DECEMBER 2015 # 14

the Pathologist

Mission: Impossible

Can pancreatic defences be breached to diagnose and treat disease?

Efficiency at your fingertips

Dissect more data from every sample with the **PAXgene® Tissue System**

- Analyze both **histomorphology** and **biomolecules** from a single tissue sample
- Preserve high-quality nucleic acids and proteins for use in downstream applications
- Transport and store specimens for up to 7 days at **room temperature**
- Benefit from an integrated **formalin-free** fixation, stabilization and processing system

For research use only. Not for use in diagnostic procedures.

Maximize your efficiency today: **www.PreAnalytiX.com**

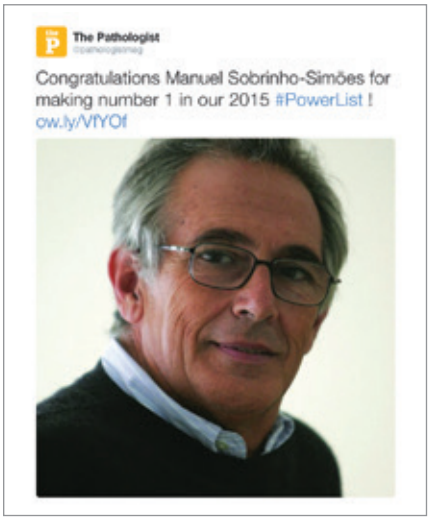
Visit our informative **Tissue Atlas** featuring stained sections of mirrored samples from Formalin-Fixed, Paraffin-Embedded (FFPE) and PAXgene Tissue Fixed, Paraffin-Embedded (PFPE) Tissue.

Explore more at **www.PreAnalytiX.com/products/tissue/tissue-atlas**

PreAnalytiX
A QIAGEN / BD Company

Online this Month

Last Month's Top Tweets
Follow us @pathologistmag



Assoc for Mol Path @AMPath
Congratulations to the multiple @AMPath members on @pathologistmag 2015 #PowerList! *http://ow.ly/Vs4EA*
11:10 PM - 3 Dec 2015

Tom Staniford @tomstaniford
@athattersley @pathologistmag @UniofExeter I think this calls for some sort of celebratory tattoo. Maybe 'power' in gothic text on knuckles?
11:26 AM - 9 Dec 2015

Jerad Gardner, MD @JMGardnerMD
@RMeunierMD thank you!
I felt very honored. And thanks to @pathologistmag for promoting our field like this!
11:51 AM - 6 Dec 2015

joener bangero @mojojoener
@pathologistmag this is so enticing! Think I shall peruse this issue for the weekend.
11:06 PM - 3 Dec 2015

Rodrigues, DN @daninava
@pathologistmag @ilovepathology That's one solid #PowerList !
4:07 PM - 3 Dec 2015

Ana-Maria Simundic @amsimundic
@pathologistmag Excited and honored to be on the 2015 #Powerlist. Thanks to all who have voted for #amsimundic!
9:50 PM - 7 Dec 2015

What's got you talking on our website this month?
www.thepathologist.com

The Invisible Doctor:
http://bit.ly/1Tvr3Np

"I concur with Dr. Lopez and add that for those of working in clinical pathology we take the same steps to make ourselves known, to get out of the lab some and insert ourselves back into the practice of clinical medicine! Clinical pathologists and clinical scientists should not just be providers of data, we should be providing more consultative service to ensure that the right diagnostic tests are performed for the right patient at the right time!"

– T. Scott Isbell, US

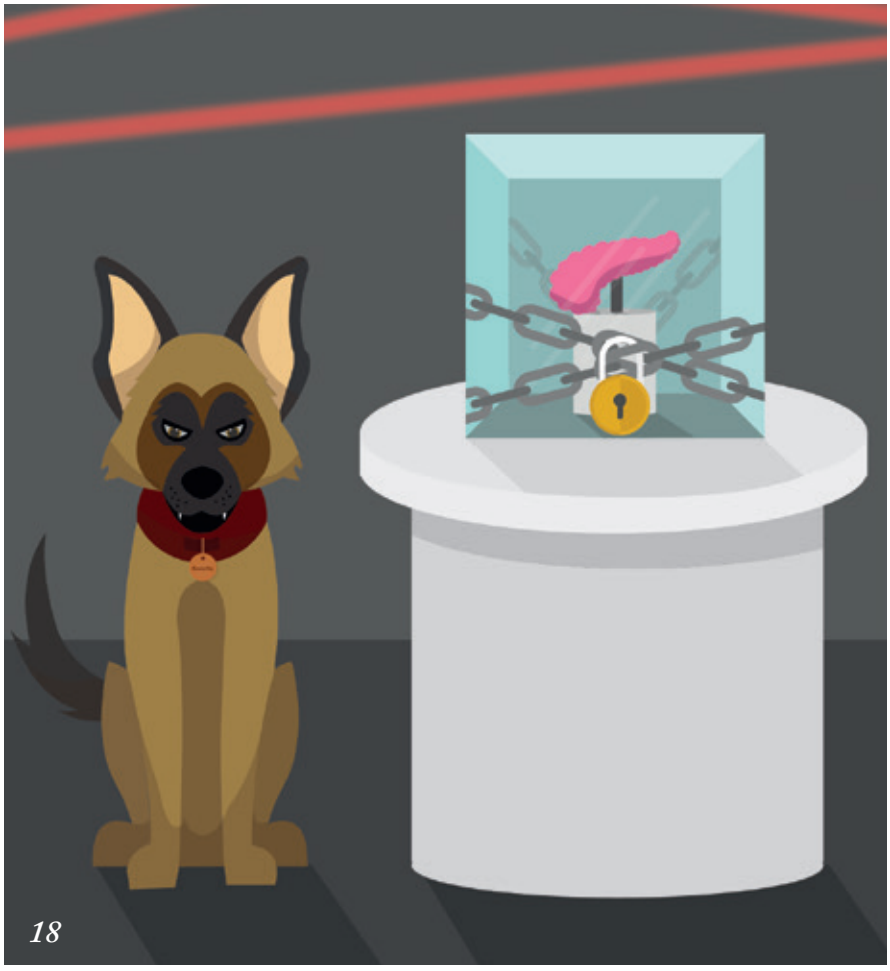
Value of Laboratory Medicine
http://bit.ly/1QeZRov

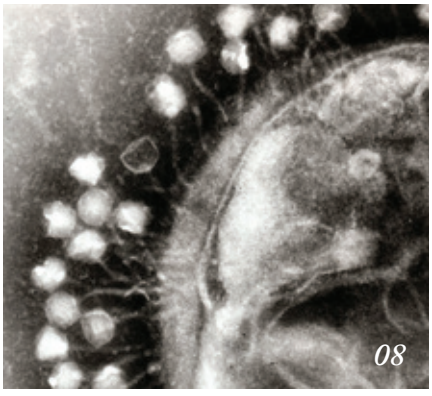
"This well written and crystal clear paper is really thought provoking. We can take from it that the laboratory professionals need some introspection on how laboratories provide services to decrease the incidence of delayed test results and of incorrect diagnostic tests. The percentages are apparently alarmingly high (%), but it would be of interest to know the absolute values? Are they in ppm or ppb?

To the comment that clinicians need our help, which is certainly true, I would add that we also need theirs as arms-length champions to make the laboratory value know to decision makers."

– Edgard Delvin, Canada

Contents





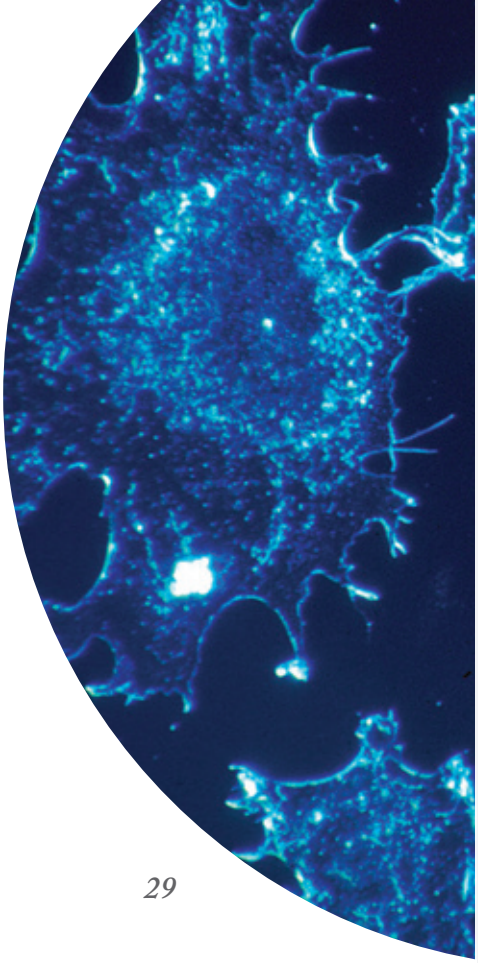
On The Cover

A reference to the Mission: Impossible film series; scientists take on the grueling task of cracking the case of the cancerous pancreas.

Upfront

In My View

Feature



29

In Practice

Next Gen

Profession

Sitting Down With

the Pathologist

ISSUE 14 - DECEMBER 2015

Change of address
tracey.nicholls@texerepublishing.com
Tracey Nicholls, The Pathologist, Texere Publishing Limited, Haig House, Haig Road, Knutsford, Cheshire WA16 8DX, UK. Single copy sales £15 (plus postage, cost available on request tracey.nicholls@texerepublishing.com) Annual subscription for non-qualified recipients £110.

General enquiries:
www.texerepublishing.com
info@texerepublishing.com
+44 (0) 1565 745 200
sales@texerepublishing.com

Distribution:
The Pathologist (ISSN 2055-8228) is published monthly except July, by Texere Publishing Periodicals Postage Paid at Piscataway, NJ and additional mailing offices POSTMASTER: Send US address changes to The Pathologist, Texere Publishing Ltd, C/o 1637 Stelton Road B2, Piscataway NJ 08854

texere publishing ltd
Available on the App Store

HUMANITY IN SCIENCE AWARD

phenomenex®
...breaking with tradition℠
&
the Analytical Scientist

2015 Winners
Andreas Seidel-Morgenstern (left)
and Peter H. Seeberger (right),

Now the judging begins...

Nominations are now closed for The Humanity in Science Award 2016.

Analytical science has the power to change human lives for the better, but rarely receives the same fanfare as other scientific disciplines. The Humanity in Science Award was launched to recognize and reward a recent breakthrough in analytical science that has truly made the world a better place.

The 2016 award will be presented on May 10 in Munich, Germany.

www.humanityinscienceaward.com

humanityinscienceaward @humanityaward humanityinscienceaward@gmail.com

Show Me the Money!

Wanting to go digital, but struggling with justification? Do not fear, help will soon be at hand...

I've noticed that more conferences on digital pathology are starting to pop up; no surprise when you consider manufacturers clearly believe there is a commercial benefit to investing in new technologies, otherwise they wouldn't make the effort. Moreover, radiology has successfully gone digital, so why not pathology? Based on the growing attendance at these niche events, it's clear the appetite among lab professionals is there. But you know what isn't? Money.

Digital technology isn't a new phenomenon, but its implementation in pathology is – and it certainly is slow. Why? Probably because the return-on-investment argument is unproven. As scientists, we know that to "prove" (or at least propose) anything, we must provide supporting evidence. And cost versus benefit evidence is severely lacking – undoubtedly a huge barrier.

I was at a digital pathology congress in London this month, and though there were the usual informative presentations on the value of digital technology, they were balanced by some refreshingly honest talks on the very real challenges. In his keynote lecture, Paul van Diest stated (more than once), "The business case is thin". Strong words. However, van Diest also believed the efficiency gains were obvious and said "digital pathology is good pathology" – in other words, the decision to digitize is a no brainer for a high quality lab. Sadly, "to build a business case, you need to use a lot of words and a lot of paper to convince people to pay for it. So I just didn't bother and we're doing it anyway." But finding alternative funding isn't so easy for everyone; what about those labs – probably the majority – that need to grovel to the purse-string holders?

Rest assured, evidence is on its way. Recently, I was chatting with Alexi Baidoshvili, pathologist and project director of the digital pathology team at LabPON (1). Baidoshvili's lab in the Netherlands was the first to run a 100 percent digital histopathology service, and he tells me that he's begun gathering the evidence to build a strong argument in favor of digitization. In fact, he's hoping to present it for the first time at a London event in May 2016. We'll be sure to report on it as soon as the data are available, so watch this space.

In the meantime, the benefits of digital technology will continue to wow conference-goers and hopefully supply many a good reason to force fund-holders to part with their cash because, in the words of van Diest, "pathology is worth it [so] show me the money!"

Fedra Pavlou
Editor

Reference

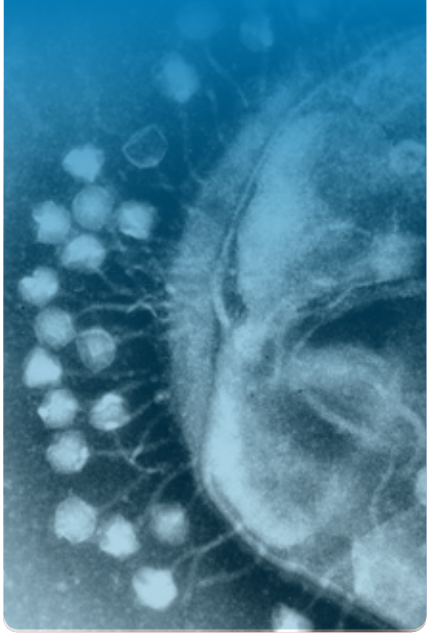
1. *A Baidoshvili, "Making the Move to 100 Percent Digital", The Pathologist, 13, 40–42 (2015).*

Upfront

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

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

Email: fedra.pavlou@texerepublishing.com



The Dark Virome

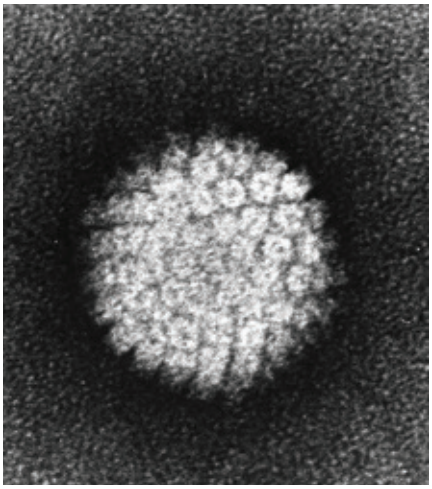
Over 90 percent of the human cutaneous virome is made up of unclassifiable "dark matter"

Studies of the human microbiome have gained popularity in recent years, but just how much do we really know about the helpful (and harmful) microbes living in the human body? A recent study by a team of dermatologists and microbiologists at the University of Pennsylvania (UPenn) in the US, has revealed we have a lot left to learn – during a viral DNA analysis of 16 healthy volunteers, the team found that over 90 percent of the DNA viruses on human skin are "dark matter" that isn't identifiable (1).

"There has been a real need for a better understanding of these viruses, given their potential effects on our skin cells as well as on our resident bacteria," says Elizabeth Grice, senior author of the paper and assistant professor of dermatology. "Until now, relatively little work has been done in this area, in part because of the technical challenges involved. For example, a skin swab taken for analysis will contain mostly human and bacterial DNA, and only a tiny amount of viral genetic material—the proverbial needle in the haystack," she adds.

To overcome the problem, Grice and her colleagues used viral DNA purification techniques on virus-like particles (VLPs) before performing shotgun metagenomics sequencing on the purified samples. A database search showed that over 90 percent of the detected DNA did not match any known genomes.

Much of the viral material identified belonged to the *Caudovirales* order (tailed bacteriophages), although most were not classed to family level. This suggests a strong link between the viruses and bacteria found on the skin, as phages can alter the phenotype of their host bacteria. The team found evidence of transmissible genes which could confer antibiotic resistance, virulence and pathogenicity, implying that the skin virome may play a significant role in health and disease. As for the viruses that could be identified, the most abundant, perhaps unsurprisingly, was the skin-cell infecting HPV.



The UPenn team hope that their work will help to establish a baseline for future study of the skin virome and the ways it changes during disease, and encourage other researchers to design their own studies of the virome – to this end, they have made their methods, including the algorithms they devised to analyze their data, freely available. They also plan to continue their work, and are now using the methods they have developed to study the genomic variability of skin viruses, and to observe how the skin virome changes in response to influences, such as UV radiation exposure and antibiotic treatments. *RM*

Reference

1. *GD Hannigan et al., "The human skin double-stranded DNA virome: topographical and temporal diversity, genetic enrichment, and dynamic associations with the host microbiome", mBio, 6, e1578–15 (2015). PMID: 26489866.*

Driving Discovery

A big data approach to cancer driver genes yields insights into how these mutations affect protein interaction

A collaborative effort between researchers in Europe and the US has resulted in the combination of two publicly available "omics" databases, to create a catalog of cancer drivers. The study has discovered over 70 new candidate cancer driver genes (1), and could help to explain how the same affected gene can lead to different outcomes or therapy responses in patients.

The computational program, called E-driver, uses tumor data from ~6,000 patients from the Cancer Genome Atlas (TCGA), and more than 18,000 three-dimensional protein structures from the Protein Data Bank. An algorithm then analyzes the information to see if structural alterations of protein-protein interaction (PPI) interfaces are enriched in cancer mutations, therefore identifying candidate driver genes.

The motivation behind the study? It was based on the theory that, as genes can have a variety of functions, information on the structures, pathways, and protein complexes involved in disease would give insight into how mutations in different genetic regions may produce different characteristics in the resulting cancer.

"I was surprised that almost all existing tools for the analysis of cancer mutations are not using available structural information on proteins, which happen to be my main field of interest," says co-author Adam Godzik. "Insights can be gained from even very rudimentary structural analysis, and we set out to do this on a large scale".

As well as identifying possible new cancer drivers, the study has given further insight into this area of oncology, adds Godzik. "We have learned two things: mutations in different regions of a gene can have different, sometimes opposite effects on cancer and treatment outcomes. And the growing list of cancer driver genes is slowly eroding the current model of driver vs. passenger mutations. It is clear now that as well as a small number of major drivers, there are a lot of genes that play a role of 'enablers' or 'mini-drivers', which when mutated, could provide an important advantage to a growing tumor, but may not be able to start cancer by themselves", he says.

