenitor cell reprogramming.

The use of immunocompromised animals increases the range of available reagents for IHC and results in less off-target, non-specific, or background staining. While naturally immunocompromised strains of mice are readily available, their small size may render them unsuitable depending on the site of administration, size of dosing apparatus, or nature of the therapeutic agent. Large animal species may be better suited to your study and can be immunosuppressed. Immunocompromise was previously thought essential in ensuring the continued viability of the cells, but experience suggests that this is less critical than previously believed.

IHC markers – to confirm the cell of origin – may target the nucleus or nuclear matrix, the mitochondrial proteins, or cell-surface markers. There is no "one size fits all" answer here; a carefully developed method is an essential component of the early development program. The viability of administered cells can be proven by demonstrating continued (but controlled) mitotic activity among the cell population; Ki67 or PCNA may be successfully used in this scenario. Markers used to assess cell differentiation will depend on the target cell type; it can be challenging to develop a staining method that is species-specific for the administered cells with antibodies compatible with the research animal species.

"The ability to demonstrate co-localization of staining can be a great advantage when presenting your proposed markers to the FDA."

I would recommend considering the use of the same antigenic determinants to target cell detection by qPCR. This can be invaluable as a highly sensitive screen for biodistribution of the cells. Relying on 5 µm sections and conventional IHC to scan a liver for a few migrating cells, for example, is rather like searching for a needle in a haystack. qPCR allows for a much larger volume of tissue to be examined. If qPCR is negative on the non-target tissues 28 days after cell administration, it is not unreasonable to store the tissue samples but drop the assay from the intermediate time points – a repeat assay can be performed at the final time point. Should any wandering cells be found at this time, the stored tissues are still available.

Pigment problems

Many of the more successful recent cell therapy programs have centered on ocular conditions. But the retinal pigment epithelium (RPE) can be a trying anatomical feature for the pathologist! The species-specific size and character of melanin granules should allow distinction between RPE cells and therapeutic cells on H&E-stained sections even if the cell line is pigmented, but such close scrutiny will prolong interpretation. Even with a non-pigmented cell line, confusion is likely when interpreting IHC sections; DAB precipitate is a similar hue to melanin, and bleaching the sections is likely to impact the sensitivity of the subsequent IHC stain. Immunofluorescence (IF) might be the answer (were it not for the fact that melanin shows autofluorescence!). Have you read a large GLP-study with IF? The only way to keep a permanent record of the staining is to photograph every section. An albino strain may just save your sanity.

Of course, IF can play a very important role during method development. The ability to demonstrate co-localization of staining can be a great advantage when presenting your proposed markers to the FDA. Having proved the robust and specific nature of your various markers in small-scale pilot studies using IF, you can justify using the same markers as conventional IHC stains on adjacent serial sections. This will give stable stained slides for archiving and negate the need to record each section photographically. Experience in our lab suggests that

evaluating a study by IF takes three times as long as conventional IHC.

Model selection

When studies include an efficacy component, an animal model of the disease condition is required. Consider the suitability of your animal model carefully. For example, the commonly used mouse model for retinal degeneration is the rd1 mouse.

Homozygous mice have lost all photoreceptors by weaning – how do you ensure that the presence of administered cells is unlikely to be detrimental to adjacent surviving photoreceptors in a clinic situation? Be sure that you can predict the course of the condition in all animals: if the condition presents only in homozygous individuals, those animals must be identified prior to group allocation to avoid the awkward situation of having all the diseased animals in control (or treated) groups. In the case of retinal degeneration, the Royal College of Surgeons (RCS) rat may be a better model than the rd1 mouse, having a very predictable course of retinal degeneration.

Tissue triage

Because many of the therapies work on the premise that the cell line will continue to be viable in the patient for many years – or even a lifetime – a core tenet of preclinical study design for cell-based therapies is that all tissue should be preserved to allow for further scrutiny if problems arise further down the line. Tissues can be stored as wet tissues, in block, or on slides. To allow reconstruction of tissues, all sections cut should be appropriately labeled and retained. Unfortunately, this can create a logistical nightmare; serial 5 µm sections cut through a 1 mm block of tissue produce 200 sections. Although we may only stain 10 percent of the slides, the unstained slides must be stored for possible later evaluation; being uncovered, these sections must be stored in slotted slide boxes to protect the tissue. A 1 mm sample now occupies the space of two slide boxes.

So how can we control the number of slides? Either we need to reduce the number of tissues examined, the number of blocks processed, or the number of slides that we cut.

Firstly let's consider the tissue list. These studies do not require examination of a full tissue list. Obviously you need to examine the site of implantation, the drainage lymph node, and any directly connected tissues (e.g. the brain has a direct connection to the eye via the optic nerve). You should also examine a variety of well-perfused organs (liver, kidney, spleen, lung, gonads). At a minimum you need to examine a single level of these off-target tissues to look for any evidence of toxicity, and to screen them for the presence of cells. If the cells remain at their site of implantation, or have only a transient lifespan in the host (operating through endogenous stem cells or progenitor cells), they are less likely to cause lesions at distant sites. Abnormal masses must also be examined to check for tumorigenicity. These could be screened using qPCR, but it may be more efficient to add them to the administration site IHC staining run for their time point. IHC is preferred for the site of administration, demonstrating the exact pattern of cell integration. Serial sections should be sequentially numbered. A predetermined pattern of slide selection identifies sections for H&E staining. The "best" level for the demonstration of the cells can then be selected by the pathologist (with a default level used if no cells are seen). Sections to either side of that level are then identified for IHC staining. Larger volume sites of administration could be trimmed into several portions and embedded in a single block, allowing a greater area to be examined on one slide, and the bulk of the tissue to be archived in the block.

Not all animals are equal

Consider treating control groups differently to cell-dosed animals. If no cells have been injected into a control group, there is no point doing an exhaustive tissue search to look for the presence of cells. You just need sufficient tissue to show both the background staining pattern – H&E and markers – and the background lesions if using a disease model. The remaining tissue from these animals can be stored wet, or taken to block without sectioning.

Does your cell line need a scaffold?

Several therapies are administered in the form of a sheet of cells on a matrix, adding another dimension to method development. The scaffold is essentially a medical device, and must be shown to be made of a biocompatible material. Ideally a scaffold may biodegrade after the cells have implanted but fragments may remain in situ, and it is unlikely that all traces of your scaffold will have disappeared from the tissues prior to the first time point; sections will include scaffold material. How does the material react with fixatives or histology processing reagents? Is the embedding medium sufficiently dense to avoid tearing of tissue sections by the scaffold fragments? These considerations should be thoroughly investigated prior to embarking on a large-scale preclinical study.

To summarize, I would say that examining the appropriate tissues at the conclusion of each time point is necessary to reap the full benefit of this "triaged" system. And this, together with DNA technologies, carefully selecting biomarkers and animal models, while creating more work in the lab and for the pathologist, could provide significant cost savings over the course of a study.

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Webinars

Virtual Events

Elucidating Tumor Heterogeneity in Prostate Cancer by Combined IHC & Novel RNA ISH

Event Overview:

Assessment of molecular heterogeneity in tumor is a challenging task. For the first time, Palanisamy et al. have demonstrated the existence of a rare subset of prostate cancer with heterogeneous molecular aberrations involving ETS family genes and SPINK1 expression utilizing both standard immunohistochemistry (IHC) and RNA in situ hybridization (Advanced Cell Diagnostics RNAscope® Technology). The presenter will discuss the application of RNA ISH technology to reveal hitherto unidentified molecular subtypes of prostate cancer.

Learning Objectives of Webinar

1. Strategies for interrogating interpatient and intratumor heterogenity
2. RNA biomarker analysis of fusion genes, non-coding genes and pseudo genes
3. Applications of RNAscope ISH along with other methods such as IHC to characterize molecular profiles of tumors

Feb 24, 2015, 8am Pacific, 11am Eastern US time
http://tp.txp.to/0115/ACD/webreg

About Us:

Advanced Cell Diagnostics is a provider of RNAscope® Technology, the most sensitive in situ hybridization technology commercially available. Based on ACD's unique patented probe design strategy which enables simultaneous signal amplification and background noise suppression, RNAscope technology represents one of the most significant advances in ISH technology in over 40 years. In 2014, applications of this new technology appeared in 83 peer-reviewed publications.

ACD
Advanced Cell Diagnostics

Speaker
Nallasivam Palanisamy, PhD
Associate Scientist

My research interests are on the discovery and characterization of gene fusions in cancer and understanding their role in carcinogenesis from a translational research perspective. Using genomic technologies such as high-density array comparative genomic hybridization, advanced molecular cytogenetic technologies including FISH, CGH, spectral karyotyping, gene expression microarrays, and next generation sequencing technology, my laboratory investigates the transcriptional and genomic architectures of solid cancer genomes. In-depth analysis of genomic amplifications provided an unprecedented view and identified rare gene fusions formed at the boundaries of amplification and deletions. I also pioneered the application of next generation sequencing technology for the discovery of new recurrent gene fusions in cancer.

NextGen

Research advances
New technologies
Future practice

Benchmarking PSA

What does analysis of the last five years of literature on prostate specific antigen tell us about the priorities of the field and the major contributors to it?

By Mark Hillen

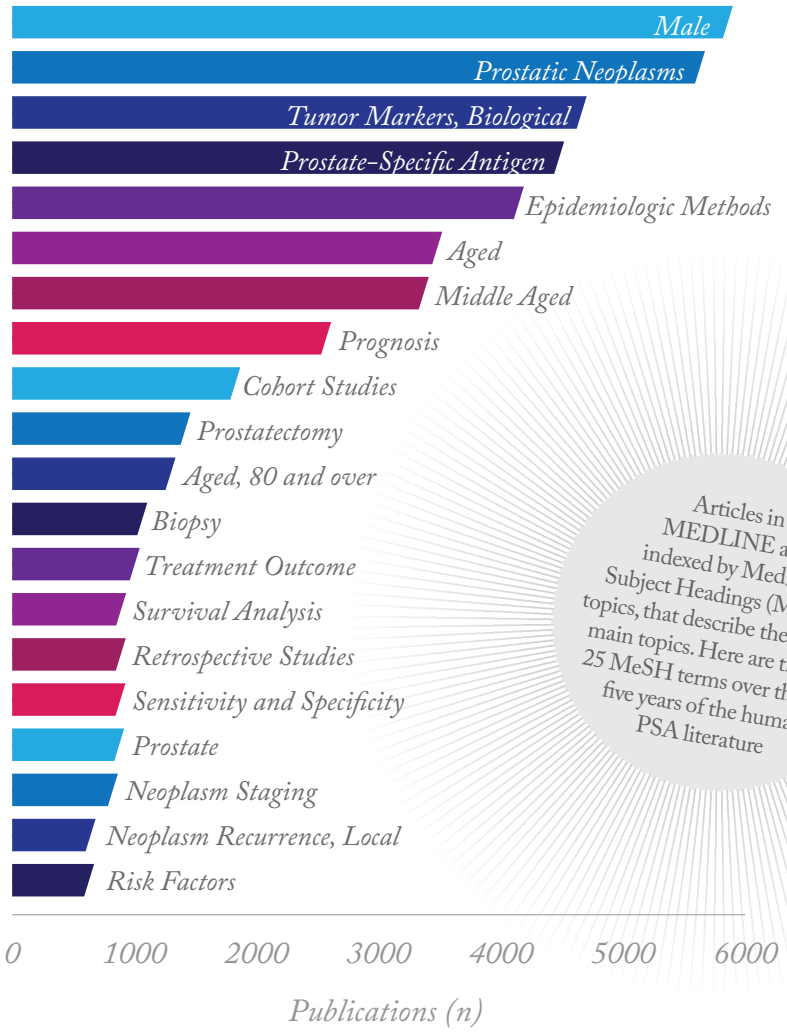
Prostate-specific antigen (PSA) is a glycoprotein enzyme secreted by the epithelial cells of the prostate gland. Its main role is to liquefy semen in the seminal coagulum, thus allowing sperm to swim freely. Small amounts are also present in the serum of men with healthy prostates. Crucially, many prostate disorders elevate serum PSA levels, one of which is prostate cancer. Our cover feature draws attention to the big debate over the value of PSA screening for the detection of prostate cancer.