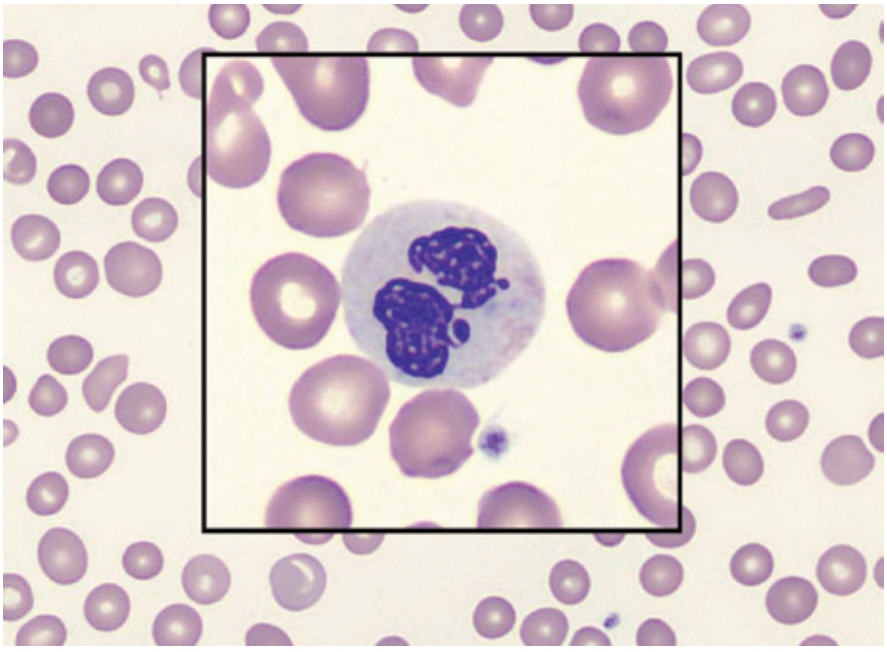
Godzik admits that more analysis is needed to better understand the entire landscape of molecular events in cancer in order to identify optimal treatments and predict patient outcomes. However, at this point it remains unclear if these newly-discovered drivers are likely to become targets for therapy, as many are relatively rare. *RM*

Reference

1. *E Porta-Pardo et al., "A pan-cancer catalogue of cancer driver protein interaction interfaces", PloS Comput Biol, 11, e1004518 (2015). PMID: 26485003.*

Illuminating Idiopathic Cytopenias

Genetic testing could shed light on unexplained low blood counts



Diagnosing the cause of cytopenia is often challenging. Even after bone marrow biopsy to identify a cytopenia of unknown etiology, the root of the issue may remain elusive. Many cytopenias that can't be easily characterized are eventually attributed to myelodysplastic syndromes – a mixed bag of malignant hematopoietic stem cell disorders that feature over- or underproduction of blood cells, morphological abnormalities in the cells, changes to the overall blood cell count, and sometimes a risk of transformation into a more aggressive leukemia.

But diagnosing myelodysplastic syndrome (MDS) requires the identification of dysplastic features and blast cells, which are notoriously hard to quantify accurately, even for experienced hematopathologists. Patients who don't meet the diagnostic criteria for MDS and whose cytopenia remains unexplained are usually given the designation of "idiopathic cytopenia of undetermined significance (ICUS)". As ICUS can progress to more serious conditions, including MDS or acute myeloid leukemia (AML), this uncertainty is far from ideal – so researchers from the University of California set out to discover whether genetic testing might give patients with ICUS a more useful diagnosis.

"We know that some ICUS patients will go on to develop overt malignancies like MDS or AML, disorders characterized by the presence of genetic mutations," says study author Rafael Bejar. "We wanted to understand the incidence and pattern of acquired mutations in patients with ICUS." The researchers found that 35 percent of ICUS patients have somatic mutations or chromosomal abnormalities associated with MDS, and they propose that this subset of patients should be described as having "clonal cytopenias of undetermined significance (CCUS)" – potentially leading to new diagnostic criteria (1).

"Our working hypothesis is that cytopenic patients with somatic mutations are more likely to develop blood cancers like MDS and AML," explains Bejar. "Having a diagnosis of CCUS not only gives a name to a patient's condition, it may prompt their physician to be more vigilant in case their disease does progress." He also believes that a formal definition of CCUS will help standardize the way investigators classify patients with those cytopenias. That would allow independent studies conducted by different groups to be more easily compared or combined, thanks to a clearer definition of the target populations.

However, Bejar cautions that the study did not track the long-term progression of the patients, and more research will be needed before new diagnostic criteria can be considered. "We do not have a clear understanding of what happens to CCUS patients over time. The presence of an MDS-like gene mutation in a patient who does not otherwise meet the standard diagnostic criteria cannot be used as presumptive evidence of the disease and should not be used to justify treatment with MDS-specific drugs," he says.

Bejar suggests extending the current prospective research to assess how CCUS patients progress over time. "This will allow us to learn which mutations can foretell what is likely to happen to patients in the future. We will also start to learn how the stepwise evolution of CCUS into more overt blood cancers occurs at the molecular level, potentially identifying opportunities for intervention." *JS*

Reference

1. *R Bejar et al., "MDS-associated somatic mutations and clonal hematopoiesis are common in idiopathic cytopenias of undetermined significance", Blood 126, 2355–61 (2015). PMID: 26429975.*

Demystifying Metastasis

Integrin expression by tumor exosomes could help oncologists pinpoint which patients will present with metastatic disease – and to which organs

The ability to predict which patients will develop metastatic disease, and where, would undoubtedly be a huge coup for oncology – but much of metastasis still remains a mystery. A common theory is that tumor cells dictate metastasis, but a recent study published in Nature, shows that this may be a misconception.

"Tumor cells only indirectly influence metastasis. The tumors are excreting exosomes, and these are preparing future sites of metastasis, not the cells, so this is very different from the accepted dogma," says co-author of the Nature paper (1), David Lyden.

Lyden and his colleagues found that tumor-excreted exosomes have distinct expression patterns of integrins, and it appears that the exosomes prepare organs to host tumor cells, by causing responses such as inflammation and vascularization, forming a metastatic niche (see Figures 1 and 2). They looked at 10 tumor cell lines which usually metastasize to specific organs – for example, pancreatic cancer often spreads to the liver – and analyzed the proteins being expressed by the cancer exosomes. Analysis showed that they could link the expression of different integrin proteins with metastatic disease in the lung, liver and brain (see Table 1).

Now, the team plan to continue their research in a larger study group, looking at different organs, and different patients, in order to further understand how metastasis works. They have a particular interest in bone cancer, as they found that patients with this secondary cancer had no integrin expression.

"Our work illustrates that we can now do a very easy blood test and predict who will develop metastatic disease, and importantly to what organ they will metastasis to. So this is really changing our traditional way of treating metastasis. We can potentially start treatment much earlier, and make a bigger impact," says Lyden.

"This study could in the future also be applied to be used for follow-up of the patients after surgery or chemotherapy. Importantly, our work shows that preparation of future metastasis sites by the exosomes starts long before the cancer cells arrive – they indicate the true start of metastasis," adds the study's first author, Ayuko Hoshino. *RM*

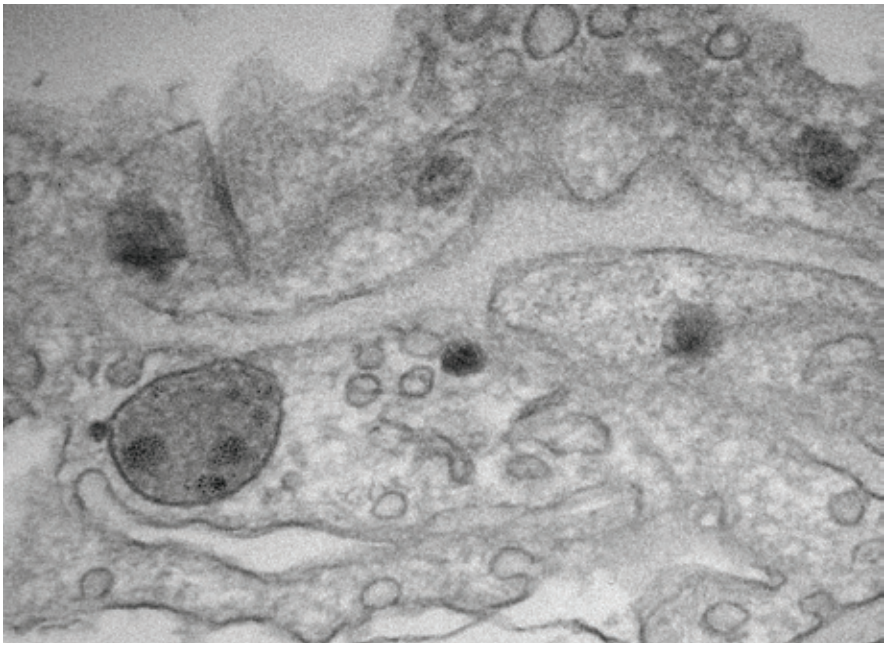


Figure 1. Electron microscopy of mice lung section. Exosomes derived from lung tropic cancer cell are labeled with dye (appearing here as black dots).

Integrin(s)	*Associated With*
ITGα 6, ITGβ 4, and ITGβ 1	Lung Metastasis
ITGβ 5	Liver Metastasis
ITGβ 3	Brain Metastasis

Table 1. Integrin expression profiles were linked with future metastasis in specific organs.

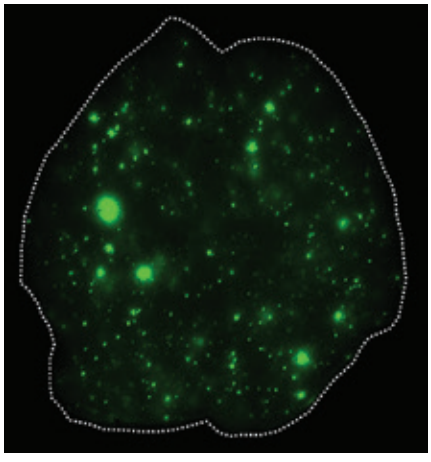


Figure 2. Dye labelled tropic exosomes, localized in a mouse lung.

Reference

1. *A Hoshino et al., "Tumor exosome integrins determine organotropic metastasis", Nature, 527, 329–335 (2015). PMID: 26524530.*

A Crystal Ball for Cancer Spread?

αB-crystallin expression could identify breast cancer patients at risk of developing brain metastasis

Breast cancer is one of the most common sources of brain metastasis (BM), and affects 10-30 percent of patients (1). It goes without saying that this has a significant effect on mortality, and the ability to predict which patients will go on to develop BM could be used to better manage the disease. Now, a team of US researchers have found a biomarker which may help oncologists predict which breast cancers will spread to the brain – αB-crystallin expression.

"BM is associated with the shortest survival time compared with other sites of metastatic spread. If, using primary tissues, we can identify a subgroup of patients who may be of higher risk to develop BM, this could potentially be very valuable for clinical management," says co-leader of the associated study (2), Maggie Cheang.

The researchers analyzed αB-crystallin protein expression in 3,987 specimens from breast cancer patients using immunohistochemistry, and tissue samples were recorded as positive for the protein if there was any staining above background levels. They found that, in women whose cancer had already begun to spread, those who tested positively were around three times more likely to develop BM, and that the protein was also linked with poorer survival rates. A test of this kind could have clear applications in both disease monitoring and management, and in selecting patients for clinical trials.

However, it remains unclear whether gene or protein levels of αB-crystallin provide the best predictive power, Cheang warns. "We hope to add αB-crystallin to established clinical trials in metastatic breast cancer to validate our findings. We also plan to see if expression of this protein contributes to BM in other types of solid tumors, like lung cancer and melanoma, which commonly metastasize to the brain," she says. *RM*

References

1. *MJ Gil-Gil et al., "Breast cancer metastases: a review of the literature and a currently multidisciplinary management guideline", Clin Trans Oncol, 16, 436–446. PMID: 24277572.*
2. *KD Voduc et al., "αB-crystallin expression in breast cancer is associated with brain metastasis", npj Breast Cancer, 1, 15014 (2015).*

Multitalented Marker

Cardiac troponin can be used as an independent early indicator of end-stage kidney disease, and death, new study suggests

Cardiac troponin T (cTnT) is an extremely valuable biomarker, most well-known for its use in detecting damage to the heart muscle. But the protein can also come in useful in a range of conditions that may have a link to cardiac injury – and a recent study has shown for the first time that cTnT can independently provide prognostic information on end-stage renal disease (ESRD) and mortality in people with hypertension (1).

The study involved 3,050 individuals with a family history of hypertension, and looked at both African Americans and Caucasians – an important point, as previous studies have found that African Americans have much higher kidney disease rates compared with Caucasians (2). The researchers found that regardless of race, or baseline kidney function, cTnT accurately indicated an increased risk of kidney failure and death (1).

In study participants with elevated cTnT, the cumulative estimated risk of death at 10 years was 47 percent, compared with 7.3 percent among those with normal cTnT levels – and incidence of ESRD was 27 percent, compared with 1.3 percent in those without elevated cTnT levels. "This study demonstrates to physicians everywhere that we are getting closer to accurately predicting future disease and death by examining this one marker. This is important, because, as with many diseases, accurate, early detection means we can more quickly recognize and efficiently treat the disease before it fully manifests – potentially improving a patient's quality and quantity of life," says co-author of the paper, LaTonya Hickson.

Despite these promising findings, further study is needed, as abnormal cTnT levels are not prevalent enough in the group studied to significantly improve risk prediction. However, the authors note that it may be possible to identify subgroups of patients who could potentially benefit from this type of screening. *RM*

References

1. *LJ Hickson et al., "Troponin T as a predictor of end-stage renal disease and all-cause death in African Americans and whites from hypertensive families", Mayo Clin Proc, 90, 1482–1491 (2015). PMID: 26494378.*
2. *National Institute of Diabetes and Digestive and Kidney Diseases, "Race, Ethnicity and Kidney Disease", (2014). Available at: http://1.usa.gov/1MRXU0g. Accessed November 9, 2015.*

Plastic Pays

An all-plastic microscope for fast staining and analysis of white blood cells could allow cheap, point-of-care blood work

Point-of-care (POC) tests developed for use in resource-poor countries are often centered on rapid, user-friendly ways to diagnosis a single disease, such as HIV. But if the causative disease is uncertain, or you need to monitor a patient's progress, this approach might not always be the best one. Now, a team from Rice University, Texas, USA, have developed an all-plastic, 3D printed, digital fluorescence microscope to allow POC white blood cell (WBC) differential measurement (see Figure 1).

The low-cost microscope allows analysis of lymphocytes, monocytes and granulocytes in whole blood samples stained with acridine orange, which can be applied directly to wet, undiluted blood samples. The device also requires no manual optical adjustment between samples, allowing for preliminary imaging of a sample in just 10 minutes (1). These factors could be particularly useful in helping healthcare workers better diagnose a range of conditions, such as bacterial or viral disease, or, for example, a change in total WBC count.

Other POC systems for analyzing WBCs do exist, but with a purchase cost of over US$1,000, and a per-test cost of over $3, which is unfeasible for many resource-poor regions. Though their prototype cost over $3,000 to create, if mass-produced, the team estimate this could fall to around $600, with each test costing only a few cents. "One of the driving aspects of the project is the cost of the sample or sample preparation. Many systems which work for point-of-care applications have quite expensive cartridges. The goal of this research is to make it possible for those in impoverished areas to be able to get the testing they need at a manageable price point," says Tomasz Tkaczyk, an associate professor at the Department of Bioengineering, Rice University, and part of the team who developed the microscope.

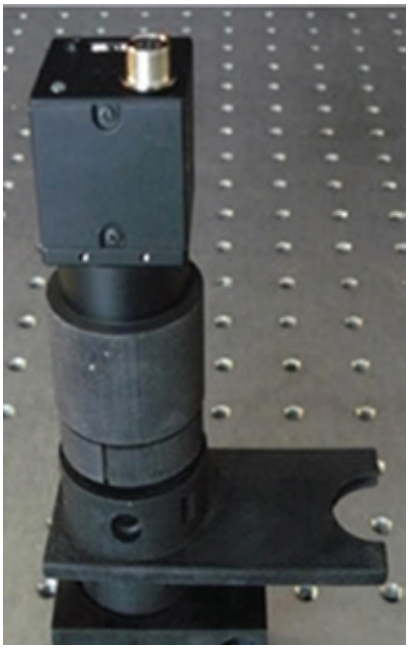


Figure 1. The prototype 3D printed microscope.

Future plans for the device include comparing the differential counts obtained with the plastic microscope to results from conventional benchtop WBC analysis. The team also hope to develop an automated algorithm for WBC identification, with the overall aim of making blood work cheaper, faster and easier in settings where expensive lab equipment may not be an option. *RM*

Reference

1. *A Forcucci et al., "All-plastic, miniature, digital fluorescence microscope for three part white blood cell differential measurements at the point of care", Biomed Opt Express, 6, 4433 (2015).*

In My View

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

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

Contact the editor at fedra.pavlou@texerepublishing.com

What's in a Name?

Can we accept that meningothelial hyperplasia is a true preneoplastic proliferative, not a simple hyperplastic reactive, lesion, and is it time to change our approach to it?

By Sanja M. Milenkovic, associate professor of Pathology and Oral Pathology at the Faculty of Dentistry, Pančevo, Serbia, and the chief of the Clinical Pathology Department, at the University Clinical Hospital Center Zemun, Belgrade.

The history of meningioma can be traced back to 1614, when Felix Paster first described the tumor, but it had not been officially named yet (1). It was Harvey Cushing who first coined the term "meningioma" in a monograph written with Louise Eisenhardt in 1938 (2).

Where are we today? Well, knowledge of the condition is still lacking. When I ask radiologists to tell me how they might go about identifying meningioma, they might say: "Ok, I see a dural tail sing, it's meningioma!" When I ask general pathologists what they know about the condition, there's a good chance they would say, "Meningioma? It is a typically benign and slow growing tumor, appearing mostly in the later decades of life!"

But when I ask neuropathologists about meningiomas, they tend to be a lot more enthused: "Fascinating tumor … the wide-ranging biologic and histologic continuum of meningiomas has been impressive since the beginning of the 20th century…!" Thankfully, times have changed. Molecular pathology is here to stay, and over the course of the last century, scientific advancements have allowed us to identify many important facts about meningioma. We now know that the most common tumor suppressor genes associated with its development are NF2, DAL-1, and various tissue inhibitors of matrix metalloproteinases (TIMPs) (3,4).

> *"From the time when I was working on my PhD thesis in 2005 until now, there have only been a few articles about it, and even less that have attempted to define it."*

It's great that we've amassed this knowledge. But have we forgotten meningothelial hyperplasia (MH)? "From the time when I was working on my PhD thesis in 2005 until now, there have only been a few articles about it, and even less that have attempted to define it." What do we know? We know that it is most commonly diagnosed incidentally during surgery or at autopsy. We know in clinical practice, MH has been reportedly associated with advanced patient age, chronic renal failure,

trauma, hemorrhage, and neoplasia (particularly prominent adjacent to optic nerve pilocytic astrocytomas, where it has the potential to be misdiagnosed as an orbital meningioma) (5).

Overall, MH represents a poorly characterized entity and we continue to be challenged by it. In particular, as pathologists, we are faced with the following conundrums: 1) When small meningothelial nests are found within a submitted surgical sample, usually labeled as "dural margin", it is really difficult to conclude if it is normal or hyperplastic meningothelial foci, or tumor spread. This is especially difficult if the meningioma radiologically shows dural tail sing; this sign histologically is nothing more than hypervascular fibrous tissue. 2) In rare cases, we have seen patients presenting with thin layers of meningeal enhancement of unknown etiology, where biopsy revealed thickened meninges with nests of arachnoidal cap cells. In these cases, it is very difficult to distinguish hyperplasia from "meningothelial tumorlets" or meningioma en plaque (5).