To provide insight into what research has been performed on this topic, a series of metrics were applied to the last five years of the published literature. We asked:

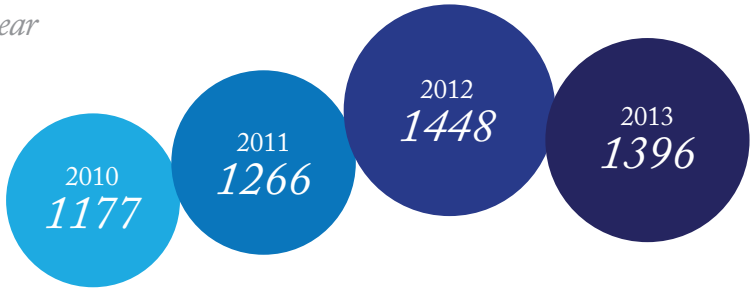
- What are the major topics for the field?
- Which publications have the greatest impact?
- How is the knowledge available online?
- Who are the most prolific authors?

PubMed, was searched for prostate cancer AND "prostate specific antigen" with results limited to the last five years, in humans (for a clinical focus). The data were analyzed in Microsoft Excel 2013.

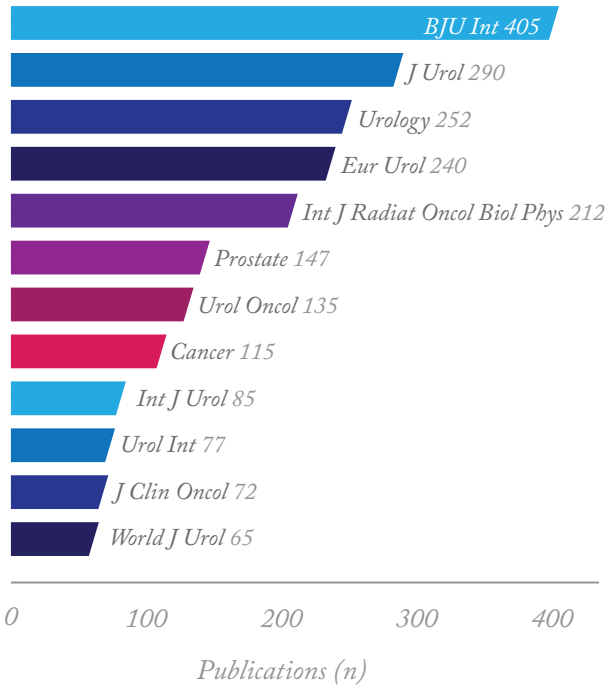
Most frequent topics on PubMed



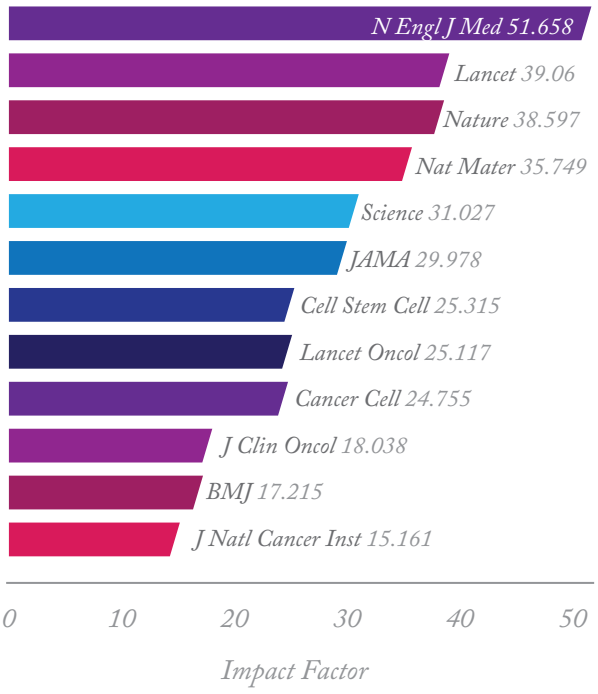
Publications per year



Top 12 journals (by number of publications)



Top 12 journals by Impact Factor



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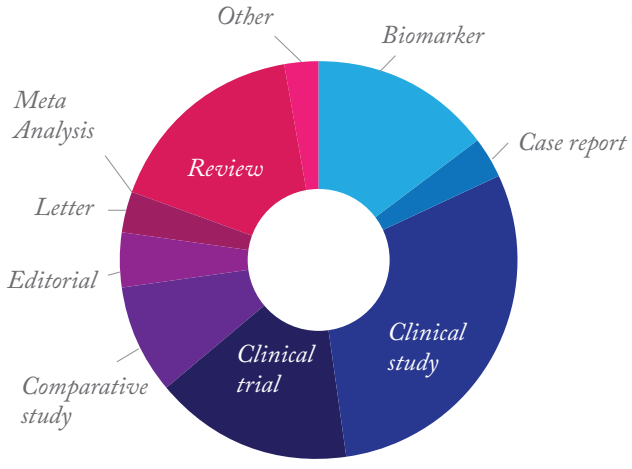

Articles are categorized according to PubMed criteria. Clinical study represents a clinical evaluation of a drug, device or technique that was not a clinical trial.

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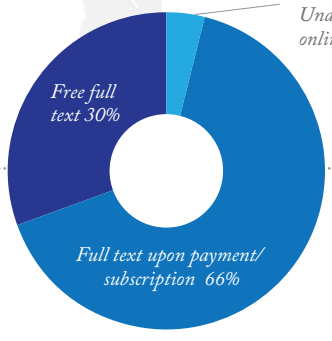
Fee or free?



A Vision of Our Mobile Future

How smartphone power, coupled with the scale of its adoption globally, offers a compelling platform for diagnostics – and a chance to level the playing field for researchers in resource-poor countries

By Aydogan Ozcan

Smartphones represent an enormous opportunity for the creation of field-portable, compact and cost-effective instrumentation of the type that you would normally find only in laboratories or hospitals. Such applications have the potential to tackle the lack of diagnostic capability in certain parts of the world or in field settings. The absence of such services is not just because advanced instruments are very expensive; beyond the initial expense, there is also the requirement for solid infrastructure that, in developing countries, is often lacking.

At a Glance

- *The cost of smartphone technology is rapidly decreasing, but the technological capabilities are increasing at an equally high rate*
- *A smartphone that fits in your pocket and costs under $500 now has some of the capabilities of full-sized laboratory instruments*
- *Smartphones are inherently connected, meaning that data gathered on mobile platforms can be accessed from anywhere in the world at a moment's notice*
- *Smartphone advances, particularly in fields like microscopy, will benefit pathologists – especially those working in developing countries or with limited resources*

Big numbers and big challenges

No one could have predicted the current status of mobile telecommunications 10 or 15 years ago. The numbers are simply staggering: more than 15 billion cell phones have been sold and there are currently seven billion cell phone subscribers worldwide, more than 75 percent of whom are in developing countries – despite a lack of basic infrastructure or even roads in some cases. In such countries, cell phones are the most advanced technology that you will encounter; phone towers, communication networks, and mobile power stations for charging cell phones appear to have found their way into every corner of the globe.

Cell phones are extremely cost-effective. The sheer economy of scale and fight for market share have driven unprecedented strides in technological advancement and capability at amazingly low cost. Let me illustrate exactly how cheap this technology has become: if you were to somehow magically remove three zeros from either the number of cell phones sold or the number of subscribers (that is to say, replace billion with million), the cell phone in your pocket would cost you the same amount as a high-end car.

The megapixel count of cell phones has been doubling every two years for the last 10 years (from 0.2 to more than 40 megapixels). So if, like me, you're a researcher who's interested in developing portable high-end microscopes, the constant improvement in cell phone performance offers regular opportunities to push for more and more functionality. We can now routinely see single viruses, sub-100-nanometer fluorescent particles and even single DNA molecules using cell phone-based microscopes. Admittedly, these cell phones are very high end, but they have enabled us to expand the boundaries of mobile imaging, sensing and diagnostics. Virus or DNA imaging is no simple task, so it is a real milestone that proves the worth of our approach and the potential that the technology has in areas besides pathology, for instance environmental monitoring or materials science.

One of the next steps is commercialization and deployment of existing instruments and designs, and it's already happening to a degree. There are commercially available applications and hardware to convert cell phones into laboratory instruments. For example, I cofounded a company called Holomic LLC (www.holomic.com, see sidebar "Introducing Holomic") that develops devices to image and quantify lateral flow immunochromatographic assays. Such cell phone-based systems can quantify analytes at concentrations in the parts-per-million or even parts-per-billion range, depending on the test of interest.

Once this and other devices gain regulatory approval, it's not hard to imagine the rapid rise of "off-the-shelf" consumer products for a number of different applications from health monitoring to food analysis.

Ironically, one of the biggest barriers to the development of cell phone-based technologies is the rapid rate at which cell phones are evolving in terms of the hardware and software that they use. This is, of course, at the heart of the business model for providers and carriers. In diagnostic applications, however, stability is a major requirement. If we wanted to develop an application for, say, the Samsung Galaxy S4, we need to know that it would still be available in its current guise for at least the next five years. The time is required to develop, test, gain regulatory approval and market our application while users still have access to the relevant phone model. However, the Galaxy S range is likely to evolve significantly over the two years – the S5 has already been on shelves for over seven months with rumors of an upcoming S6 release – and there is no real end in sight to this marketing strategy.

There is an old saying that "every challenge is an opportunity." New ventures could take advantage by taking control of the billions of used handsets and smart devices, communicating with the industry, discovering its needs, and offering a regulated supply chain to ensure that biomedical device manufacturers have access to the smartphones they need. Forget recycling – how about diagnostic upcycling? In this way, used phones become the hearts and brains of new portable analytical systems rather than add-on devices being made available to consumers.

Another solution to the problem could present itself, if whispers in certain circles about phone modularization come to fruition: imagine that rather than constantly changing phones, we could simply upgrade or change modules within an endoskeleton. Google has already staked out this potentially fertile field with Project Ara (www.projectara.com) – a forum that aims to bring together module developers with that exact aim. Clearly, from a diagnostic device point of view, this is a very positive development.

"There is a nonlinear threshold beyond which machine learning becomes very powerful – something that Google has taught the entire world. To breach that threshold requires progress on both the technology side and in terms of deployment"

Big data and big players

One of the reasons why mobile health tools are better than laboratory-based instruments that perform the same tasks is the collection and use of data: mobile tools are inherently connected. The wireless connectivity of cell phones coupled with smart and secure servers means that rather than working with a single disconnected instrument or sensor, an entire network of instruments from all over the world can be accessed. Reference libraries would virtually self-assemble and databases would get richer and richer, enabling increasingly sophisticated analysis, such as the self-classification of images or signals, and automatic flagging of risk signatures.

There is a nonlinear threshold beyond which machine learning becomes very powerful – something that Google has taught the entire world. To breach that threshold requires progress on both the technology side and in terms of deployment. By bringing analysis to the masses at a fraction of the cost and by stabilizing the technology, the output of big data (and the analytics and machine learning that will result) will benefit not only the users, but also those who collate information for large-scale studies to discover wider patterns and trends. The new opportunities presented by such large amounts of networked analytical data and the potential size of the overall impact is hard to exactly predict right now. But perhaps a simplistic musical analogy is in the difference between only being able to access your own CD collection in the 1990s and having the ability to listen to almost any song ever recorded today…

Google, Apple, Samsung and others are all building collaborations in the medical diagnostics area and working on products and business models behind closed doors. They have the cash and the muscle to make waves, and the outcome may look like the phone market: who's winning or losing at any given time will depend on the user interface, the relevance of the data captured, the level of integration into the consumer's life, and the "coolness" factor. If you remember when the iPhone was introduced, it was a different kind of phone and a different way of interfacing with a computing device – and that lit the fuse for an explosion of innovation.