MH is pretty incredible in nature; immunohistochemically it has a profile like normal arachnoidal cap cells, but with progesterone receptor (PR) positivity. On the other hand, we know that MH shows 64 percent similarity to meningiomas. We don't see any deletions of NF2 or 4.1B by FISH analysis in MH, which we do see in classic meningiomas, and MH cells retain merlin and protein 4.1B expression (5).

In a bid to shed some light, scientists have created a genetically engineered model, using conditional mutagenesis to specifically inactivate mouse Nf2 gene in arachnoidal cells and the results of these studies have confusingly shown formation of intracranial MH and meningiomas. The data suggests that MH may represent a preneoplastic lesion in some cases. To add further confusion, some important differences exist between the human and animal lesions. In humans MH is a reactive process, but in a mouse model (although the lesions are small), they are not reactive, but neoplastic (6).

Is there a border between these two entities and if so, where is it? In my view, I think it's better to talk about meningothelial proliferation in general, rather than MH specifically. And moving forward, I believe there is a need to develop additional studies and apply a rigorously controlled multidisciplinary approach to study this disease and, eventually, to define a suitable approach to its diagnosis and to its treatment. Until this time comes, the disease will be shrouded with question marks, uncertainty and, unfortunately, missed diagnoses.

References

1. *MG Netsky, J Lapresle, "The first account of a meningioma", Bull Hist Med, 30, 465–468 (1956).*
2. *H Cushing, L Eisenhardt, "Meningiomas: Their classification, regional behaviour, life history and surgical end results," Bull Med Libr Assoc. 27, 185 (1938).*
3. *M Shibuya, "Pathology and molecular genetics of meningioma: Recent advances", Neurol Med Chir (Tokyo), 55, 14–27 (2015). PMID: 26236799.*
4. *DH Gutmann et al., "Molecular biological determinations of meningioma progression and recurrence", PLoS ONE, 9, e94987 (2014). PMID: 24722350.*
5. *A Perry et al., "Meningothelial hyperplasia: A detailed clinicopathologic, immunohistochemical and genetic study of 11 cases", Brain Pathol, 15, 109–115 (2005). PMID: 15912882.*
6. *M Kalamarides et al., "Natural history of meningioma development in mice reveals: A synergy of Nf2 and p16Ink4a mutations," Brain Pathol, 18, 62–70 (2008). PMID: 17924978.*

Be Ready for IQCP

The USA will implement a new approach to laboratory QC on January 1, 2016, but what is "The Individual Quality Control Plan (IQCP)" and should test sites adopt it?

By Sharon Ehrmeyer, professor of pathology and laboratory medicine, School of Medicine and Public Health, University of Wisconsin, Madison, Wisconsin, USA.

On the first day of 2016, the US Centers for Medicare and Medicaid Services (CMS) will replace the current Equivalent Quality Control (EQC) QC option for meeting Clinical Laboratory Improvement Amendments (CLIA) standard §493.1256(d) with IQCP (1,2). What this means is that test sites, typically providing point-of-care testing and wanting to implement or continue to use a nonwaived testing device and rely solely on the device's built-in quality assessments to meet daily QC requirements, will need to develop and follow an IQCP. Nevertheless, note that this is a voluntary option!

Test sites not wanting to develop an IQCP will need to perform external QC for each analyte on patient testing days. While IQCP is a CMS mandate, many tests choose to meet CLIA requirements through professional US accrediting organizations' (AOs) standards, such as those from the College of American Pathologists (CAP), The Joint Commission (TJC), and Commission on Office Laboratory Accreditation (COLA). These AOs standards now include some form of the CMS IQCP option.

IQCPs and risk management

What's the difference? IQCP expands the definition of QC to include the assurance of quality in all three phases of the testing process – preanalytical, analytical and post-analytical. For example, a test result will not accurately reflect a patient's condition when the sample is not drawn or processed correctly.

IQCP is founded on risk management concepts (see Sidebar "Defining Risk"), which include the systematic application of policies, procedures, and practices to analyze, evaluate, control, and monitor risk (3,4). Test sites continually make decisions based on risk management, although many do not realize this when they develop standard operating procedures (SOPs) for the three phases of the testing process.

Developing the IQCP

CMS offers a step-by-step IQCP development guide that details the process (5). According to the guide, all IQCPs must be based on facts – clinical, regulatory, test setting and organizational requirements – while ensuring quality test results. The testing device's approach to evaluating quality and its error detection and mitigation features are essential to consider when making appropriate decisions in developing the QC plan as part of the whole IQCP.

CMS requires test sites to identify and assess potential errors (risks) that ultimately can influence quality in each phase of the testing process. In its August 2013 memo, CMS provides insight into what should be included in the process (1). The memo mandates that at least five components of testing be included in the risk assessment process: specimen, operator (personnel), testing environment, reagents, and test system. Once the test site identifies the potential errors, it needs to review its current SOPs, practices, and test device's features to determine whether the identified risks are eliminated and/or detected.

In reality, the risk assessment process is another check on the validity of a test site's practices. For those potential errors identified but not eliminated by current practices, test sites need to determine whether they are significant (6,7). If they are, the test site needs to modify and/or add additional practices accordingly for quality improvement.

"IQCP expands the definition of QC to include the assurance of quality in all three phases of the testing process – preanalytical, analytical and post-analytical."

The quality control plan

While the quality control plan focuses only on the analytical phase and, therefore, analytical quality, CMS assumes that test sites can make more appropriate decisions for what practices are necessary by first ensuring "quality" in the pre- and post-analytical processes.

The quality control plan section of the

IQCP must at least include the number, type, and frequency of testing controls; the criteria for acceptable results(s) for each of the QC approaches used; and assurances that the QC performed meets the minimum specified by the manufacturer (1). Test sites need to be explicit on what QC is performed daily, weekly and monthly as well as what response is acceptable for each. CMS requires the laboratory director to take responsibility for the proper development and implementation of the IQCP, which includes the quality control plan. The director can delegate this responsibility in writing, but there must be documented evidence that the director approves.

Quality assessment

Test sites are accustomed to continually evaluate their many activities and make changes when necessary for quality assessment. Specifically, CMS requires a test site to establish a review system for the ongoing monitoring of its quality control plan effectiveness. Most sites use a typical "plan-do-check-act" cycle. With this approach, test sites first develop and then implement the quality control plan. Once the plan is followed (do), it is monitored (check) to verify effectiveness. When problems are identified, the plan should be improved (act), starting the quality assessment cycle again.

Putting the IQCP pieces together

The final step in the development process is the IQCP itself, which summarizes the findings of the development process and demonstrates compliance to CMS mandates. Although site policies, procedures and practices will detail the specific changes resulting from the risk assessment process, a summary of these changes for all three phases of testing together with the location of these details will need including in the IQCP (7). CMS provides no specified or "official" format for the IQCP, so test sites have flexibility in presenting the information. However, CMS inspectors will look for evidence, so test sites will need supporting documentation.

Will you adopt IQCP?

While CMS rationalizes the IQCP option by identifying a series of benefits, each test site needs to decide whether it "fits" its particular testing device and situation (7,8). For me, the primary benefit is the risk assessment because it forces sites to evaluate their current policies, practices, and testing device thoroughly for possible risks and risk mitigation and then to make appropriate decisions for quality improvement based on the information they collect.

Undoubtedly, there will be downsides. IQCPs should, in theory, improve test sites' testing processes and ensure test result quality. However, when test sites do not properly design a thorough risk assessment to identify problems and make appropriate changes for quality improvement, the "quality" of all testing processes will be in question. The verdict for IQCP effectiveness is still out. Only time will tell what the impact will be.

References

1. *US Centers for Medicare & Medicaid Services, "Individualized quality control plan (IQCP): a new quality control (QC) option", see: http://go.cms.gov/1N4c4VZ. Accessed June, 2015.*
2. *US Centers for Medicare & Medicaid Services, "Code of Federal Regulations. Part 493 – Laboratory Requirements". http://1.usa.gov/1X98kxp. Accessed June, 2015.*
3. *Clinical and Laboratory Standards Institute, "CLSI EP23A. Laboratory quality control based on risk management", http://clsi.org/edw/ep23/. Accessed June, 2015.*
4. *ISO, "ISO14971:2007. Medical devices – application of risk management to medical devices", http://bit.ly/1cafc6S. Accessed June, 2015.*
5. *US Centers for Medicare & Medicaid Services, "IQCP individualized quality control plan: developing an IQCP a step-by-step guide", http://go.cms.gov/1SVZBZ2. Accessed June, 2015.*
6. *JO Westgard, "Six Sigma Risk Analysis", Westgard QC, Inc., Madison, Wisconsin, USA (2011).*
7. *SS Ehrmeyer, "The new poor lab's guide to the regulations", Westgard QC, Inc., Madison, Wisconsin, USA (2015).*
8. *US Centers for Medicare & Medicaid Services (CMS), "CLIA individualized quality control plan (IQCP) benefits", http://go.cms.gov/1lEOh9b. Accessed June, 2015.*

Defining Risk

Risk is the chance that an "error" will cause harm or loss to a patient. It is estimated from the probability of error occurrence and the severity of the harm or loss to the patient when the error is present.

The risk assessment process systematically evaluates available information to identify and estimate the significance or acceptability of each potential risk identified.

Risk mitigation is the elimination, reduction, or detection of the significant potential risks identified. Typical practices for mitigation include modifying current quality control and quality assurance practices, developing new practices and/or selecting a different testing device with more mitigation features. The goal is to have mitigation mechanisms in place to eliminate all significant risks.

Mission: Impossible

Pancreatic cancer is tough to understand, diagnose and treat. Undeterred researchers are on a quest to crack the case.

By Michael Schubert

Although only the 12th most common cancer worldwide (1), pancreatic cancer has gained increasing attention over the last few years. High-profile figures like Steve Jobs and Randy Pausch have shone a bright spotlight on the disease, but despite the increase in research interest, progress remains slow. Why? A combination of factors: the potential causes of the disease are not well understood, screening techniques are imperfect, chemotherapy and radiation treatments have limited success, and the mortality rate is high – only about 6 percent of patients survive five years (2), and that number drops to 1 percent after 10 years (3). But these are not just dismal statistics – they are a call to arms for researchers, and lately, that call has been answered extensively, with new ideas for diagnosis, prognosis and treatment seeming to arrive every day. Here, we speak with some of those scientists at the forefront of this research to learn more about what's being done. Will we soon see those survival statistics improve? It's still early days for the new wave of pancreatic cancer breakthroughs, but one thing's for sure – the promise is most certainly there.

References

1. *J Ferlay et al. "GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11", (2013). Available at: http://globocan.iarc.fr. Accessed November 23, 2015.*
2. *M Sant et al., "EUROCARE-4. Survival of cancer patients diagnosed in 1995-1999. Results and commentary", Eur J Cancer, 45, 931–991 (2009). PMID: 19171476.*
3. *Cancer Research UK, "One-, five- and ten-year survival for pancreatic cancer", (2014). Available at: http://bit.ly/21d4tyO. Accessed November 23, 2015.*

Tapping a Rich Vein

Circulating tumor cells may hold information crucial for better pancreatic cancer diagnosis and treatment – but locating them needs special tactics

By Christopher Chapman

At the moment, it's difficult to catch pancreatic cancer early. There are often few or no symptoms in the early stages of the disease, and there are no screening tests recommended by any professional gastrointestinal society. Even in high-risk populations where regular screening could catch disease early and make treatment more effective, the recommendations are controversial. It's unclear which patients need screening and how best to do it – whether by cross-sectional imaging (like MRI or CT scans) or by endoscopic ultrasound.

Even after pancreatic cancer is diagnosed, there are still problems. The radiologic imaging we use to determine a patient's eligibility for curative surgery has limited ability to determine postoperative risk of recurrence. Not only that, but we can't use it to identify whether or not patients would benefit from invasive surgery or harsh neo-adjuvant chemotherapy – and that's vital, because it helps us determine where the benefits of aggressive treatment may outweigh the risks.

Our goal at the University of Chicago Center for Endoscopic Research and Therapeutics is to pioneer innovative approaches to diagnose and treat gastrointestinal disease. We consider pancreatic cancer a primary focus because it's a devastating disease that's difficult to cure – often due to local advancement or distant metastasis. Recently, it has become increasingly clear that not all pancreatic cancers are equal. This offers the possibility of personalized treatment, but it's still a challenge to adequately assess tumor molecular heterogeneity and perioperative risk of recurrence. That's where our new test may be able to help.

The power of CTCs

Circulating tumor cells (CTCs) have been explored as a minimally invasive tool for assessing solid tumor burden. But unless the tumor burden is very high, CTCs in peripheral blood are extremely rare in patients with pancreatic cancer – possibly due to biophysical factors like platelet adhesion or cluster size and shape, which might trap the CTCs in smaller vessels. So we hypothesized that collecting blood from a different site – the portal vein – might yield a differential CTC count and provide us with the necessary biomarkers to personalize treatment.

To do that, we devised a technique that makes use of endoscopic ultrasound and a small needle to sample the portal venous blood.

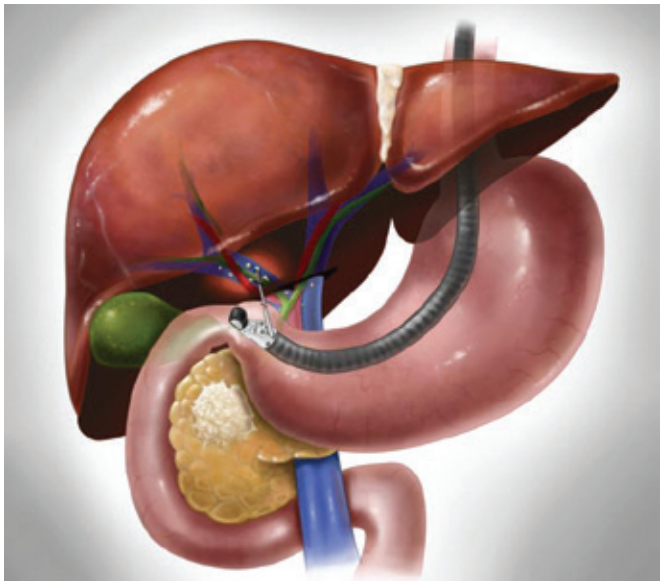


Figure 1. A 19-gauge EUS-FNA needle was advanced transhepatically into the portal vein to aspirate blood samples.

Our technique involves inserting a linear echoendoscope into the stomach or proximal duodenum via the mouth. Under ultrasound guidance, we identify the left and right portal veins and verify flow signal. Then, we advance a 19-gauge EUS-FNA needle transhepatically into the portal vein and take two to four 8.5 mL aliquots of blood (see Figure 1). Once aspirated, we can count and characterize the CTCs in the blood, helping us to define the patient's diagnosis and make better treatment decisions.

What makes our method better than the existing ones? It gives us access to the portal venous blood, which contains CTCs even in gastrointestinal tumors where the peripheral blood does not. Because we can isolate sufficient numbers of those cells, we're able to perform genomic and proteomic tumor profiling to personalize pancreatic cancer treatment. Not only that, but the technique has a good safety profile – minimally invasive, incisionless, and with no complications seen in the patients who have undergone this type of testing.

So far, we've used portal venous sampling to evaluate 18 patients with suspected pancreatobiliary cancers (1). We were able to detect CTCs in the portal vein blood of all 18 – but when we sampled peripheral blood, only four of the patients had detectable CTCs. On average, portal vein blood contained 118.4±36.8 CTCs per 7.5 mL, whereas peripheral blood contained only 0.8±0.4 CTCs in the same volume. For patients with less invasive disease, the technique is particularly effective; peripheral blood revealed a mean of 0.4 CTCs and a median of 0, but our portal vein samples had a mean of 83.2 CTCs and a median of 62. It

seems clear that it's much easier to locate CTCs in portal venous samples – and that portal vein blood contains enough cells to enable further analysis.

A promising prospect

Of course, CTC analysis is still in its infancy. The results we recently published came from a pilot and feasibility study; we wanted to ensure that portal venous CTC sampling could be done without excessive risk to the patient. Our next step is to conduct a prospective study, which will let us determine what role portal vein CTC isolation, enumeration and molecular profiling can have on prognostic significance, including postoperative recurrence, length of survival, or response to neo-adjuvant chemotherapy. So the test is not yet ready for clinical use – but because CTC enumeration has already been validated and is US Food and Drug Administration-approved for other solid tumors, we're optimistic about the prospects of this new method.

The most immediate potential of portal venous CTC sampling is to provide clinical utility in stratifying risk of postoperative recurrence, as well as helping to identify patients who may benefit from more aggressive treatment. Ultimately, I hope it will supplement imaging to help doctors determine personalized cancer treatment plans for their patients. I have an additional hope for the test, too – I think it has a future role in screening high-risk patients whose cancer may not yet be visible on imaging studies.

And pancreatic cancer isn't the end of the story. My colleagues and I believe that portal venous sampling can be applied to many different gastrointestinal cancers – for instance, colon cancer, where intraoperative surgical access has already confirmed the presence of CTCs in the portal vein. Any cancer with a high rate of metastasis to the liver is likely to have CTCs arriving via the portal vein, which also broadens the applicability of our test. In the future, we'll need to clarify what types of CTCs harbor the most risk for seeding metastases and determine what kind of molecular profiling we can use to characterize them. In the meantime, we've shown the feasibility of portal vein access for effective liquid biopsy – and hopefully, this will soon translate into better research, diagnosis and treatment of pancreatic cancers.

Christopher Chapman is a gastroenterology fellow at the University of Chicago Hospitals, Chicago, USA.

Reference

1. *DV Catenacci et al., "Acquisition of portal venous circulating tumor cells from patients with pancreaticobiliary cancers by endoscopic ultrasound", Gastroenterology, 149, 1794–1803 (2015). PMID: 26341722.*

New Ab-ilities in Imaging

Pancreatic cancer is a challenge to stage because there's no good molecular imaging method – but three new antibody conjugates may change that

It's well-known that one reason pancreatic cancer is so difficult to defeat is that it's usually diagnosed in the late stages, after surgical resection – the only curative option – is no longer a possibility. But of the small fraction of patients who do qualify for surgery, less than one-fifth survive for five years or more (1). That's because of the high incidence of undiscovered metastases. It's difficult to accurately stage pancreatic ductal adenocarcinoma (PDAC), because no molecular imaging tools are up to the task. But that might soon change; a group of scientists at Memorial Sloan Kettering Cancer Center have described and evaluated three new immunoconjugates that can be used for positron emission tomography (PET) and near-infrared fluorescent (NIRF) optical imaging of PDAC tumors.

To make the new imaging agents (2), researchers began with the human monoclonal antibody 5B1, which recognizes CA19.9 – the most highly expressed antigen in PDAC tumor tissue. To

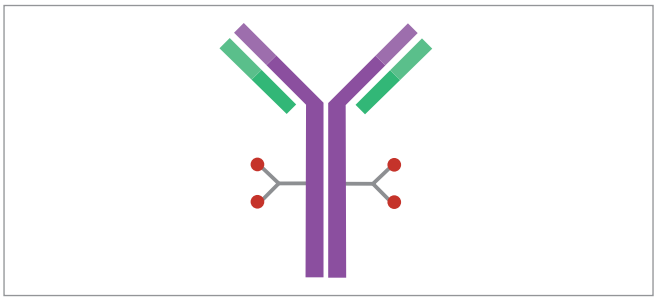


Figure 1. A schematic of DFO or fluorophore conjugation to the antibody heavy chain. Purple: heavy chain of the antibody, green: light chain of the antibody, red: DFO or fluorophore, grey: glycans.