I anticipate a fragmented market, which means that we will see leapfrog advances from these giant companies driven by the desire to be the first to present the next "big thing." The next couple of decades will be a frantic struggle to be increasingly involved in the consumer's daily life and routine – to the point of monitoring the bodily fluids

"The idea that we could replace medical personnel with gadgets and algorithms is a dangerous and misleading one that goes against the fundamental and centuries-old philosophy of medicine – which is all about feeling empathy for the patient."

as well as biochemical and physical signals that we leak over the course of the day – and making insightful and actionable "sense" out of the resulting data.

Where is health care in all this?

Our technological future should not strive to replace pathologists or other medical experts. Rather, it should improve their performance by providing better, faster diagnostics and more in-depth patient data. Mobile diagnostics will simply bring in new complementary tools for the medicine of the future, driving us closer towards preventative health care.

The idea that we could replace medical personnel with gadgets and algorithms is a dangerous and misleading one that goes against the fundamental and centuries-old philosophy of medicine, which is all about "feeling empathy for the patient". We have to be very aware of our continued need for the human touch. I certainly don't want to live in a world where we replace doctors and other healthcare providers entirely with robots, no matter how advanced artificial intelligence and machine learning becomes. But surely such a view is not in conflict with the fact that health care delivery can be significantly improved with technology and new instruments that significantly assist professionals with their medical practice.

Certainly, regulatory agencies will be strict with new diagnostic devices. Where there have been attempts to skirt around the rules, the US Food and Drug Administration (FDA) has been quick to point out the requirements. I don't think the FDA is going to fight against change, rather they will continue to set and monitor appropriate safety and performance standards. In the case of Holomic's platform, which is actually a diagnostic reader for many kinds of tests, the approval process for the cell phone-based system as a whole will be much shorter because similar (non-cell phone-based) systems already exist, which allows us to go down the 510(k) route of proving equivalent performance to a validated bench-top instrument.

Getting on board

The automation of signal reading is a no-brainer; it makes tests more robust, improving accuracy, sensitivity and repeatability. The cell phone provides everything needed for automated reading of a signal or image: an advanced camera for imaging, powerful processing capabilities for computational tasks, and a high-resolution screen to display data, all within a compact package. Even though not every application will make full use of all these abilities of the cell phone, anyone interested in developing field-portable devices who fails to utilize these advantages will quickly fall behind. Or at the very least they will find it extremely costly to improve specifications at the same rate as cell phone technology, which is simply not sustainable in the long run: how can a small biotech company compete with Samsung or Apple on those terms? Instead of competing with emerging consumer devices, we must accept them and leverage their power for our own applications.

Using the power of consumer electronics to bring the advanced functions normally found in a hospital or laboratory into field settings empowers applications in a whole range of areas, from environmental monitoring to material science to health care in developing countries. It also helps build research capacity in developing countries. Insufficient infrastructure or funding can make it impossible to buy and maintain expensive laboratory instrumentation or perform some research; however, the innovation landscape generated by the coupling of consumer electronics with diagnostic tools changes the dynamic. Through democratization of measurement toolsets using mobile phones and other ubiquitous and cost-effective devices and interfaces, researchers in developing countries will be capable of generating high-quality scientific output, matching that of their colleagues in developed countries. Not only that, but mobile analytics will also have a big impact on the democratization of science in general. Right now, the research world is highly polarized in terms of output; there is a close correlation between a country's GDP and the number of significant papers published.

In education, the same holds true. But the recycling of cell phones or their components to make innovative, high-end analytical devices will boost science and engineering education. Hands-on education experience is very important, especially for science, technology and engineering fields; it enables skills in solving problems, the testing of hypotheses, and prompts students to ask the right questions. In developing countries,

where even basic instrumentation is lacking, education suffers. And, in fact, it's unlikely, even in developed countries, that we would happily use a US$50,000–$100,000 microscope to show a kid what a HIV virus looks like – but now we can use a phone that costs less than $500 to do the same thing. That's a game changer.

The term "citizen science" is a little fuzzy – but it certainly hints at another facet of the current direction of innovation. Acquisition of high-quality data from large numbers of cell phones or other consumer electronics devices all over the world will enable us to discover patterns and trends that would be impossible to find otherwise.

What's coming up for pathologists?

There are a number of recent smartphone applications that specifically serve the needs of the pathologist. For instance, we have developed a lens-free microscope that can be used to detect cancer or other cellular abnormalities as accurately as a full-scale optical microscope (1). It's a particularly significant milestone because it is the first lens-free on-chip microscope that can be used for high-throughput 3D tissue imaging. The scope uses a laser or a light emitting diode to illuminate a sample that has been placed into the device on a slide. A microchip sensor array (the same chip that all our camera phones and webcams use for digital image acquisition) then captures the shadow patterns cast by the sample and processes them as a series of holograms, allowing the construction of a 3D specimen image. The image is several hundred times larger than those captured by conventional microscopes and can even be color coded by algorithms, highlighting the contrasts to make abnormalities easier to detect. Through a board-certified pathologist, we have run blind tests on our device for analyzing Pap smears, tissue specimens and blood samples – with accurate diagnoses 99 percent of the time.

We have also managed to image and measure the size of individual DNA molecules with a smartphone-based fluorescence microscope of our own design (2). The device is 3D printed, so it can be produced at low cost, and it features an attachment that creates a high-contrast darkfield imaging setup, thin film interference filters, a miniature dovetail stage and a laser diode to excite fluorescently labeled DNA. After fluorescent labeling, the DNA molecules are stretched on disposable chips that fit into the attachment. The included app transmits the raw images to our server, where the lengths of the individual molecules are rapidly measured; the results are recorded both in the app and on remote computers linked to the server. Devices like these can have widespread positive impact on diagnostics and pathology practices in resource-limited areas.

To conclude, the various benefits of mobile phone-based diagnostics, for example, improved implementation of healthcare and more widespread environmental monitoring, are immediately obvious. The slow-burning transformation in the behavior of researchers and educators in resource poor countries is less obvious – but it too is almost inevitable.

Aydogan Ozcan is the Chancellor's Professor at the Departments of Electrical Engineering and Bioengineering, University of California, Los Angeles, USA , an HHMI Professor at the Howard Hughes Medical Institute (HHMI), and founder of Holomic LLC.