5B1, they conjugated either desferrioxamine (DFO, which enables radiolabeling with 89Zr for PET imaging), a fluorophore for NIRF imaging, or both. They used a site-specific strategy that involves affixing molecules via the heavy chain glycans of the antibody (see Figure 1), which produces well-defined, robust results and simplifies the development of dual-modal immunoconjugates.

The uptake of the antibodies was exceptional both *in vitro* and *in vivo*, particularly in the case of the dual construct – and they were retained much better in tumor than in non-target tissue. When applied to a mouse model of PDAC, the conjugates provided

a clear image of the malignancy, including NIRF-visualized abdominal micrometastases that couldn't be seen by the naked eye or in PET imaging.

These are only early steps toward a better way of imaging pancreatic cancer. Further studies need to be conducted – first in a better mouse model that constitutively expresses CA19.9, and then, if successful, in clinical trials. But if the new immunoconjugates work as well in future studies as they have to date, doctors may eventually be able to gain a clearer picture of a tumor's invasiveness, leading to better staging and, of course, more targeted treatment.

References

1. *JL Cameron et al., "One thousand consecutive pancreaticoduodenectomies", Ann Surg, 244, 10–15 (2006). PMID: 16794383.*
2. *JL Houghton et al., "Site-specifically labeled CA19.9-targeted immunoconjugates for the PET, NIRF, and multimodal PET/NIRF imaging of pancreatic cancer", Proc Natl Acad Sci USA, [Epub ahead of print] (2015).*

Surprising Subtypes

Pancreatic cancer is often treated as a homogeneous disease, but new research reveals distinct subtypes of tumor and stromal gene expression

Exocrine tumors make up about three-quarters of pancreatic cancer, and of those, the vast majority are ductal adenocarcinomas (PDACs). Unfortunately, these are also among the most lethal pancreatic cancers, with only one in 25 patients surviving for five years. But despite this low rate of treatment success, pancreatic cancers are still treated as a single entity – although the disease has been exhaustively sequenced, very few new genetic mutations have come to light, which suggests that the tumors are very alike. So why do clinicians see such different outcomes in different patients? What guides the tumors to spread to different locations, or allows some patients to survive for many years while others progress quickly?

A hallmark of PDAC is the extensive involvement of the stroma – the tissue surrounding the tumor. This tissue is poorly understood, has very few genetic mutations, and makes it difficult to capture precise molecular information from the tumor itself. Previous researchers have attempted to surmount this hurdle by either isolating their studies to looking at tumors with high tumor cell content (1) or microdissecting for the pure tumor cell population (2). But although both of these are valid strategies for studying the tumor compartment, they fail to gather data on PDAC's defining characteristic: its low ratio of tumor to stromal content.

Prognostic power

A research group at the University of North Carolina's Lineberger Comprehensive Cancer Center wanted to better understand the nature of PDACs, which meant collecting information both on the tumors themselves and on the surrounding stroma. "In order to differentiate between stroma- and tumor-specific signals, we used a computational strategy called 'non-negative matrix factorization' (NMF) to separate out gene expression components (3)," said study leader Jen Yeh, UNC Lineberger member, associate professor and the vice chair for research in the UNC School of Medicine Department of Surgery. "This is an unbiased strategy that forced us to understand at the back end what the gene expression components mean. Once we understood what the gene expression of each compartment represented, we realized that NMF was able to separate out all the different tissue compartments – essentially performing a virtual microdissection." For instance, if they analyzed a metastatic liver sample, they were able to distinguish a normal liver compartment, a tumor compartment that was similar to the primary PDAC tumor, and some PDAC stroma.

> *"We were able to identify two different tumor-specific subtypes... and two different stroma-specific subtypes."*

The study involved using NMF to analyze 357 samples – 145 primary and 61 metastatic tumors, 17 cell lines, and 46 pancreas and 88 distant site adjacent normal samples. "After confirming that we were able to distinguish between different tissue compartments," said Yeh, "we were able to identify two different tumor-specific subtypes, the 'classical' and the 'basal-like,' and two different stroma-specific subtypes, the 'normal' and the 'activated'."

Why is this so significant? When the patient populations were divided by tumor subtype, the data revealed that those with basal-like tumors had a significantly worse median survival time (11 vs. 19 months) and one-year survival rate (44 vs. 70 percent) than those with classical tumors. The news isn't all bad for basal-like tumors, though; the subtype also showed better response to adjuvant therapy, with a hazard ratio of 0.38 compared with 0.76 in the classical subtype. Interestingly, the researchers found that all of the tested cell lines were classified as basal-like, meaning that

researchers who use pancreatic cancer cell lines may not be getting a complete picture of the disease's diversity. Stromal subtype has a similar effect; when the patient populations were divided by stromal subtype, those with activated stroma had a worse median survival time (15 vs. 24 months) and one-year survival rate (60 vs. 82 percent) than those with normal stroma.

Individual interventions

Differential gene expression allowed the research group to classify PDAC into four distinct subtypes – classical tumors with normal stroma, classical tumors with activated stroma, basal-like tumors with normal stroma, and basal-like tumors with activated stroma. Although even these basic categories have prognostic relevance, Yeh says that there are still two major limitations in the current understanding of PDAC. One is our knowledge of the stroma; although many groups have made a great deal of progress with that in the last few years, there's still much more to do. The second is our lack of understanding of metastatic disease. "It's rare that we have patient samples from metastatic sites," says Yeh, "and to truly make headway into the treatment of pancreatic cancer, we as clinicians should make a concerted effort to enroll patients in clinical studies and obtain permission to store specimens as frozen tissue or create patient-derived xenografts, so that the field will have have a library of metastatic samples for investigating biology and treatment options."

Thus far, Yeh and her colleagues are excited to have confirmed their subtypes in large external cohorts – and they now believe that, in the short-term, these subtypes can be used as diagnostic and prognostic tools. In the longer term, though, they plan to examine whether or not the subtypes can be used in decision-making to help tailor therapy types and timing for patients. This will become even more important as the repertoire of therapies for pancreatic cancer increases. Once it's possible to biologically explain the behavior of different pancreatic cancer subtypes, clinicians can be encouraged to stop thinking of it as one homogeneous disease. Pathologists will then be able to use that knowledge in conjunction with existing evaluations to inform patients' individual therapy plans.

References

1. *AV Biankin et al., "Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes", Nature, 491, 399–405 (2012). PMID: 23103869.*
2. *AK Witkiewicz et al., "Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets", Nat Commun, 6, 6744 (2015). PMID: 25855536.*
3. *RA Moffitt et al., "Virtual microdissection identifies distinct tumor- and stroma-specific subtypes of pancreatic ductal adenocarcinoma", Nat Genet, 47, 1168–1178 (2015). PMID: 26343385.*

Friend or Foe?

Screening for pancreatic cysts can help with early detection and treatment – but how can we tell which lesions are benign and which may progress to cancer?

By Anne Marie Lennon

A lot of the press around pancreatic cancer focuses on the difficulty in detecting early-stage disease and in treating advanced forms. As a result, most research focuses on ways to detect more cancers earlier in the disease, and on how to improve subsequent interventions. But not every pancreatic cyst we detect is a precursor to cancer.

Unfortunately, the currently available methods for distinguishing between harmless and precancerous pancreatic cysts are very poor. Although detecting the cysts gives us the opportunity to prevent or intervene early, there's also a risk that patients may undergo significant surgery – with a mortality rate of up to 2 percent in high-volume centers (1) and even more elsewhere (2) – without actually needing it. Recent studies have shown that the accuracy of cyst fluid carcinoembryonic antigen (CEA), currently considered the gold standard for differentiating mucinous cystic neoplasms from other cysts, is only 63 percent (3). But if we could reliably identify benign (serous) cysts and intraductal papillary mucinous neoplasms (IPMNs) with high-grade dysplasia or early invasive adenocarcinoma, we could determine which patients needed which interventions – and significantly impact patient care.

Combination is key

At the moment, we examine pancreatic cysts by endoscopic ultrasound-guided biopsy, aspirating fluid directly from the cyst and then testing it for the presence of malignant cells or proteins associated with cancer. But fewer than two-thirds of cyst fluid CEA tests are accurate, and surgical series have only reinforced the issues. In some cases, over 20 percent of resected cysts were found to be entirely benign (4), while in others up to 78 percent of branch duct IPMNs harbored only low- or intermediate-grade dysplasia (5). It seemed clear to us that a better system was needed.

The word "system" is appropriate here, because we believe that

the solution doesn't lie in a single test. To get all of the relevant information, it's important to evaluate a combination of tests reflecting various features. We began our investigations by using molecular markers to identify the cyst type – which worked very well for some cysts (like solid-pseudopapillary neoplasms), but not quite as well in others (like IPMNs). So, to fill in the blanks, we combined them with clinical features like the presence of a dilated main pancreatic duct. Overall, our multicenter retrospective study (6) incorporated 130 patients with resected pancreatic cystic neoplasms, 74 of whom were later determined not to have needed surgery. Our panel of molecular markers correctly identified 67 of those patients – which could have reduced the amount of unnecessary surgery by 91 percent. After adding in the clinical features, our testing was able to classify cyst type with 90 to 100 percent sensitivity and 92 to 98 percent specificity. That's a significant improvement in the overall accuracy of testing, and could mean a significant decrease in the number of patients undergoing risky operations they don't truly need.

Fighting false-positives

The testing still has limitations, though – this is a preliminary paper, so both the molecular markers and the clinical features we identified need to be validated in a larger series before being used in clinical practice. We're currently completing a large multicenter trial, the data from that trial should be available next year. My personal opinion is that the markers will be available in clinical practice towards the end of 2016. When they reach the clinic, we hope that they will provide additional information for pathologists, improving our ability to both correctly classify the type of cyst and detect the presence of high-grade dysplasia or invasive cancer.

We've previously demonstrated that there's a large window of opportunity for detecting and treating pancreatic neoplasms before they progress beyond the point of curability (7). We have both imaging and molecular methods for detecting curable lesions – and different research groups are making great strides forward in those areas (see "Tapping a Rich Vein"). But what we haven't had to date is a good way of distinguishing between benign and precursor lesions, meaning that a false-positive result can lead to unnecessary and potentially harmful treatment in over one-fifth of cases. We hope that our system of molecular markers and clinical features will improve the accuracy of screening and help doctors make the best decisions for each individual patient.

Anne Marie Lennon is associate professor of medicine and surgery at the Johns Hopkins University School of Medicine, and the director of the Multidisciplinary Pancreatic Cyst Clinic and an attending gastroenterologist at the Johns Hopkins Hospital, Baltimore, USA.

References

1. *JL Cameron et al., "One thousand consecutive pancreaticoduodenectomies", Ann Surg, 244, 10–15 (2006). PMID: 16794383.*
2. *RF de Wilde et al., "Impact of nationwide centralization of pancreaticoduodenectomy on hospital mortality", Br J Surg, 99, 404–410 (2012). PMID: 22237731.*
3. *M Al-Haddad et al., "Performance characteristics of molecular (DNA) analysis for the diagnosis of mucinous pancreatic cysts", Gastrointest Endosc, 79, 79–87 (2014). PMID: 23845445.*
4. *NP Valsangkar et al., "851 resected cystic tumors of the pancreas: a 33-year experience at the Massachusetts General Hospital", Surgery, 152, S4–S12 (2012). PMID: 22770958.*
5. *K Sahora et al., "Branch duct intraductal papillary mucinous neoplasms: does cyst size change the tip of the scale? A critical analysis of the revised international consensus guidelines in a large single-institutional series", Ann Surg, 258, 466–475 (2013). PMID: 24022439.*
6. *S Springer et al., "A combination of molecular markers and clinical features improve the classification of pancreatic cysts", Gastroenterology, 149, 1501–1510 (2015). PMID: 26253305.*
7. *AM Lennon et al., "The early detection of pancreatic cancer: what will it take to diagnose and treat curable pancreatic neoplasia?" Cancer Res, 74, 3381–3389 (2014). PMID: 24924775.*

(Chemo)resistance Is Futile

The epithelial-to-mesenchymal transition in pancreatic cancer may play a role in its resistance to treatment – and inhibiting it may improve treatment efficacy

While late diagnosis remains a key reason for the dismal outcome of pancreatic ductal adenocarcinoma (PDAC), the fact that it is difficult to treat using non-surgical methods also presents a big problem. PDAC tumors are often resistant to chemotherapy; only two agents are currently approved to treat advanced disease. The first, gemcitabine, increases median survival by just over one month (from 4.41 to 5.65 months) compared with the previously used drug, 5-fluorouracil (1). Adding the second, erlotinib, has an even smaller effect – increasing survival by only one-third of a month (2). Despite the many Phase III trials conducted to improve the efficacy of chemotherapy – using everything from traditional chemotherapy to experimental targeted approaches – PDAC remains stubbornly resistant to treatment (3).

Why? Because very few patients ever experience a good response to chemotherapy, we know that PDAC's resistance to treatment is primary (innate), rather than secondary

(acquired) as in most other cancers – but what we haven't known is what gives rise to this resistance. One research team from the University of Texas MD Anderson Cancer Center pointed the finger at the epithelial-to-mesenchymal transition (EMT). The EMT program plays a role in metastasis, but the researchers noticed that when cancer cells begin to migrate, they stop proliferating. Lead author Raghu Kalluri explained that "gemcitabine works primarily on cancer cells that are dividing or proliferating. When cancer cells suspend their proliferation – such as when they launch an EMT program – then anti-proliferation drugs like gemcitabine do not target them well," (4).

To examine the role of EMT in PDAC, Kalluri and his colleagues generated mouse models of PDAC that featured a deletion of either Snail or Twist – two transcription factors responsible for the EMT program. Deleting either of those proteins had no effect on tumor pathology, invasion or metastasis, but it did increase cancer cell proliferation and gemcitabine sensitivity (5). "We found that the EMT program suppressed drug transporter and concentrative proteins, which inadvertently protected these cancer cells from anti-proliferative drugs, such as gemcitabine," said Kalluri – so suppressing the influence of that program resulted in a stronger response to chemotherapy, including reduced tumor burden and significantly better survival. What does this mean for patients? No research has been conducted yet in humans, but the promising results in mice indicate that EMT suppression may be an intriguing target worthy of further investigation.

References

1. *HA Burris 3rd et al., "Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial", J Clin Oncol, 15, 2403–2413 (1997). PMID: 9196156.*
2. *MJ Moore et al., "Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group", J Clin Oncol, 25, 1960–1966 (2007). PMID: 17452677.*
3. *PE Oberstein, KP Olive, "Pancreatic cancer: why is it so hard to treat?", Therap Adv Gastroenterol, 6, 321–337 (2013). PMID: 23814611.*
4. *MD Anderson, "Study reveals why chemotherapy may be compromised in patients with pancreatic cancer", (2015). Available at: http://bit.ly/1MZsiCV. Accessed December 3, 2015.*
5. *X Zheng et al., "Epithelial-to-mesenchymal transition is dispensable for metastasis but induces chemoresistance in pancreatic cancer", Nature, 527, 525–530 (2015). PMID: 26560028.*

Breaching Cancer's Defenses

A new immunotherapy approach shows that engineered T cells are able to penetrate into pancreatic tumors and directly attack the cancer

By Ingunn Stromnes

Pancreatic ductal adenocarcinoma (PDAC) is unique among cancers for its survival mechanisms, which include the ability to survive with limited blood supply and low oxygen, and to protect itself from the immune system. The lack of angiogenesis means that it's difficult for chemotherapy to reach the cancer cells; the hypoxic tumor environment means that radiation therapy is of limited use; and the ability of the cancer to induce inflammation and condition immune cells in its favor means that it's able to avoid the body's natural defenses. As a result, despite advances both in treatment options and in our understanding of the disease, we remain unable to effectively penetrate PDAC's fortress – the majority of patients present with locally advanced or metastatic disease that is inoperable, meaning that they have only months to live, and no known therapy provides lasting benefit.

The immune advantage

In previous research, we were able to deplete a particular subset of immune suppressor cells in PDAC and unmask the ability of the adaptive immune response to target the cancer (1). Continuing on from this work, we decided to investigate a way of overcoming the immunological barriers set up by

PDAC, knowing that developing an effective immune therapy to treat this disease was likely to change the therapeutic landscape, and that the principles we learned would likely translate to other types of solid tumors. T cell therapy is not entirely new – it's currently under investigation in a variety of leukemias and lymphomas. But treating solid tumors with T cells is harder, because it's not always possible for the cells to penetrate the tumor tissue. So we knew that if we were able to develop a method that allowed T cells to attack the PDAC effectively, we might be able to broaden our horizons to include other tumors as well.

Immunotherapy is quite attractive because it's highly specific to targeting the malignant cells, leaving healthy tissue unharmed. T lymphocytes, the type of cell we engineer to target and kill cancer cells, have the ability to form memory – so their antitumor activity can be long-lived. Lastly, immunotherapy lets us take advantage of millennia of evolution. T lymphocytes naturally traffic throughout all of the body's tissues. It's conceivable that no site is off limits, including distant metastases, dormant tumor cells and desmoplastic tumors. This is particularly important in PDAC tumors because of their ability to form a dense shell around themselves, compressing blood vessels and preventing chemotherapy access.

An engineered attack

The main issue with the current treatments is that they have minimal, if any, clinical benefit. Chemotherapy is not specific, very toxic and typically has only transient or palliative benefit. It's also unable to penetrate bulky pancreatic tumors due to high interstitial pressure and compressed blood vessels. And of the minor population of pancreatic cancer patients who are able to undergo surgery, only 20 percent will survive for five years – so even surgery isn't curative in most patients. It's clear that we need a better way to attack these tumors.

Our immunotherapy method involves isolating a population of T lymphocytes and engineering them to express a particular affinity-enhanced T cell receptor. This receptor specifically recognizes an epitope of a protein overexpressed by tumor cells. We chose to target the protein mesothelin, which is highly expressed in most PDACs, as well as in several other cancers. After our T cells are ready, we expand them in culture and transfer them back into patients – who, in this preliminary study (2), were mice.

Eight days after infusing our T cells into the mice, we observed increased tumor cell apoptosis, showing that the cells were doing their job. But by day 28, that effect had been lost, thanks to the inhospitable environment of the PDAC tumors. We provided the mice with a second infusion of the cells to see whether or not the tumors remained susceptible, and saw the same effects again. Eventually, we randomized mice to receive either our T cells or a control T cell infusion every two weeks – and saw that, while control mice showed consistently progressing disease, those receiving our treatment showed objective responses, including increased tumor cell apoptosis, decreased metastatic disease and malignant ascites, and almost double the median survival time (54 days in control vs. 96 days in treated mice).

Taking T cells to trial

In these preclinical studies, the engineered T cells preferentially accumulated in the tumor and metastases, killed cancer cells, persisted indefinitely, and prolonged survival. They showed another advantage as well – they specifically targeted the cancer without toxicity to the mice. Some of the proteins that we target are also expressed at low levels in some normal tissues, which means that there is potential for some toxicity, but after extensive evaluation in our preclinical models, we detected none. So not only are these T cells able to penetrate the biophysical barriers that chemotherapy can't, they offer the chance for an improved safety profile as well.