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Introducing Holomic

Holomic is a spin-out from UCLA that has licensed more than 15 intellectual property (IP) applications created by my lab. It has funding from the US government in the form of small business initiatives from the National Institutes of Health, NASA and the Department of Defense (Army), along with some private funding. Holomic's first product was introduced in 2011, and it is currently in the process of FDA approval.

The company's main mission is mobile microanalysis. We aim to provide the complete readout solution for all diagnostic tests available, whether colorimetric or fluorometric. We have created an imaging platform that universally accepts all diagnostic tests, automatically recognizing and reading them. This functionality enables us to work with many other companies that are developing diagnostic tests. At the same time, we also provide the server end, so that when the user creates an image and diagnostic report, we offer extra analytics and mapping of the data. Essentially, we are positioning Holomic as a digital provider of field-portable, high quality data analytics for all available clinical tests.

Holomic also has an interest in microscopy and imaging. We have created a unique field-portable microscope, which may be useful in direct discovery or in imaging microarray plates among various other specimens. We are targeting mobile health, telemedicine and the research field as a whole with these high-end computational imagers.

Profession

Your career
Your business
Your life

A Rather Unconventional Career

Industry and drug development: how opportunities for pathologists extend beyond pathology

By Mai H. Le

Early on in medical school, I had two key realizations that led me to pathology. The first was my fascination with understanding the mechanisms of disease. I often found myself focused on pathophysiological mechanisms during my clinical rotations, which wasn't the most efficient way to practice medicine. I couldn't really help it, though; it's just the way I process information and think through situations. I also participated in a "year-out" program offered by the University of Rochester's Department of Pathology, which led to my second realization: I wanted a research-oriented career. During my year-out program, I learned that laboratory medicine training was the fastest route to a career in clinical research.

Most of the people I know who started out as laboratory medicine residents ended up taking the traditional route – training in both anatomic and clinical pathology (AP and CP). I think that this was driven at least in part by the reductions in grant-funded research. Without confidence in the ability to secure research funds, you need to be sure you're widely employable. Academic positions are much fewer and farther between than general pathology positions, and general pathology, of course, requires training in both AP and CP. Those colleagues who stuck with laboratory medicine tended to specialize in hematopathology because of the demand.

I did things a little differently. After completing my three-year laboratory medicine residency at the University of California, San Francisco, I went straight into an associate medical director position at a biotech company. The first several years I spent in biotech was like fellowship training in a way, so I guess you could say I specialized in drug development.

"After completing my three-year laboratory medicine residency at the University of California, San Francisco, I went straight into an associate medical director position at a biotech company."

At a Glance

- *Industry careers offer pathologists the opportunity to be directly involved in developing new therapeutics*
- *Pathologists have a firm understanding of clinical, laboratory, and translational medicine – all key skills needed in industry*
- *The ability to understand and interpret medicine through the lens of laboratory values and data means that pathologists are uniquely suited to working with clinical investigators and overseeing the execution of clinical trials.*
- *If you're considering making the move to industry, find the program you're most interested in and go for it!*

Lured into biotech

It was the opportunity to help push forward the development of a new drug – something that could improve the prognosis for an entire population – that drew me into industry. During my residency, I was fortunate enough to meet Lori Kunkel, the Chief Medical Officer at Proteolix, a small biotech company in South San Francisco. She's a hematologist/oncologist by training, and had moved into drug development. She told me about her time at Genentech and the key role she played in developing Rituxan. When I completed my residency, I was offered a position with Proteolix. Here I was, with the chance to help bring a new therapy to patients with refractory multiple myeloma. I'd reviewed some of the data demonstrating that the drug worked, and I spoke to some of the clinical investigators on studies whose patients went from being hospice-bound to getting their lives back. How could I not be attracted?

I started as an associate medical director and the primary medical monitor for a Phase II trial treating multiple myeloma with carfilzomib, a second-generation proteasome inhibitor. I was also the medical backup for this pivotal trial that was the basis for accelerated approval. I supervised the day-to-day activities of the trials and was the primary medical contact for the clinical investigators. It was also my job to review the data being generated from all of the sites, looking for any adverse events that might herald a trend. And because of my background in pathology, I was involved with a lot of the preclinical and translational research activities too.

In laboratory medicine, we're trained to

understand what's going on with patients based on lab values, progress notes and conversations with our clinical colleagues. We provide our knowledge and support as consultants to the primary medical team and work with them to understand and interpret the laboratory data in the context of a specific patient. The relationship between a medical monitor and the investigators on a clinical trial is fairly similar; we work together and use our different, but complementary, knowledge and experience to evaluate the data, both at the individual patient level and for the entire clinical trial cohort. Pathologists have the additional advantage of understanding laboratory test development; that's critical in the age of companion diagnostics.

I've been really lucky in terms of projects. I was spoiled with my first program, carfilzomib. It gave me a lot of experience in managing critical Phase II trials, as well as in launching the global Phase III trials needed for full registration. Plus, it culminated in carfilzomib's accelerated approval in 2012. I then moved to the biotech company Plexxikon Inc., where I led clinical programs for a small molecule tyrosine kinase inhibitor called PLX3397 and designed the initial proof-of-concept trials for several other inhibitors. I've also had the opportunity to launch the first set of clinical trials for a very interesting small molecule inhibitor of glutaminase, which is thought to be a key player in tumor metabolism. Now, as the Chief Medical Officer at OncoSec Medical, I'm working with a talented team of businessmen and women, scientists and engineers to develop intratumoral immunotherapies.

Since entering biotech, my role in drug development has evolved. I started out managing the day-to-day execution of clinical trials, medical monitoring, and data analysis and interpretation; now I'm the central person responsible for devising the clinical and regulatory strategy for a candidate therapy and managing diverse groups, including clinical operations, data management and clinical science. But don't get me wrong – being in a small company setting, I'm still

"In laboratory medicine, we're trained to understand what's going on with patients based on lab values, progress notes and conversations with our clinical colleagues."

heavily involved in the details of running the clinical trials.

The biggest thing I've experienced in biotech – something I couldn't have experienced if I'd stayed in laboratory medicine – is the incredible satisfaction of helping to bring new therapies to patients with cancer. It's a great feeling to know that my work is directly contributing to making treatments that may improve survival for an entire population of patients.