But this is a living cell therapy, which means it's more cumbersome to generate and requires access to an experienced good manufacturing practice (GMP) facility. And at the moment, the suppressive tumor microenvironment shuts down T cells over time – meaning that patients must receive regular infusions, increasing the burden on both patient and facility. We are currently working on how best to refine our approach so that we can sustain T cell expansion and function within the harsh tumor environment.

In the meantime, our first priority is to translate these result to patients as quickly as possible. We now have the equivalent T cell receptors for engineering human cells and hope to open a trial in the near future. My hope is that our approach will eventually significantly prolong survival in patients with advanced pancreatic cancer. The fact that it's more technically challenging to deliver is less of a

concern – if we have an effective solution, it will change how patients are treated and ultimately bypass the need for toxic chemotherapies altogether.

Ingunn Stromnes is a researcher at the Fred Hutchinson Cancer Research Center, University of Washington, Seattle, USA.

References

1. *IM Stromnes et al., "Targeted depletion of an MDSC subset unmasks pancreatic ductal adenocarcinoma to adaptive immunity", Gut, 63, 1769–11781 (2014). PMID: 24555999.*
2. *IM Stromnes et al., "T cells engineered against a native antigen can surmount immunologic and physical barriers to treat pancreatic ductal adenocarcinoma", Cancer Cell, 28, 638–652 (2015). PMID: 26525103.*

An Epigenetic Epiphany

When genetics yielded unsatisfactory answers about pancreatic cancer's persistent survival, researchers looked beyond the genome – and found telling epigenetic changes

Many researchers have investigated the genetics of pancreatic cancer, hoping to find answers to the disease's mysteries. Some studies have struck gold with oncogenic events like KRAS mutations (1), which occur in almost all cases of pancreatic ductal adenocarcinoma (PDAC) – but then discovered that treatments targeting those mutations are hampered by dose-limited toxicity or disease resistance. More recently, next-generation sequencing has revealed mutations in several genes that code for chromatin regulators (2,3), suggesting that epigenetic factors might be responsible for some properties of PDAC tumors – perhaps even their persistent survival.

Based on their knowledge of the properties of proteins in the BET (bromodomain and extraterminal) family, researchers from the Technical University of Munich and Stanford University decided to use them to investigate the possibility of an epigenetics-based therapy for PDAC. BET proteins use their bromodomains to recognize acetylated lysines on histones, the proteins involved in DNA packaging in the cell. Histone acetylation is associated with increased transcription and a more open, accessible chromatin structure – including in oncogenes like MYC, thereby increasing the survival and proliferation of abnormal cells that would otherwise undergo apoptosis.

To generate their treatment, the researchers examined the expression of BET proteins in PDAC tumors and found three proteins – BRD2, BRD3 and BRD4 – in preneoplastic and neoplastic lesions. They then used a mouse model to test a small molecule known as JQ1, which inhibits the function of those proteins (4). By inhibiting the BET proteins, the researchers were able to decrease both MYC activity and inflammatory signaling, suppressing PDAC development. But JQ1 alone wasn't effective enough – the mice still ultimately succumbed to their disease. So the researchers investigated agents that could be used alongside the small molecule to improve treatment and discovered that the addition of the small molecule SAHA – which inhibits histone deacetylation – had a synergistic effect.

"They've already begun investigating potential biomarkers – like the gene p57, which may be a key mediator of the drugs' function."

This is an especially promising start because JQ1 (as TEN-010) is already in clinical trials and SAHA (as vorinostat) has been approved by the US Food and Drug Administration for use in cutaneous T cell lymphoma. Because the researchers don't need to start from scratch, their treatment may reach the clinic more quickly than a brand new combination. With that in mind, they've already begun investigating potential biomarkers – like the gene p57, which may be a key mediator of the drugs' function and a predictor of treatment success.

References

1. *MA Collins, M Pasca di Magliano, "Kras as a key oncogene and t herapeutic target in pancreatic cancer", Front Physiol, 4, 407 (2014). PMID: 24478710.*
2. *AV Biankin et al., "Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes", Nature, 491, 399–405 (2012). PMID: 23103869.*
3. *AK Witkiewicz et al., "Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets", Nat Commun, 6, 6744 (2015). PMID: 25855536.*
4. *PK Mazur et al., "Combined inhibition of BET family proteins and histone deacetylases as a potential epigenetics-based therapy for pancreatic ductal adenocarcinoma", Nat Med, 21, 1163–1171 (2015). PMID: 26390243.*

Building a Better Mousetrap

It's often difficult to target pancreatic cancer cells while sparing healthy tissue – but a new therapy concept not only makes this possible, but also enhances the potential effectiveness of adjuvant treatments

"Minimally invasive" would not typically be a term that you would associate with pancreatic cancer treatment, in fact quite the opposite, but one team in Ireland believe they've made a breakthrough, and discovered a technique that's just that!

It's a two-part process. First, tiny, oxygen-filled microbubbles with an attached inactive chemical agent are delivered to the tumor tissues by injection. Second, the sensitized tumor is exposed to low-intensity ultrasound waves, breaking up the bubbles and activating the attached drug. This serves more than one purpose – not only is the drug delivered directly to the tumor without damaging healthy tissue along the way, but the oxygen itself also assists with treatment, improving the function of therapies like radiation that require oxygen to work.

> *"Because we can control exactly where the sound waves go, we can selectively target the tumor and spare healthy tissue."*

Ulster University's Norbrook Chair of Pharmaceutical Science, John Callan, explained, "Because we can control exactly where the sound waves go, we can selectively target the tumor and spare healthy tissue making this a highly targeted therapy with reduced side effects," (1).

The researchers have named this technique "sonodynamic therapy" (SDT) and are excited by its potential, in particular given that their initial testing has shown a five-fold reduction in tumor size on pancreatic ductal adenocarcinoma (PDAC). SDT is not the first of its kind – similar techniques, like photodynamic therapy, exist – but it has advantages over other established treatments because ultrasound waves are capable of much deeper tissue penetration than light (2). It's a uniquely beneficial approach for pancreatic cancer because of the disease's characteristic low blood supply and large tumor size at diagnosis; increasing the tumor's oxygen content can make radiotherapy and some chemotherapies more effective, while successful shrinking of the tumor can make surgery an option for larger population of patients.

Ultimately, the researchers hope to make pancreatic cancer a treatable disease, even in patients who have more advanced, or less accessible, tumors.

References

1. *Ulster University, "Ulster University scientists reveal breakthrough in fight against pancreatic cancer", (2015). Available at: http://bit.ly/210pGfj. Accessed December 7, 2015.*
2. *AP McHale et al., "Sonodynamic therapy: concept, mechanism and application to cancer treatment", Adv Exp Med Biol, 880, 429–450 (2016). PMID: 26486350.*

In Practice

Technologies and techniques
Quality and compliance
Workflow

A New Dimension in Biomarker Research

RNA in situ hybridization offers a new way to overcome the challenges of advancing RNA cancer biomarkers to the clinic

By Xiao-Jun Ma

Cutting-edge cancer research aims to unravel a tumor's complexities at the molecular level. There are many different ways to work toward this goal – we can investigate the cancer genome, transcriptome, or proteome. Each of these can provide unique information, but comes with its own set of capabilities and limitations (see Table 1).

For the last two decades, the transcriptome has been a focus in cancer research – which has led to the discovery of numerous promising RNA biomarkers for diagnosis, prognosis and prediction of therapy benefit. But identifying biomarkers is only half the battle; to validate them and develop them into clinical applications, we need improved methods for RNA analysis in clinical specimens.

	Pros	*Cons*
Genome	• Rapidly decreasing cost of genome sequencing • Detection of gross changes (amplifications, deletions and rearrangements) in the genome by fluorescence *in situ* hybridization	• Provides a static view of the genome • Does not reliably predict functional relevance
Proteome	• Can examine both proteins themselves and their post-translational modifications • Protein chip technology is rapidly advancing	• Antibody-based detection is limited; high-quality antibodies are often unavailable or vary in sensitivity and specificity • Protein-coding sequences account for less than 1.5 percent of the human genome and about one-third of known genes, so information may be missed
Transcriptome	• Whole transcriptomic profiling is practical • RNA is a versatile marker capable of reflecting the dynamic nature of cancer • RNA *in situ* hybridization technology is rapidly advancing	• Limited to transcribed material • Does not capture protein dynamics at the translational and post-translational levels

Table 1. A comparison of the pros and cons of genomic, proteomic and transcriptomic approaches to the molecular analysis of tumors.

At a Glance

- *There are many ways to explore cancer at the molecular level, but each method comes with an individual profile of advantages and disadvantages*
- *Whole transcriptomic data can be generated rapidly by microarray and RNA sequencing techniques, fueling the growth of RNA biomarkers*
- *RNA in situ hybridization (RNA ISH) can complement protein visualization techniques and provide access to information on noncoding RNAs*
- *RNA ISH allows direct visualization of RNA biomarkers in their native morphological context*

Tools of the trade

Most interrogations of the cancer transcriptome begin with a comprehensive transcriptomic discovery program, using high-throughput approaches like microarrays or RNA sequencing (RNA-Seq). The evolution of next-generation sequencing (NGS) over the past 10 years has offered researchers the ability to look at the cancer transcriptome in greater detail than ever before. Once we define a set of transcripts whose expression differs between healthy and tumor tissue, or between clinical outcomes, we can then focus on validating their functional significance and clinical relevance. That's the point where spatial information provided by RNA *in situ* hybridization (RNA ISH) and immunohistochemistry (IHC) come in. Analyzing RNA in its morphological context is highly desirable for cancer research, and the spatial resolution provided by RNA ISH adds a new dimension to gene expression analysis. For instance, it can provide us with precise localization of target RNA in single cells, allowing direct mapping of RNA biomarkers to specific cell types in the tumor tissue.

RNA ISH can be conducted using either isotopic or non-isotopic probes.

Non-isotopic probes, labeled with substances like biotin or digoxigenin, present a particularly pragmatic approach, improving turnaround time, sensitivity and safety in comparison with isotopic probes (1). But because RNA targets are short and the probes can only incorporate a limited amount of label, the technique lacks sufficient sensitivity for most expressed genes. Attempts to amplify the targets before hybridization, or the signals afterward, have met with a poor signal-to-noise ratio thanks to nonspecific binding and cross-hybridization in complex tumor sections. Only with recent technological advances has the sensitivity, specificity and ease-of-use improved enough to make non-isotopic ISH a practical choice (2–6). We can even perform robust single RNA molecule detection in routine formalin-fixed, paraffin-embedded (FFPE) clinical specimens (see Figure 1), unlocking the potential of RNA for a wide range of cancer study areas.

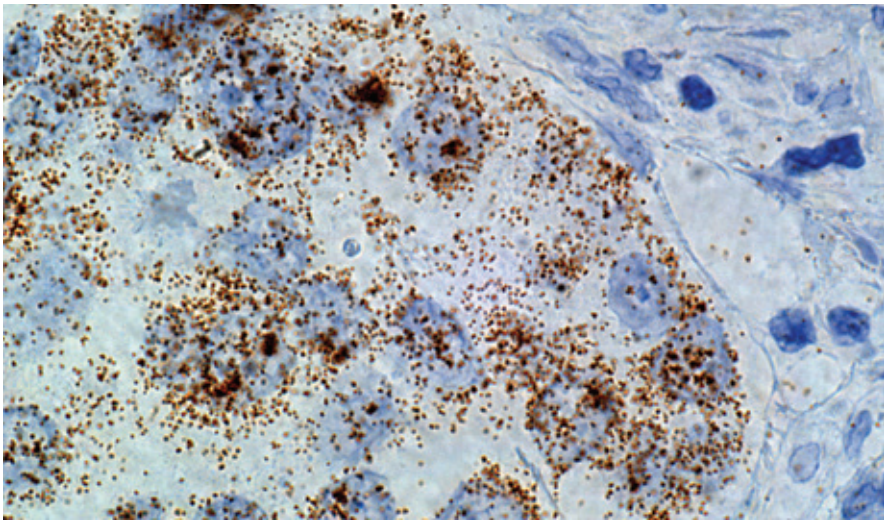


Figure 1. HER2 expression in human breast cancer FFPE tissue using RNAscope 2.0 HD Reagent Kit-Brown (Advanced Cell Diagnostics).

"Tumor molecular heterogeneity presents a significant challenge for cancer researchers and pathologists alike."

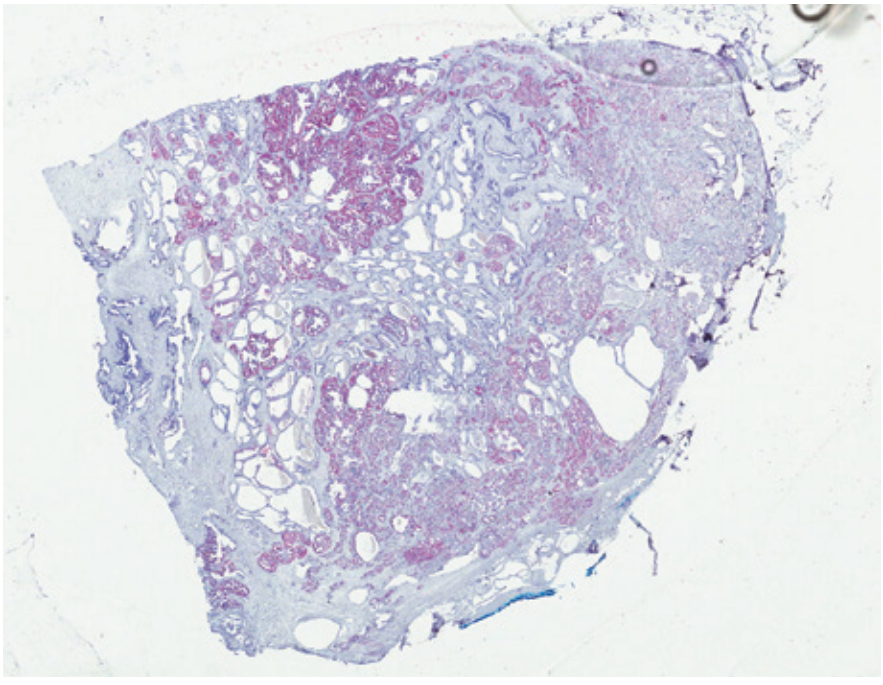


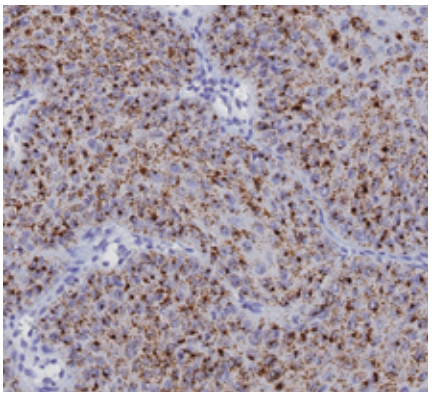
Figure 2. RNA ISH in prostate tumor tissue. This whole tissue section was probed for the noncoding PCA3 transcript using RNAscope ISH technology (Advanced Cell Diagnostics) (8).

Getting a handle on heterogeneity

Tumor molecular heterogeneity presents a significant challenge for cancer researchers and pathologists alike. Methods like RT-qPCR and RNA-Seq, which require nucleic acids to be in solution, destroy a sample's morphological context and spatial resolution. Researchers are limited to comparing RNA expression data among heterogeneous cell populations – unless they use *in situ* methods (see case study "Analyzing Prostate Tumor Molecular Heterogeneity").

No protein? No problem!

New classes of long noncoding RNAs (lncRNAs) are highly valuable as



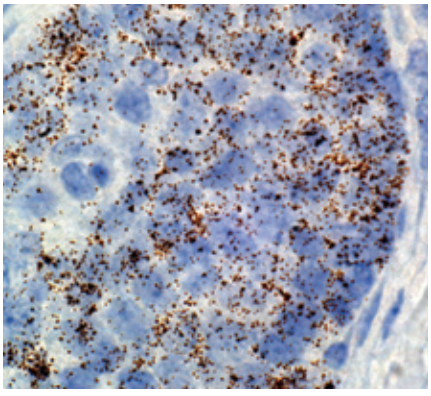


Figure 3. HPV detection using two different RNAscope ISH assay kits (Advanced Cell Diagnostics) that detect oncogenic HPV E6/E7 mRNA expression in human head and neck cancer FFPE specimens.

Analyzing Prostate Tumor Molecular Heterogeneity (7, 8)

Researcher: Nallasivam Palanisamy, associate scientist, Henry Ford Health System; associate research professor (adjunct), University of Michigan.

Research topic: Refining approaches for molecular classification to replace morphological assessment of tumors. This involves the discovery of new molecular markers in cancer, particularly recurrent gene fusions, and understanding their role in cancer development.

Methods:
Discovery – RNA-seq
Initial transcriptome sequencing presents an unbiased characterization of a given sample, identifying biomarkers in both protein-coding and noncoding genes.

Validation – RNA ISH
For subsequent biomarker profiling, RNA detection is the only option when looking at noncoding genes; even some of the markers based on protein-coding genes do not have good antibodies. Palanisamy explained, "ETV1, ETV4 and ETV5 genes are overexpressed in a small subset of prostate cancer, and RNA screening is the method of choice. Even for the genes with good antibodies, if the protein level is variable or always too low for detection, supporting protein analysis with information on RNA expression forms an unequivocal assessment."

Approach
RNA ISH followed by IHC is performed on the same slide in a sequential manner. Given the limited availability of tissue from a small biopsy, it is important to develop methods to detect more than one type of marker on the same slide. Palanisamy commented, "Development of combined protein and RNA detection methods may overcome many concerns for accurate detection of biomarkers, and I can see this being the standard practice in future molecular cancer profiling."

biomarkers capable of uncovering a specific biological trait or measurable change directly associated with a physiological condition or disease status (9). But because these RNAs are not ultimately translated into protein counterparts, however, their detection relies entirely on our ability to detect RNA in cancer biopsy samples. With several such candidates showing diagnostic and prognostic promise (7, 10), and others vital for understanding a tumor's underlying biology, RNA ISH allows for effective and reproducible *in situ* detection of lncRNA biomarkers (see Figure 2 and case study "Realizing the Potential of Long Non-Coding RNA as a Cancer Biomarker").

From bench to bedside

It's clear that the future of RNA biomarkers in the clinic is promising with the advent of modern RNA ISH technologies. In particular, companion diagnostics – vital in guiding cancer therapeutics – are central to the personalized medicine revolution. A prime example of RNA ISH application is in determining HER2 status in the management of breast carcinoma – where single-cell quantitative *in situ* RNA has been shown to be advantageous in resolving equivocal HER2 status and tumor heterogeneity (13). Viruses can also contribute to tumor development, and routine detection of viral genes demands a specific, sensitive and accessible technique. Human papillomavirus (HPV), for instance, is a causal agent in head and neck squamous cell carcinoma. Evidence for transcriptionally active HPV oncogenes E6/E7 is the gold standard for determining the presence of clinically relevant infections, but it can be challenging to detect E6/E7 mRNA using conventional techniques. PCR amplification of HPV DNA is more sensitive, but still less specific than DNA ISH – and RNA ISH has been found to provide both sensitive and specific detection (see Figure 3), facilitating a potential diagnostic standard in the future (14).