Making the move

I think the biggest hurdle to getting pathologists into biotech and pharma is that so few of them are aware of the available opportunities. Before spending time at Proteolix, I had no idea these jobs even existed.

But pathologists bring a very different skill set to the table – one that's very important to understanding the mechanisms by which novel therapeutics can impact the course of a given disease. Pathologists' knowledge of pathophysiology and what diseases look like under the microscope and in laboratory assays is invaluable. We can provide a context for evaluating the findings in preclinical toxicology animal studies and have a key role in the evaluation of arguably the most critical marker of a new drug's potential success – target engagement and pharmacodynamic activity. Pathologists have a very natural role in industry – bridging the gap between the industry's research laboratories and the clinical investigators.

I think that the key challenges for pathologists trying to move into industry, outside of diagnostics, include the lack of exposure to opportunities and the fact that industry professionals don't always understand our knowledge base. Often, clinical opportunities in industry are aimed at those who have trained in internal medicine or, in the context of cancer therapeutics, hematology/oncology. Pathologists are often overlooked, I think, because people don't realize that our medical knowledge extends beyond what we do at the microscope or in the clinical lab. Hiring managers don't necessarily realize that our knowledge base essentially spans all of medicine because we serve as consultants to all clinicians. This is not to say that we're experts in every field, but that isn't really what industry needs. Industry needs people who have a broad understanding of medicine and diagnostic technologies and who know when and how to work with their clinical colleagues to answer specific questions.

Succeeding in industry certainly takes a person who isn't afraid to tackle issues they've never previously managed. You have to be prepared to learn on the fly and make decisions based on very imperfect or partial information. But these challenges are not unique to anyone who has been trained in medicine and they can be overcome. Don't be afraid to ask for opinions. Ask your colleagues; ask your clinical collaborators; ask for advice from people whose intellect you respect, even if it isn't in their area of expertise. They may have great ideas.

Finding opportunities in industry is about actively seeking out the people working on your program of interest. Find out what research is going on at your institution and try to get involved; make an effort to speak to people at scientific or medical conferences. Don't let an advertised job description discourage you from seeking out an opportunity you want, either; the descriptions tend to be generic and very few of them will be explicitly for pathologists. If you find out about an opportunity that interests you, talk to people and figure out ways to speak to the hiring manager for that position. Finally, never be shy about contacting someone directly.

A world of opportunity

Pathology training provides a firm foundation for understanding clinical, laboratory, and translational medicine. These are all key skills needed in the drug development industry.

Consider that the knowledge base of someone with pathology training is very broadly applicable. Don't limit yourself to positions that are specifically posted for pathologists; find the program you're most interested in and go for it!

Don't be afraid to reach out to people directly. Most hiring managers are very open to people with alternative training or expertise. They're primarily interested in someone who is motivated, intellectually creative, scientifically critical and, for these positions, medically competent.

Do I have any professional regrets? Nope – none at all.

Mai H. Le is Chief Medical Officer at OncoSec Medical, Inc., San Diego, California, USA.

The RNA Revolution

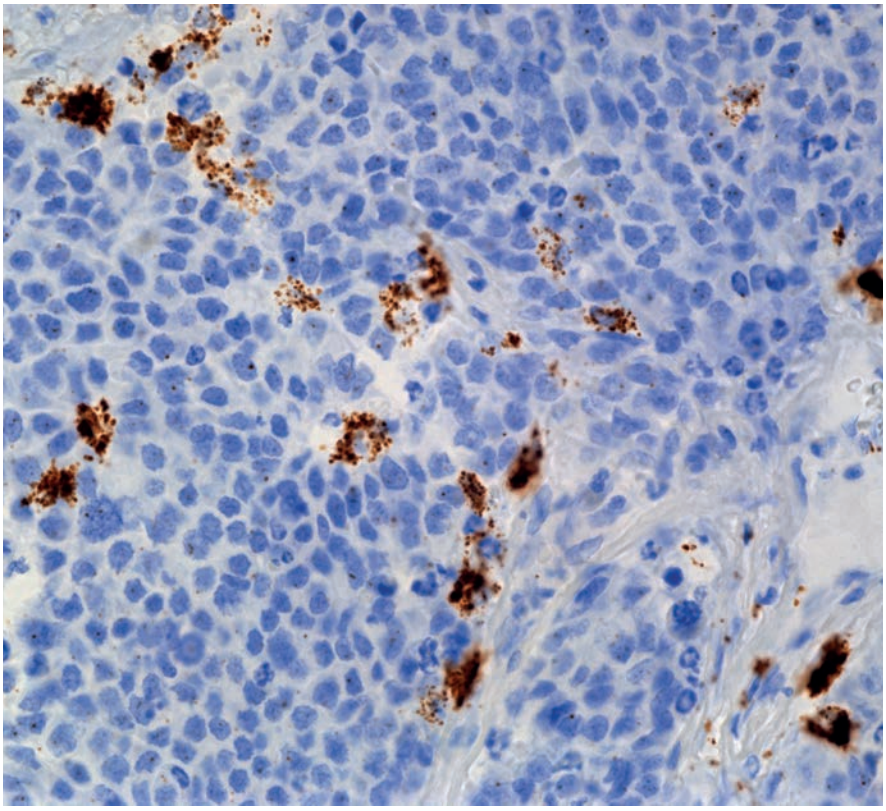
A guide to RNA as a biomarker and its detection

Gene expression profiling yields many insights into the disease state, particularly in discovering those molecular indicators known as biomarkers. Indeed, the widespread application of transcriptomic techniques in cancer research over recent years has proven that, like protein, RNA is a rich source of clinically valuable biomarkers for diagnosis, prognosis and predicting therapeutic response. Although such approaches may identify many potential biomarkers, translating these discoveries into the clinic for routine measurement has traditionally been hindered by established analytical technologies. While it is commonplace to detect and visualize DNA and proteins in their native context within single cells, until now the best routine measurement tools for RNA have been those that detect and quantify RNA in solution, losing all morphological context. Times are changing, however, and the 'RNA Revolution' is here.