Realizing the Potential of Long Non-Coding RNA as a Cancer Biomarker (10–12)

Researcher: Rohit Mehra, clinical assistant professor of pathology at Michigan Center for Translational Pathology.

Research topic: Long non-coding RNA (lncRNA) plays an important role in the pathogenesis of genitourinary cancers, especially prostate cancer. Of the several lncRNAs important in prostate cancer, one in particular – SChLAP1 – may have clinical utility as a prognostic or diagnostic biomarker. For this, accessible methods for routine *in situ* lncRNA detection are vital.

Methods:
Discovery – RNA-seq
Comprehensively profiling the transcriptome of over 100 prostate cancer tissues and cell lines revealed that ~20 percent of RNA transcripts in prostate cancer represent novel, uncharacterized lncRNA genes (12). From this set, 121 candidate lncRNAs were nominated for further investigation.

Validation – RNA ISH
One of these candidates was re-named SChLAP1, and in the cohort studied, RNA ISH for SChLAP1 effectively stratified patient outcomes by predicting more rapid biochemical recurrence, clinical progression to metastatic disease (defined by a positive bone scan), and prostate cancer-specific mortality. Mehra commented, "This technology allows us to directly visualize gene expression in the target tissue of interest – for example, within the same sample we can tell whether gene overexpression occurs in benign prostate glands, high grade prostatic intraepithelial neoplasia (HGPIN – a precancerous state) or prostate cancer."

Because cancer is a disease of gene expression gone awry, understanding the tumor's dynamic transcriptomic landscape is invaluable for basic and translational research. From characterizing tumor heterogeneity to studying noncoding transcripts, innovative RNA ISH methods are proving their worth in modern-day cancer research. By providing morphological context as well as detection, these methods join a set of powerful approaches enabling cancer researchers to discover, develop and implement a new generation of tissue- and cell-based techniques integral to the promise of personalized medicine.

Xiao-Jun Ma is chief scientific officer at Advanced Cell Diagnostics, Hayward, California, USA.

References

1. *LI Larsson, DM Hougaard, "Detection of gastrin and its messenger RNA in Zollinger-Ellison tumors by non-radioactive in situ hybridization and immunocytochemistry", Histochemistry, 97, 105–110 (1992). PMID: 1559841.*
2. *A Raj et al., "Imaging individual mRNA molecules using multiple singly labeled probes", Nat Methods, 5, 877–879 (2008). PMID: 18806792.*
3. *HMT Choi et al., "Programmable in situ amplification for multiplexed imaging of mRNA expression", Nat Biotechnol, 28, 1208–1212 (2010). PMID: 21037591.*
4. *C Larsson et al., "In situ detection and genotyping of individual mRNA molecules", Nat Methods, 7, 395–397 (2010). PMID: 20383134.*
5. *KH Chen et al., "Spatially resolved, highly multiplexed RNA profiling in single cells", Science, 348, aaa6090 (2015). PMID: 25858977.*
6. *F Wang et al., "A novel in situ RNA analysis platform for formalin-fixed, paraffin-embedded tissues", J Mol Diagn, 14, 22–29 (2012). PMID: 22166544.*
7. *JI Warrick et al., "Evaluation of tissue PCA3 expression in prostate cancer by RNA in situ hybridization--a correlative study with urine PCA3 and TMPRSS2-ERG", Mod Pathol, 27, 609–620 (2014). PMID: 24072184.*
8. *LP Kunju et al., "Novel RNA hybridization method for the in situ detection of ETV1, ETV4, and ETV5 gene fusions in prostate cancer", Appl Immunohistochem Mol Morphol, 22, e32–e40 (2014). PMID: 25203299.*
9. *MW Pfaffl, "Transcriptional biomarkers", Methods, 59, 1–2 (2013). PMID: 23312615.*
10. *JR Prensner et al., "The long noncoding RNA SChLAP1 promotes aggressive prostate cancer and antagonizes the SWI/SNF complex", Nat Genet, 45, 1392–1398 (2013).PMID: 24076601.*
11. *R Mehra et al., "A novel RNA in situ hybridization assay for the long noncoding RNA SChLAP1 predicts poor clinical outcome after radical prostatectomy in clinically localized prostate cancer", Neoplasia, 16, 1121–1127 (2014). PMID: 25499224.*
12. *JR Prensner et al., "Transcriptome sequencing across a prostate cancer cohort identifies PCAT-1, an unannotated lincRNA implicated in disease progression", 29, 742–749 (2011). PMID: 21804560.*
13. *Z Wang et al., "Automated quantitative RNA in situ hybridization for resolution of equivocal and heterogeneous ERBB2 (HER2) status in invasive breast carcinoma", J Mol Diagn, 15, 210–219 (2013). PMID: 23305906.*
14. *JA Bishop et al., "HPV-related squamous cell carcinoma of the head and neck: An update on testing in routine pathology practice", Semin Diagn Pathol, 32, 344–351 (2015). PMID: 25724476.*

Portrait of a Dying Cell

Confocal Raman microscopy is a new, noninvasive way of obtaining morphological and chemical information about cells that may lead to better cancer research

By Katherine Lau

Autophagy is normally a good thing in a healthy host – it's a self-degradative process important for homeostasis and stress survival. Cells begin by sequestering intracellular components like proteins, lipids, micronuclei and damaged organelles in double-membraned structures known as autophagosomes; then, the autophagosomes fuse with lysosomes in the cell, allowing lysozyme to digest those components into their basic units. Autophagy recycles the basic units of intracellular components to aid in cell survival, and helps to suppress cancer and promote genome stability by removing damaged DNA, protein and organelles. But what happens when cancer is already present? In that case, the ideal scenario is to remove the tumor with minimal cytotoxicity imposed on the surrounding healthy cells – for instance, by inducing apoptosis, a kind of cell death that minimizes cytotoxicity to surrounding healthy cells.

Malignant tumor tissue is a stressful environment for several reasons: rapid proliferation makes high metabolic demands on the cells, the tumor interior is hypoxic, and cells far from the vasculature have little access to nutrients. This stress can induce autophagy – but in this case, it's bad news; autophagy can aid the survival of malignant cells by exerting cytoprotective effects and antagonizing chemotherapy-induced damage (1). That's why many successful cancer treatment regimens involve combining chemotherapy drugs with autophagy-inhibiting agents. But those are just the first steps toward addressing the complex relationship between autophagy and cancer, and we need to learn a lot more about the interplay between them (2) – so it comes as no surprise that autophagy is a hot topic in cancer research.

At a Glance

- *Autophagy normally helps healthy cells survive – but in cancer, it can aid in the survival of malignant cells*
- *An essential part of understanding cell death processes in cancer is understanding the nature of autophagy and apoptosis, and their effects on the cell*
- *Raman spectroscopic imaging measures molecular light scattering to provide a rich variety of morphological and chemical information*
- *By applying Raman imaging to autophagic and apoptotic cells, we were able to observe their features in a label-free, non-contact, nondestructive way*

Raman explained

Raman spectroscopy is a technique that provides chemical information by measuring light scattering. How does it work? Molecules vibrate at a particular set of frequencies. When light interacts with a molecule, it's usually scattered without any change in energy – called "elastic scattering." But one in a million photons scatters with an energy change, and when that happens, it's known as "inelastic" or "Raman scattering." This change in energy is specific to the individual molecules, so by measuring it, we can identify the materials in a sample. And to generate Raman images, we take the technique one step further – collecting spectra for an array of points on or in a sample and plotting pictures based on the chemical information in those spectra.

Raman images offer numerous opportunities for spatial analysis. We can use them to determine the presence of a particular material in a sample, detect unknown materials, examine the variation in a sample, or analyze the size, distribution or relative concentration of any given material. The images are powerful communication tools, too; not only can they clearly display complex visual information, but that information can be used for quantitative measurements as well.

> *"Not only can [the images] clearly display complex visual information, but that information can be used for quantitative measurements as well."*

We wanted to explore the possibilities of Raman imaging in understanding autophagy, apoptosis and cancer. There are several advantages to examining tissues with Raman imaging. For instance, it requires minimal sample preparation, you can use it on live cells to obtain both chemical and spatial information simultaneously. It reveals the complete chemical profile of the sample, meaning that there's no need to select specific targets. It's a label-free technique, which reduces

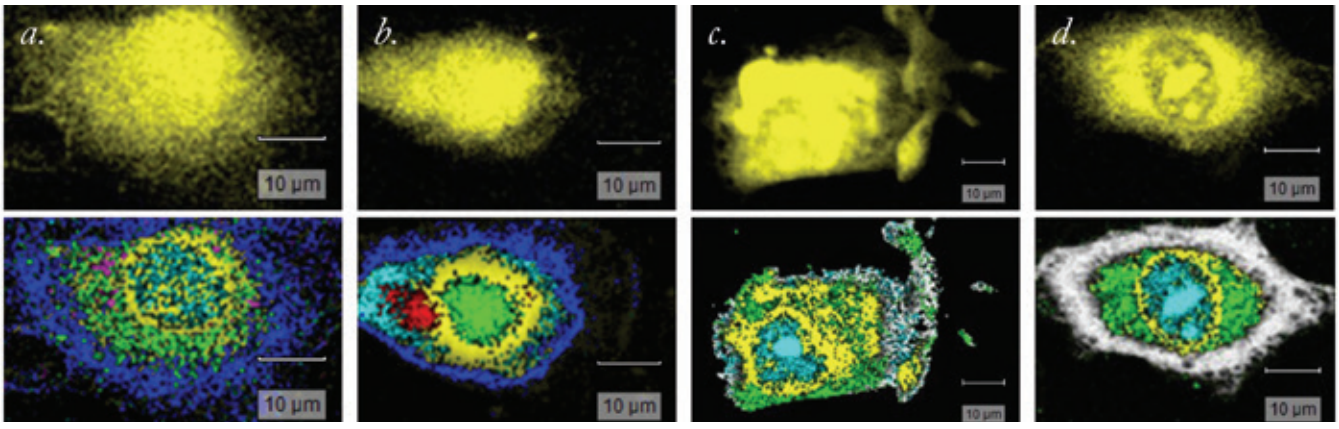


Figure 1. Phenylalanine intensity (top) and principal component analysis (bottom) images for a. normal, b. autophagic, c. early (4-hour) and d. late (24-hour) apoptotic MG-63 cells. Cellular components are shown in different false colors (cyan: nucleic acids, green: membrane-rich organelles, magenta: lipid droplets, blue: membranous areas).

the potential for artifacts. And because it's a non-contact, nondestructive method, it's possible to analyze samples multiple times – allowing the use of downstream parallel techniques to generate correlative and complementary information. Because of its many benefits, we hoped to use this relatively unknown technique to examine the morphological and chemical changes associated with autophagy and apoptosis, revealing new information on the life and death of a cancer cell.

To represent our Raman data visually, we use a technique called principal component analysis (PCA). We established the outlines of the cells and then indicated each subcellular component – nucleus, nucleoli, membrane-rich organelles and lipid vesicles – using a different color.

Autophagy or apoptosis?

In autophagic cells, we were able to spot an increased number of vesicles in the membranous areas and identify them as putative autophagosomes. Raman imaging also allowed us to examine the contents of those vesicles without extraction or staining. We were able to see autophagosomes whose color was the same as that of the cytoplasm (due to containing mainly proteins and lipids) and those that were the same color as the nuclei or nucleoli (due to the presence of nucleic acids) – results we confirmed by analyzing the original Raman spectra to verify the vesicles' contents. We also observed aberrant nucleic acid distribution in some cells, where the DNA was scattered or pushed to one side of the nucleus.

Dysregulated DNA, as we saw in our autophagic cells, eventually leads to cell death – but how does this differ from apoptosis? To answer that question, we looked at early and late apoptotic cells. Our images revealed membrane blebbing in the early apoptotic cells, but not in the 24-hour apoptotic cells. In early apoptotic cells, we also observed the fragmentation of DNA and its localization to the cell membrane. There, we could see its being packaged in readiness for expulsion as apoptotic bodies – a neat way of disposing of unwanted cellular contents! Close to the cell membrane, we saw a ceramide lipid fraction consistent with our understanding of *de novo* ceramide formation as part of the early apoptotic process. In the late apoptotic cells, we not only saw DNA fragmentation and packaging into apoptotic bodies, but we were also able to spot those apoptotic bodies outside the cell after expulsion. The location of nucleic acids and ceramide to the cell periphery are features unique to apoptosis and aren't detected in normal or autophagic cells.

The differences between autophagic and apoptotic cells are very clear – and we were able to observe them without labeling, tissue destruction, or extensive sample preparation. That makes Raman imaging a very powerful tool for cell analysis, particularly when combined with statistical evaluation methods. It can reveal a multitude of morphological and chemical information, and in our study, it has helped us to gain a better understanding of autophagy, apoptosis and cancer – hopefully, to eventually lead us to better strategies for cancer prevention and treatment.

Katherine Lau is a Raman applications scientist specializing in the life sciences at Renishaw plc, UK.

References

1. *JW Lee et al., "Dendropanoxide induces autophagy through ERK1/2 activation in MG-63 human osteosarcoma cells and autophagy inhibition enhances dendropanoxide-induced apoptosis", PLoS One, 8, e83611 (2013). PMID: 24358301.*
2. *DA Gewirtz, "The four faces of autophagy: implications for cancer therapy", Cancer Res, 74, 647–651 (2014). PMID: 24459182.*

the Pathologist App

The Pathologist iPad edition is available for free on the Apple Newsstand and offers a rich and engaging multimedia experience in a stylish, purpose-built app. The ability to read content onine and download the past issue archive make it a must-have for frequent flyers.

https://thepathologist.com/app

Available on the iPhone App Store

Check out our social channels

thepathologistmag

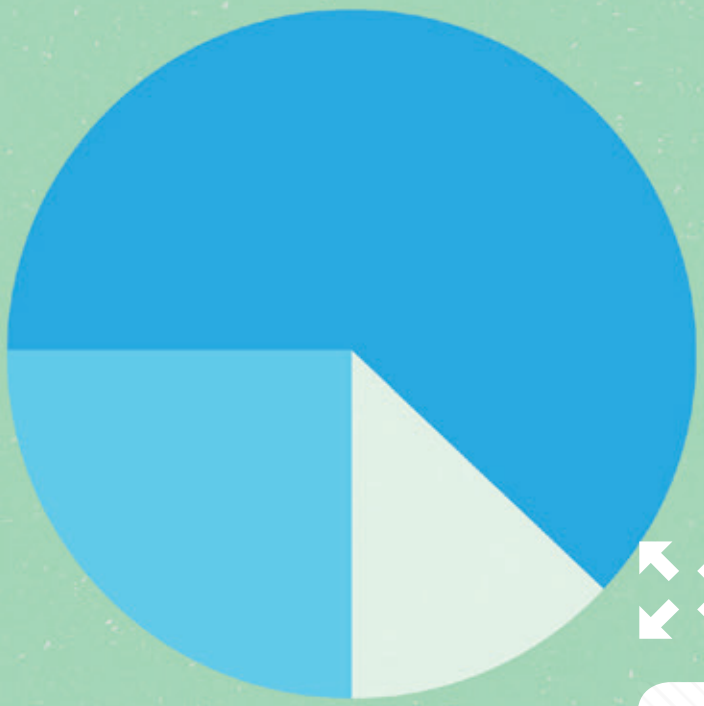
@pathologistmag

The Pathologist

thepathologistmag

NextGen

Research advances
New technologies
Future practice



Killing Cancer From the Inside

New super-resolution microscopy techniques give us better insight into how our immune cells fight cancer, and how we can help

By Kathryn Lagrue and Daniel M. Davis

There are 10,000 natural killer (NK) cells in every drop of blood (1) – and yet most people haven't heard about them. That's a shame, because these white blood cells are particularly good at killing cancer cells as well as virus-infected cells. They have the unique ability to detect cells under stress and can respond to abnormal states much faster than other components of the immune system. They can even detect cells that, due to a lack of MHC protein expression, are effectively hidden from surveillance by other lymphocytes.

Because these cells are so fascinating, much of our work has focused on trying to see what happens at the point of contact between NK cells and other cells. How do NK cells decide whether or not another cell is diseased and should be killed? What occurs at that point of contact? Some years ago, we (along with other researchers like Avi Kupfer at Johns Hopkins and New York University's Mike Dustin) learnt to our surprise that the contact immune cells make with other cells are reminiscent of what happens between neurons – and coined the term "immunological synapse" to describe it (2).

It has been difficult to find answers to a lot of questions that have arisen about the immunological synapse, though, because we simply haven't had the technology to investigate them. But recently, super-resolution microscopy has come to the fore, especially with the 2014 Nobel Prize in Chemistry awarded "for the development of super-resolved fluorescence microscopy," (3). This technology gives us the power to break through the diffraction limit and see structures as small as individual molecules. The techniques that our team use include photoactivated localization microscopy (PALM), stochastic optical reconstruction microscopy (STORM), structured illumination microscopy (SIM) and stimulated emission depletion (STED). Most of these methods rely on allowing only a few molecules in a sample to fluoresce and applying a mathematical model to find the positions of those molecules. We keep repeating the process, a few molecules at a time, until we've located every molecule in the sample. By using these techniques to study NK cells in multiple myeloma, we have been able to understand more about immune responses in disease states (Figure 1).

At a Glance

- *Natural killer (NK) cells fight cancer and other abnormal cell states*
- *Super-resolution microscopy techniques are giving us the ability to take a closer look at the interface between NK cells and target cells*
- *We can now see how activated NK cells open up their cortical actin meshwork to release lytic granules, and how their surface receptors are organized*
- *The next step: to actively reorganize those receptors and observe the effects of nanoscale structural changes on downstream immune signaling*

The two aspects of activation

Multiple myeloma is a cancer of the plasma cells, a specific type of B cell that produces antibodies. NK cells, which specialize in killing abnormal cells, are initially very good at dealing with multiple myeloma – but over time, the NK cell response decreases. One of the "blockbuster" drugs used to treat myeloma patients is lenalidomide, an immunomodulatory compound derived from thalidomide. We already knew that the drug worked to inhibit cancer cell growth directly, but whether or not it could also help NK cells attack the cancer cells was less clear.

> *"It has been difficult to find answers to a lot of questions that have arisen about the immunological synapse, though, because we simply haven't had the technology to investigate them."*

We started by asking whether or not lenalidomide directly impacts on the NK cells' ability to deal with myeloma. We looked at the release of interferon gamma (IFNγ), a small compound produced by NK cells that is involved in activating the immune system, in relation to the lenalidomide dose. We found that when we increased the dose of lenalidomide, we saw an increased IFNγ response from the NK cells. After verifying that it was a direct effect on the NK cells, we noticed something interesting – that lenalidomide actually seems to have two effects: not only does it make more cells produce IFNγ, but the amount of IFNγ secreted by each cell also increases (4).