The intriguing molecule of RNA is no longer viewed as merely the 'messenger', especially with new classes of non-coding RNAs being discovered on a regular basis that have a hand in genetic regulatory control and a wide range of cellular activities. The discovery of this "new world" of RNA has sparked an unprecedented drive towards better tools to characterize the complexity of RNA – in terms of quantity, function and spatial distribution. In particular, pinpointing the localization of specific RNAs within cells and tissue architecture is an important factor in realizing its true potential as a biomarker.

Exploring how RNA presents an ideal biomarker, especially in light of novel RNA analysis methodologies, the new whitepaper from Advanced Cell Diagnostics (ACD) will discuss:

- **The Biomarker:** What makes a valuable biomarker, and how a direct path from RNA biomarker discovery to the clinic is vital, avoiding the use of DNA or protein surrogates.
- **The Method:** A biomarker is only as good as its routine analysis methodology, but what constitutes the optimal biomarker method? Advantages and pitfalls of existing methods for routine biomarker analysis will also be discussed.
- **The Future:** How the utilization of RNA as a biomarker is achieved through the latest RNA analysis methods, such as ACD's RNAscope®.



Revealing the RNA expression signature of individual cells within the tissue architecture. The above image shows human breastcancer FFPE tissue probed for MMP9 mRNA expression using ACD's RNAscope® technology

Novel RNA analysis technologies are unlocking the potential of RNA as a clinically valuable biomarker. This new whitepaper examines the utility of RNA as a biomarker, and how this is profoundly linked to the methods now available for its validation, detection and localization.

To read the full whitepaper, please visit the website: www.acdbio.com/whitepapers
info@acdbio.com

ACD
Advanced Cell Diagnostics

Uro Star

Sitting Down With… Antonio López Beltrán, Professor at the University of Cordoba, Spain, and Director of Anatomic Pathology at the Champalimaud Cancer Center, Lisbon, Portugal.

What inspired you to specialize in uropathology?

Actually, I made that decision because of the high frequency of bladder cancer in Spain. As I was interested in developing a research-based career, I thought bladder cancer would be a good model to learn about the field of cancer in general.

What is the most groundbreaking research in your field in recent memory?

Major advances in uropathology include the definition of the urothelial carcinoma-related molecular pathways. Three primary genetic alterations are associated with the pathogenesis pathway of non-muscle invasive bladder cancer (the Ras-MAPK and PI3K-AKT pathway alterations, and mutations in upstream tyrosine kinase receptor FGFR3), all of which are responsible for promoting cell growth in urothelial neoplasia. On the other hand, muscle-invasive urothelial carcinoma primarily involves alterations in tumor suppressor genes related to cell cycle control.

Understanding the pathogenesis of kidney cancer (from *VHL* mutation to up-regulation of hypoxia-inducible factor and the consequent stimulus of neovascularization, cell proliferation, energy supply, and metastasis) clearly represents an important breakthrough. And it has modified the way we approach the pathology of renal cell carcinoma.

Major advances in pathology of prostate cancer include the discovery of the *TMPRSS2-ERG* rearrangement, which once again significantly improves our molecular understanding of the disease.

What do those advances mean for you and pathology in general?

Our new molecular understanding – together with the refinement of classic histopathology approaches to these tumors – offers a translational approach to urologic cancer, making uropathology an important discipline in uro-oncology practice and research. How have these advances modified the practice of anatomic pathology? Well, the answer is a little disappointing – no major molecular breakthrough has been incorporated in practice. I guess the integration of knowledge gained on the molecular level remains challenging, but should at least motivate uropathologists to find the right balance between classic histopathology and the molecular approach to uro-oncology practice.

What current research initiatives hold most promise – and why?

Diagnosis of bladder cancer via urine biomarker detection is really promising and could be put into practice in the coming years. And using FGFR3 tyrosine-kinase alterations to target non-muscle invasive bladder cancer is another major field of research. Finally, there are a few good examples of how the uropathology field might evolve in the future; for instance, rationalizing therapy of prostate cancer based on *TMPRSS2-ERG* rearrangement. The general consensus is that translational research will be incorporated in practice via novel tests that ultimately select the patients who can benefit from upcoming new therapies.

What about your own research?

Over the last decade, we have observed that cyclin D3 (cell cycle regulator, *CCND3*) is altered in bladder cancer and seems to be detected in aggressive tumors. We have subsequently developed models to use *CCND3*, either alone or in combination with FGFR3, as a potential predictive biomarker of response to immunotherapy in high-grade urothelial bladder cancer – an aggressive form of the disease. We have hypothesized that *CCND3* and FGFR3 protein alterations could form the basis of a urine detection test of recurrent bladder cancer after therapy. Our data, which is currently at prepublication stage, appears to match information from more conventional invasive clinical approaches, such as cystoscopy, and so holds promise.

Is any area of uropathology neglected by research?

When looking at the resources dedicated to cancer research in the USA and Europe, I notice that uropathology projects are somewhat limited compared with "popular" cancers. So I feel the whole field is somewhat neglected. This trend is particularly true with bladder cancer research funding – and that must change. For instance, an organ pathology like bladder cancer, which is considered the most expensive single solid tumor in the USA and Europe, needs more attention.

Is uropathology a growing field or struggling to attract new talent?

Uropathology is a relatively new field in practicing pathology, mainly stemming from the complexity of prostate pathology and its correlation with different therapeutic modalities. In this context, it's relatively easy to get new pathologists and residents interested. In fact, the large number and lower age of current uropathologists attending scientific activities clearly reflects an increasing interest. That's great because it will help improve research in urologic oncology and it will also support implementation of future translational tests that can modulate the therapy of urologic tumors – a flourishing area of modern uropathology.

What do you like most about your job?

I guess my diagnostics and counseling help guide proper treatment of patients with urologic disease – I really love that aspect of my job. The opportunity to teach junior pathologists from an academic perspective is also very rewarding.

I have to say that there is something special about signing out cases on the microscope – I enjoy that very much. And the fact that I learn something new, curious – or both – every day keeps me professionally happy.

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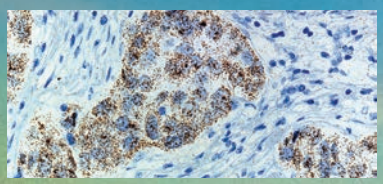

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