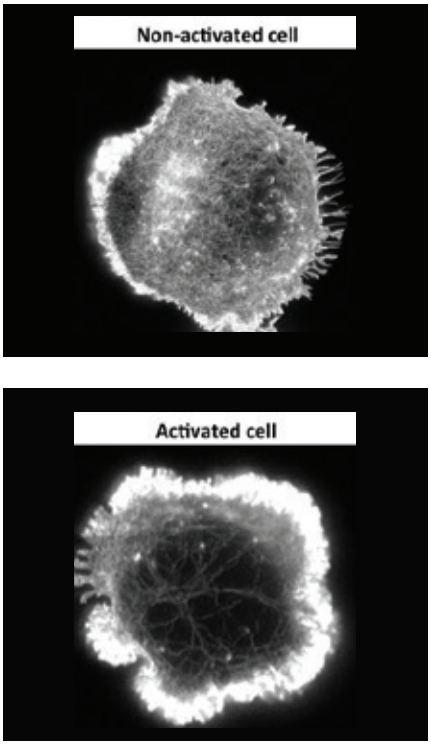


Figure 1. Super-resolution microscopy images of an inactive (top) and an activated (bottom) NK cell.

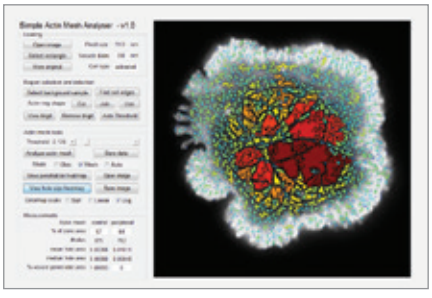


Figure 2. A screenshot of the Davis laboratory's Simple Actin Mesh Analyzer, showing a heat map of hole sizes in the actin mesh. The darker the color, the larger the hole and the more likely that area of the cortical meshwork is to be penetrable to vesicles.

The first effect – an increase in the number of cells responding – was easy to explain. At the point of contact, ligands on a cancer cell bind to receptors on the NK cell's surface. Once enough ligands have bound, the NK cell is activated and attacks the cancer cell. We titrated the number of activating ligands versus the NK cells' responses and found that when we added lenalidomide, the threshold shifted downward. And because fewer ligands were needed to activate each NK cell, more of them could respond to the same number of cancer cells. Lenalidomide is very effective at lowering this threshold. But that only explained one of the drug's effects. How is it that each NK cell also secretes more IFNγ after lenalidomide treatment? To try and understand this, we turned to super-resolution microscopy to watch what happened.

Imaging inside immune cells

Underneath the surface membrane, NK cells have a dense cortical meshwork of actin. That raises a big question: how do vesicles containing cytokines, or proteins that kill diseased cells, move through this meshwork to exit the cell? We thought we had the answer in 2009, when we saw that the actin moves out to the periphery of the cell. It looked like the center of the synapse was entirely cleared of actin. But we were wrong, because our microscopes weren't good enough. In 2011, we revisited the problem with super-resolution microscopy and saw that, in fact, the actin doesn't entirely clear from the center of the cell. We wrote some software to analyze the periodicity of the meshwork and discovered that when the NK cell is activated, the periodicity changes and the holes in the mesh enlarge (5) (Figure 2).

We then wanted to know where in the cortical meshwork there were holes large enough to allow vesicles (which average about 250 nm in diameter) to pass through. Using a super-resolution microscope, we determined that, even after activation, the granules can't pass through most of the meshwork. Only in small penetrable regions (about 4 percent of the total surface area), where the holes are largest, can they fit through (6). Lenalidomide increases the degree to which the meshwork opens up in activated NK cells, so that there are more regions from which vesicles and granules can be secreted. But it doesn't affect cells that have not been activated; it just augments their actin remodeling response, which may be important in increasing the overall cytokine release. In general, lenalidomide may be able to enhance the immune response to allow the patient's own immune system to fight the cancer.

Kathryn Lagrue is co-author of this research study, which she conducted during her PhD.

Daniel Davis is director of research at the Manchester Collaborative Centre for Inflammation Research and a professor of immunology at the University of Manchester. He's also written the popular science book The Compatibility Gene (Penguin Books, 2014).

References

1. *RM Aspalter et al., "Deficiency in circulating natural killer (NK) cell subsets in common variable immunodeficiency and X-linked agammaglobulinaemia", Clin Exp Immunol, 121, 506–514 (2000). PMID: 10971518.*
2. *DM Davis, "Intrigue at the immune synapse", Sci Am, 294, 48–55 (2006). PMID: 16478026.*
3. *Nobel Media AB, "The Nobel Prize in Chemistry 2014", (2014). Available at: http://bit.ly/1y5svPr. Accessed on October 26, 2015.*
4. *K Lagrue et al., "Lenalidomide augments actin remodeling and lowers NK-cell activation thresholds", Blood, 126, 50–60 (2015). PMID: 26002964.*
5. *AC Brown et al., "Remodelling of cortical actin where lytic granules dock at natural killer cell immune synapses revealed by super-resolution microscopy", PLoS Biol, 9, e1001152 (2011). PMID: 21931537.*
6. *AC Brown et al., "Super-resolution imaging of remodeled synaptic actin reveals different synergies between NK cell receptors and integrins", Blood, 120, 3729–3740 (2012). PMID: 22966166.*

Benchmarking Amyotrophic Lateral Sclerosis

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

By Roisin McGuigan

Amyotrophic lateral sclerosis (ALS), also known around the world as Lou Gehrig's disease, Charcot disease or motor neurone disease, is a neurodegenerative condition that causes motor neuron death in the brain and spinal cord. There is no cure, and the origin of disease is unclear in the vast majority of cases. As a field in desperate need of better therapies, ALS researchers received a windfall last summer, when the "ice bucket challenge" significantly raised their profile, and their funding. Although it remains to be seen if this funding boost can be sustained, one thing is certain – researchers have been working to better understand ALS before it found fame, and their work will continue even if the condition returns to its previous semi-obscurity.

To provide insight into past research priorities, and predictions for the future of the field, 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 "amyotrophic lateral sclerosis" and "diagnosis" (for a diagnostic focus) with results limited to the last five years, in humans (for a clinical focus). The data were analyzed in Microsoft Excel 2013.

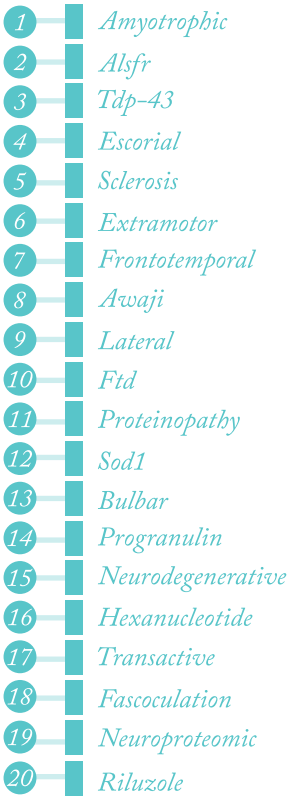
Top 20 Authors by Number of Publications

Fee or Free?

Full Text Upon Payment/Subscription 63%
Free Full Text 26%
Unavailable Online 11%

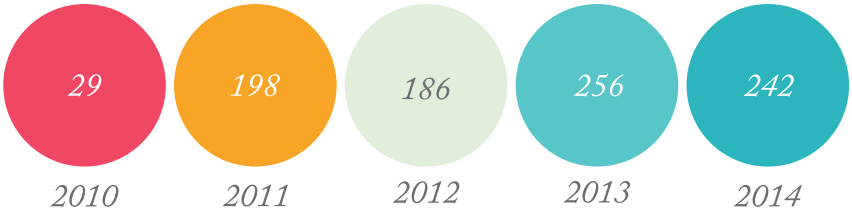
Proportion of articles by availability online.

Top 20 Important Words



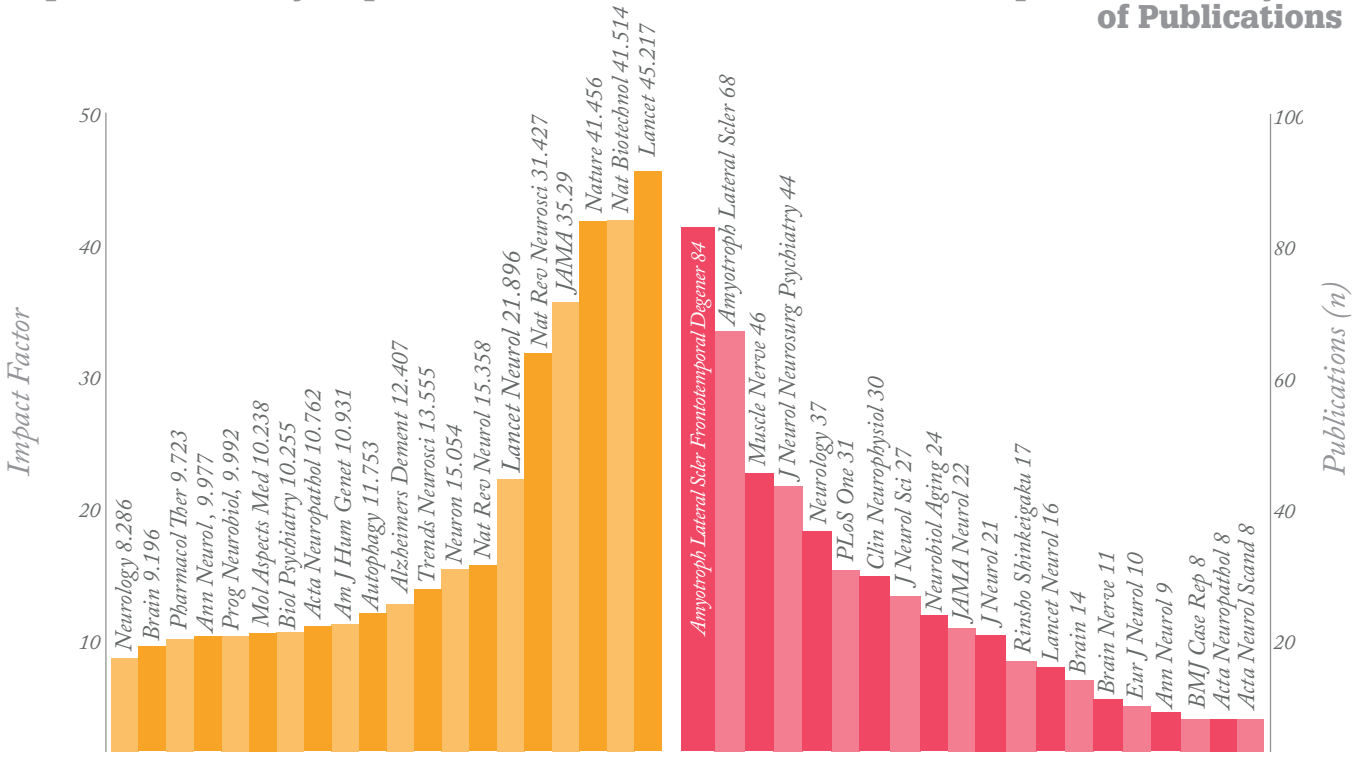
Here are the top 20 important words found in the literature. Important words have more frequent occurrences in the result subset than in the MEDLINE as a whole; therefore they distinguish the result subset from the rest of MEDLINE.

Publications per Year

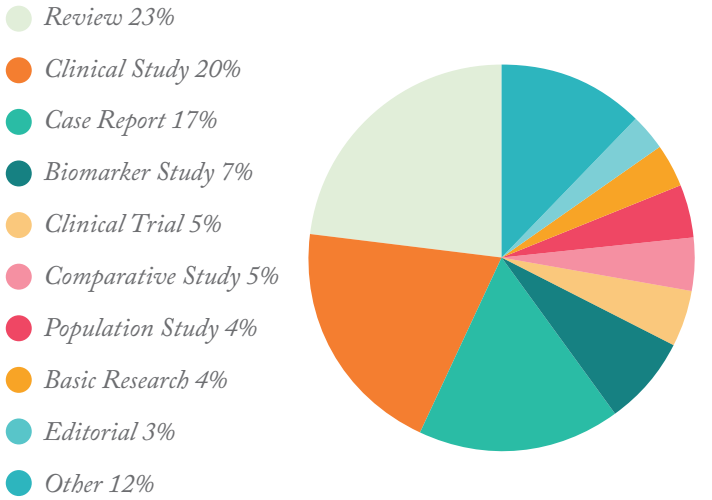


Top 20 Journals by Impact Factor

Top 20 Journals by No. of Publications

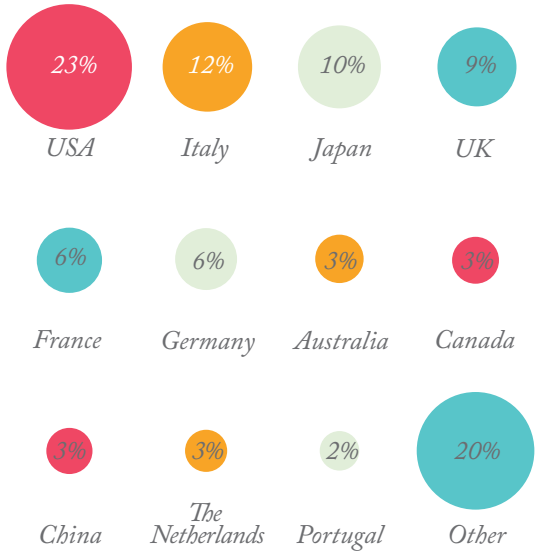


Categorization of Articles



Articles are categorized according to PubMed criteria.

Publications by Country



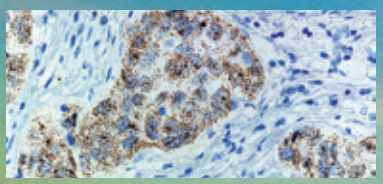
NO ANTIBODY. NO PROBLEM.

Breakthrough multiplex RNA *in situ* hybridization for any gene with RNAscope® technology.

Be Amazed.

Because up to 76% of protein-coding genes have no reliable antibody for immunohistochemistry (IHC), your research often comes to a screeching halt while you wait for a new antibody to be developed. We say forget your antibody problems. Choose RNAscope® technology. With rapid probe design and universal assay workflows for any gene, RNAscope® frees you from the hassles of antibody screening, saving you precious time and effort, while driving publication quality data.

Learn more about RNAscope® at www.acdbio.com



HER2 expression in human breast cancer FFPE tissue using RNAscope® 2.0 HD Reagent Kit-BROWN

ACD
Advanced Cell Diagnostics

Profession

Your career
Your business
Your life

An African Alliance

UK laboratory medicine professionals team up with their counterparts in Africa to address quality standards, and improve health outcomes

By Peter Chimkupete

Credit: Royal College of Pathologists

Laboratory services often lack recognition for their crucial contributions to healthcare. In many African countries, where medical resources are commonly low, this has resulted in a lack of investment, training and education, and a "bottleneck" in the healthcare system – without the right diagnosis, the appropriate treatment cannot follow, and many people face missed or inaccurate diagnoses, inappropriate treatments, and serious or even fatal consequences.

At a Glance

- *Inadequate investment in laboratories and staff training in many African hospitals has caused a diagnostic bottleneck, resulting in many delayed, inaccurate or missed diagnoses*
- *LabSkills Africa is an initiative established by the RCPath to improve the standards and quality of diagnostic and laboratory medicine services in Africa*
- *Similar to many other countries, African laboratory professionals also face the problem of recognition, with other areas of medicine being prioritized over diagnostics*
- *The more professional bodies that join initiatives like these, the greater the benefit to resource-poor areas, and the overall improvement of public awareness of lab medicine globally*

This prompted the Royal College of Pathologists (RCPath), working in partnership with the College of Pathologists of East, Central and Southern Africa (COPECSA), the British Division of the International Academy of Pathology (BDIAP) and the East, Central and Southern Africa Health Community (ECSA-HC) to initiate the LabSkills Africa (LSA) project: a collaboration aimed at improving diagnostic speed and accuracy in labs around Africa, and therefore healthcare overall. Having trained in Africa myself, I saw this as an opportunity to give something back, and to contribute to improved diagnostic services in the region.

Targeting health and mortality

The pilot of the project involved 20 laboratories; four each in Kenya, Tanzania, Uganda, Zambia and Zimbabwe. Together, these laboratories serve a combined population of 100 million people, and perform over 1.7 million tests every year.

The aim of the project is to improve the diagnosis and management of health conditions related to the United Nations Millennium Development Goals (MDGs) of reducing child mortality, improving maternal health, and combatting HIV/AIDS, malaria and other diseases, by focusing on improving seven key tests (see Figure 1). As well as increasing diagnostic accuracy and proficiency, the project hopes to shorten turnaround times, strengthen front-line services, and improve clinical decision-making based on test results.

Understaffed and invisible

Like in many other places around the world, the visibility of pathologists and laboratory professionals is a huge issue in Africa. The profession is competing with more well-recognized areas of medicine such as pharmacy and radiography, and although patients and doctors alike understand why we need medications and X-rays, they often don't realize the crucial importance of lab work. Because it functions quietly in the background, the laboratory simply isn't prioritized, and as a result is often left behind when it comes to decision making and resource allocation.

Another challenge is the lack of a

1	Rapid HIV antibody testing
2	Rapid malaria testing
3	Hemoglobin/hematocrit determination
4	Urine dipstick for sugar and protein
5	Malaria smear testing
6	Tuberculosis smear microscopy
7	Peripheral blood film smears

Figure 1. The laboratory tests LabSkills Africa focused on improving.

trained, adequately qualified workforce. Particularly in district hospitals, there is a huge deficiency in fully trained staff. Often, the most qualified people leave for other positions, leaving many medical lab assistants (MLAs) and very few qualified scientists and doctors to do the job. This lack of training, and a lack of understanding of the importance of diagnostic testing, means that often clinicians must diagnose their patients without adequate evidence.

Seeing stars

As a biomedical scientist, my aim during my time working with RCPath on LSA was to improve quality standards in the labs I visited. This didn't come without challenges – in short-staffed, resource-poor hospitals, time is very precious. It can be hard to get people to dedicate time to quality issues when they are trying to focus on taking care of patients – understandably, extra training can seem like an additional time burden. But with very few exceptions, we found that people were willing to get behind the initiative.

I found that our two-pronged approach to quality improvement worked well: the project trained pathologists, senior biomedical scientists and laboratory managers in leadership and quality management – and provided hands on technical skills development training for 40 laboratory technicians and technologists. As time went on, it was truly gratifying to see the labs I worked with improve their compliance with international standards. We were able to see real changes in standard operating procedures, and guideline and policy documents. As members of the World Health Organization Stepwise Laboratory Improvement Process Towards Accreditation (SLIPTA) program, the labs were able to see how they'd improved in meeting the essential standards – one in particular even moved from two to three stars on the WHO scale.

All in this together

For me, the most memorable moment of this project was watching one of the participating countries hold a meeting between the four hospitals we visited, and seeing them sharing their quality control (QC) methods. For example, the staff were having trouble getting a control sample to use for urinalysis – they couldn't afford to buy it. But one lab had started using saline spiked with various analytes as a cheaper alternative, and they were able to share this method with the other hospitals. Seeing them working together and supporting each other, and showing initiative, was extremely rewarding.

But with any project of this nature, it's important to question what the long-term impact will be. By choosing four key hospitals in each of the countries, we hope our work will have a knock-on effect, as they form supportive networks and continue to help each other. Some of the people we worked with have already begun preparing their own QC materials, which they are now sending on to other labs. This is great to see! The staff will also continue to have access to their UK mentors, and can ask for any advice and assistance they might need. These measures mean that both the hospitals we visited, and others around these countries, can continue to learn and improve.

The importance of mentorship

I believe one of the keys to our success in this program was that we provided mentorship, as opposed to simply training, or "aid". In a donor-recipient relationship, I don't think you get the best results – the donor may choose what to provide, and often the training or equipment received in this way is not relevant to many of the hospitals. Africa is a very big place, and you simply can't generalize.

But a mentor-mentee dynamic is different; it isn't patronizing, and fosters understanding. It's a friendly relationship with someone who wants to assist you – they've been through these issues before, and they can guide you along the path to improving. It allows the training to be led by the mentee, who can say "these are the problems we have, and this is the help we need."

Getting involved

Although my work with LSA has been, in my opinion, a great success, there is still a lot to do, and we need to get more people involved in mentoring. Professional bodies could really help, by mentoring professional bodies in Africa, and using their wealth of experience to assist with a wide number of issues. I'd love to see the different societies involved in lab work get behind this – from hematologists to chemists – to help us all move forward together. This will help to raise awareness of the important work we do as lab professionals, whether we do it in the UK, Africa, or anywhere else in the world.

Peter Chimkupete is a senior lecturer in biomedical sciences at De Monfort University, Leicester, UK.

Track or Treat?

Considering the impact of the consumer health revolution on pathology

By Shane Brown

The consumer health revolution is already sending shockwaves through the health sector. Between health apps, monitoring devices, and personal portals, consumers are more engaged in their health than ever before and they're creating a growing community centered around wellness and preventative care. Consumers are also wanting as much information and control over their own health as possible; a trend that is exemplified by the growing interest in self-ordered tests.

When you look at the size of the consumer healthcare market – $500 billion+ (1) – and the fact that consumers today are tracking everything from exercise, to sleep patterns, to blood pressure and calorie intake, this generation are potentially the most engaged patients in the history of health.

In the US, data shows a staggering 75 percent of adults already own a health and fitness device and of these, some 60 percent plan to purchase other fitness consumer products in the next year – more than four times the number in 2012. Similarly, some 80 percent of patients proactively seek out information regarding their treatment, and are increasingly preferring digital channels as a means of communication (2)

Whilst as healthcare professionals we might dismiss this trend as nothing more than a fascination in the latest devices or a self-indulgence fueled by social media, the reality is the consumer health revolution isn't happening in a vacuum. It's set to disrupt the entire health sector, including pathology. In fact luminaries in the field are already advocating the use of social media to advance academic rigor on key topics of interest to their colleagues (3).

What will this consumer-driven era in pathology look like?

In more recent times, the direct connection between pathology and patients has been limited. Referring doctors have predominantly owned that relationship, and as a result have had direct exposure to the behaviors of patients and the expectations of this group. They have also been the arbiters of control over what tests are requested, as well as translating data into lay terms and determining what information is shared with their patient. In essence, the control has been squarely with clinicians.

Given we are now in an era where consumers want to be in control of every aspect of their life, and want as much information as possible to inform their own decision making, gatekeepers will not be tolerated. So if consumers are not given more control, alternative channels will be sourced to enable them to access what they want, when they want it.

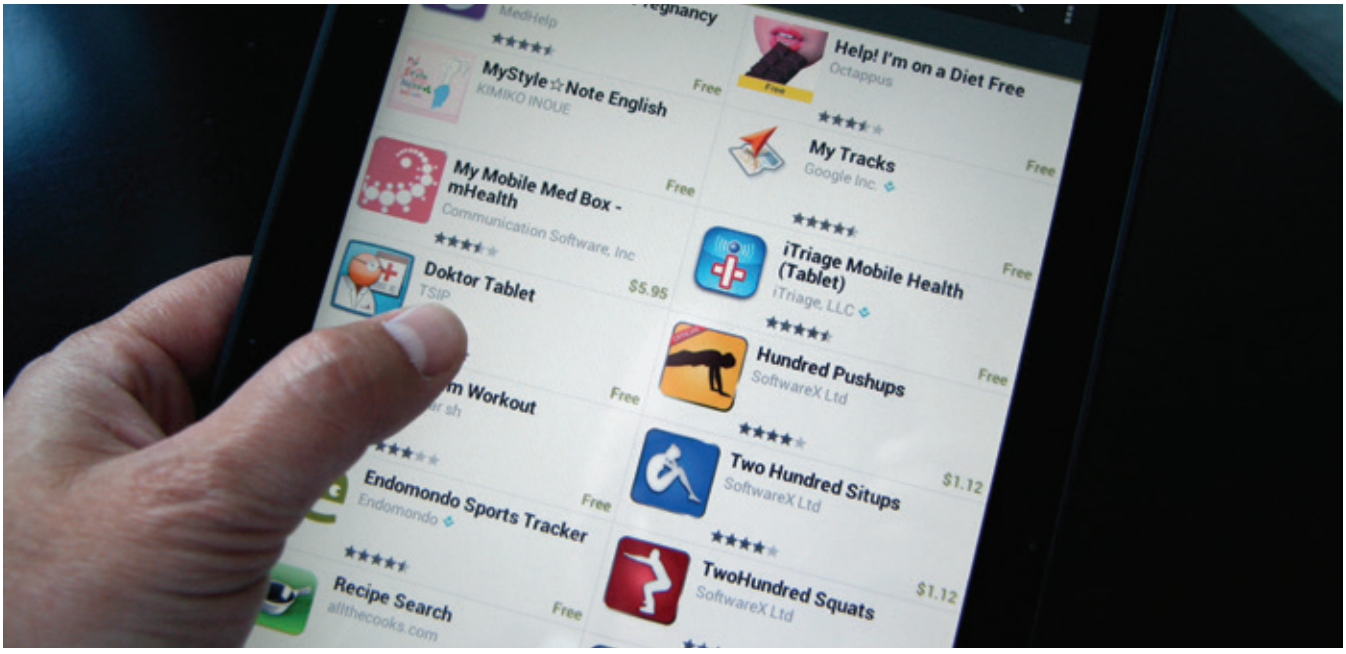
> *"The consumer health revolution isn't happening in a vacuum. It's set to disrupt the entire health sector, including pathology."*

This desire will likely to lead to a restructuring of existing relationships and may result in pathology moving closer to the patient. With impatience over access and time delays, we are already seeing a growing trend in patients ordering their own tests, and wanting their own results, without having to physically visit their doctors. Already, 55 percent of millennials have said they would trust a health app in preference to consulting health professionals, signaling a significant change as far as trusted relationships are concerned (4). A recent report states clearly that the "era of direct patient access to test results has arrived", and that this "major impact on laboratory practice" is going to necessitate development of new ways to "engage directly with patients in a way that will contribute maximally to safe and effective care" (5).

Independent of this shift, pathologists will also be confronted with unprecedented amounts of patient data generated from data rich molecular biology analyses. This will be further

At a Glance

- *The rapidly expanding use of healthcare apps, monitoring devices and websites among consumers is changing the face of healthcare*
- *With this change, comes a growing demand among the public for rapid access to personal health information, and a growing trend towards patients ordering their own tests*
- *This is having a major impact on laboratory practice; pathologists are expected to be confronted with unprecedented amounts of patient data from this new trend, as well as from molecular biology analyses*
- *Pathologists should see this as an opportunity to modernize their laboratories to better support this highly engaged community, giving access to a new revenue stream*



supplemented by complementary data fueled by the increased use of health apps, devices and portals. While some of this complementary data may be deemed irrelevant, it will help form a more comprehensive view of the patient which can assist in the speed and accuracy of diagnosis if accessed and interpreted at the right time. As the immediate past president of the European Society of Pathology Han van Krieken said recently, "For a long time, I've felt that the era of the general pathologist who knows everything is over… Even areas that used to be fairly simple have become more complicated in the sense that we can gather much more information about our patients and make precise, accurate diagnoses," (6).

Beyond trying to manage and interpret this huge volume of referential information, the insatiable desire for information and personal monitoring will likely translate into an increase in test requests that are focused more on tracking as opposed to treating. Whereas currently tests are ordered when health episodes occur or there is a degradation in a condition, today's consumer will drive a trend towards wellness tests which is more around monitoring personal health, as opposed to testing related to diagnosis and treatment.

With a growing interest in personal health portals – where individuals can aggregate and track their own health information – there has been an increased desire in gaining accurate insights into their overall health against which they can measure their performance. This will create an entirely new customer base for pathology and will likely also lead to novel test menus offered by organizations and altered reporting paradigms.

As a result, pressure will increase on laboratories to provide immediate access to test results and intelligible personalized reports as soon as the results become available. Furthermore, the new generation of consumers will expect their information to be automatically synchronized with their existing applications and portals, so they have a "one-stop-shop" for all of their health information.

Challenge vs. opportunity

As with any major change, this new era in consumer-driven pathology presents both a challenge and opportunity for laboratories.

Much like the disruption caused by the internet, the true challenge with the consumer health revolution will come from underestimating its impact, or ignoring it completely. Whilst to some it may not appear to be a welcome change, it is a change that is already underway and will become more pervasive as time goes on.

With the growth in self-ordered tests, and development of point-of-care and home monitoring devices, vital signs and a range of substances can now be analyzed by consumers. This has fundamentally changed the dynamics of how consumers engage with healthcare processes and information traditionally controlled by clinicians and pathology. The continued expansion of this trend

presents a challenge to pathology to avoid the risk of becoming disconnected from the patient care process.

Conversely, for those who embrace this change and become an early adopter in providing the type of services that meet or exceed consumer expectations, an opportunity exists to drive real change within pathology to not only better position laboratories for this new world of modern health, but to create a point of differentiation and potentially secure a significant portion of this market.

What should be done to prepare?

Modern pathology requires a modern mindset and practices, and there are three main areas which need to be addressed to prepare laboratories for the consumer health revolution:

- Understand your customers – patients versus consumers
- Modernize and personalize your systems
- Prepare your people.

To truly prepare for this change, laboratories need to first understand the difference between the modern patient and the modern consumer. By understanding the differences between tracking and treating patients – including behaviors, preferences, and expectations – laboratories can design structures, processes and resources that are aligned to support these new expectations and deliver a good service.

To be able to execute against these consumer expectations, modern laboratories will need to ensure their systems are able to support, adapt and potentially predict changes in requirements. This will require an ability to aggregate and interpret large volumes of data, to personalize and automate the results delivery, and to ensure information is shared in a timely manner in a format that is requested and easily understood by consumers themselves. Modern laboratories should also consider how their systems can support integration with other relevant channels such as portals and alerts to mobile devices, without compromising either the quality of the data or its security.

Finally, pathology staff themselves need to be organized and equipped to manage a new customer base, changes in test types and request sources, and processes for receiving, analyzing and reporting results. This might even need to be extended to provide services "whereby laboratory clinicians and scientists are available by telephone or email to discuss test results" (5).

"The Fitbit generation are changing expectations, behaviors and even business models."

Embrace and modernize

With 75 percent of patients wanting digital health services (7), the Fitbit generation are changing expectations, behaviors and even business models. While it might even be tempting to ignore the wishes of a group who source most of their information from non-traditional sources such as Google or Wikipedia, the reality is that this group could be responsible for driving a disruptive change within healthcare that is both meaningful and sustained.

When you consider that many chronic diseases are preventable by changed behaviors (8), by supporting a generation who are already engaged and informed when it comes to their health, we could not only improve the health outcomes of entire populations, we could also significantly reduce the resource strain on global health systems.

For pathology this presents a huge opportunity. For those that embrace this change, and modernize their laboratories to better support this highly engaged community, they not only have an opportunity to differentiate themselves and access new revenue streams, they can move closer to the patient and help to directly improve health outcomes.

Shane Brown is Medical Director, Pacific Knowledge Systems, Sydney, Australia.

References

1. *Accenture Consulting, "The changing future of consumer health". Accessed November 20, 2015. http://bit.ly/1X2xaiz*
2. *F Paul, "What's the market size for wearables? Bigger than you think, says CES expert", Broadcom. Accessed November 20, 2015. http://bit.ly/1l9hVTs*
3. *B Friedman, "Time for Academic Medicine to Embrace Social Media and Blogging", LabSoftNews. Accessed November 20, 2015. http://bit.ly/1NGVbBg*
4. *J Pennic, "55% of millennials would trust a health app over health professionals", Healthcare IT Consultant. Accessed November 20, 2015. http://bit.ly/1KW00qA*
5. *M J O'Kane, "Direct patient access to test results: implications for the laboratory", Ann Clin Biochem, 52, 525–526 (2015). PMID: 25995286.*
6. *M Schubert, "The changing face of pathology", The Pathologist, 7, 46–49 (2015).*
7. *iHealthBeat, "Poll: more than 75% of patients want to use digital health services", Accessed November 20, 2015. http://bit.ly/1tQv2f6*
8. *Australian Institute of Health and Welfare, "Health behaviours and their role in the prevention of chronic disease". Accessed November 20, 2015. http://bit.ly/1OVcBAc*

idylla
powered by BIOCARTIS

Biocartis' Idylla™

high precision diagnostics for high precision medicine

Idylla™, Biocartis' fully automated, real-time PCR based molecular diagnostics system, is designed to offer fast and easy access to clinical molecular diagnostic information.

The Idylla™ platform covers the entire process from sample-to-result in a time frame of 35 to 150 minutes with less than 2 minutes hands-on time. Idylla™ is applicable for a wide range of clinical sample types and can analyze both RNA and DNA. The fully integrated system enables clinical laboratories to perform a broad range of applications in oncology, infectious diseases and beyond. Today the Idylla™ KRAS Mutation Test and Idylla™ BRAF Mutation Test allow detection of KRAS or BRAF mutations directly from FFPE.

KRAS

- Detection of 21 relevant KRAS mutations in codons 12 and 13 (exon 2), codons 59 and 61 (exon 3) and codons 117 and 146 (exon 4)
- Approx. 120 minutes total turnaround time from sample to result
- Highly sensitive & standardized

BRAF

- Detection of BRAF V600E, E2, D, K, R and M mutations
- Approx. 90 minutes total turnaround time from sample to result
- Highly sensitive & standardized

If you would like a personal demonstration at your laboratory, send us your details to **seeing-is-believing@biocartis.com** and we will set-up a demo at your convenience.

BIOCARTIS
www.biocartis.com

Plus Ça Change!

Sitting Down With… Professor Michael Wells,
Emeritus Professor of Gynecological Pathology, University of Sheffield Medical School, consultant histopathologist, Leeds Teaching Hospitals NHS Trust, UK, Past President of the European Society of Pathology.

You've been a pathologist for 38 years – how has the profession changed?

First, the number of hoops we have to jump through on a regular basis has increased, including accruing CPD/CME points, and participating in EQA schemes, appraisals, and revalidation. None of these things are necessary for dedicated professionals – they are for identifying substandard practice or unprofessional behavior. But in my opinion, the UK's National Health Service (NHS) is singularly hopeless in handling this kind of issue when it does arise.

Second, increasing subspecialization has led to more staff, and in some cases, considerable disparities in workloads between consultants, and although it is beneficial to patient care, it has eroded the collective ethos of cellular pathology departments. Often, consultant pathologists will interact more with clinicians than with pathologists in their own department who are pursuing other subspecialties. In academic pathology, the pursuit of research themes has seen pathologists ally themselves with other members of their "theme", resulting in an institutional diaspora of pathologists.

That said, even after almost 40 years of practice, I still begin my morning or afternoon of work with a pile of slide trays full of glass, which I must shift from one side of the microscope to the other. My essential work has stayed much the same, but all the peripheral aspects have become more complicated.

How has gynecological pathology changed?

Without a doubt, the single most important advance in my working lifetime has been the introduction of a vaccine against human papillomavirus (HPV), though it will be several more years before its full impact is realized. It represents the culmination of 30 years of HPV research, to which I made my own modest contribution.

Molecular diagnostics are increasingly important too – for example, the identification of *FOX-2* mutations in granulosa cell tumors. However, the application of molecular pathology is incremental, and despite claims to the contrary by pundits, is not going to supplant histological diagnosis any time soon. That being said, I believe that cellular pathologists in all subspecialties must embrace the burgeoning developments in this field, and be responsible for integrating molecular diagnostic information into their cellular pathology reports.

Diagnostic gynecological pathology itself has changed surprisingly little. The mainstay is still the H&E stained section. There have been regular, often unnecessary changes in terminology in the spurious belief that by engaging in such activity the subject is being advanced.

What have been some of the highlights of your leadership work?

I have been President of several societies over the years, including the European Society of Pathology and the British Division of the International Academy of Pathology. I tried with each society to make a difference, but I consider myself a diplomat rather than a politician – I only had influence, not power!

I also found my three years as vice president of the Royal College of Pathologists an enjoyable challenge. I introduced digital pathology into the College, enhanced its influence in the rest of Europe, and fostered the increased inclusion of molecular pathology in the cellular pathology curriculum.

Tell us about the UK Pathology Summer School for medical students that you started?

I wasn't alone in noticing that we do not attract enough of the brightest graduates to pathology, as a result of the erosion of pathology in the medical undergraduate curriculum. The first Summer School was held in 2014, and was enjoyed by the participants and faculty alike. It is too soon to talk of "outcomes", but I guess the most gratifying early outcome is that the second Summer School was held earlier this year, and the proportion of those considering a career in pathology has increased.

If you could travel back in time, what would you tell yourself at the start of your career?

When I was interviewed for my Chair in Sheffield, I said I had tried to advance on the three fronts of diagnosis, teaching and research, and that I had never regarded one aspect as more important than another. Regrettably, this is an increasingly anachronistic approach. I have had a wonderfully enjoyable career that has taken me round the world with more than 400 invitations in 58 countries, and I realize that I could never have been a full-time researcher – I would always have questioned why I had a medical degree. Nevertheless, it's a brutal fact that academic careers flourish because of research performance. Teaching and diagnostic work actually count for little in terms of academic advancement, and I think it's now impossible to do all three well.

With that in mind, I would tell myself to be more ruthlessly calculating about my career steps, particularly in relation to research. The longest period of full-time research I ever had was six months at the University of Nice, where I published three papers on reproductive immunology. I was promoted too quickly, too young, but I suppose it all came out in the wash. Now I'm happily working part-time in Leeds, where I started 35 years ago. Plus ça change, plus c'est la même chose!

The Pathology Company

Leica BIOSYSTEMS

During Surgery, When Minutes Count, Choose Aperio ePathology Solutions

Remote intraoperative consultation:

Frozen section preparation and review without an on-site pathologist.

Improved Productivity

- Share expertise between locations

Fast Turnaround

- View remotely scanned slides immediately

Total Oversight

- Oversee specimen cut-up from anywhere

Find out more:
www2.LeicaBiosystems.com/Pathologist

*Aperio AT2 and Aperio CS2 scanners are members of the ScanScope family and are registered CE IVD for primary read on-screen. For more information go to LeicaBiosystems.com. Copyright Notice:

95.13307 Rev A

Aperio®
ePathology Solutions

Capture | Integrate | Analyze | Collaborate