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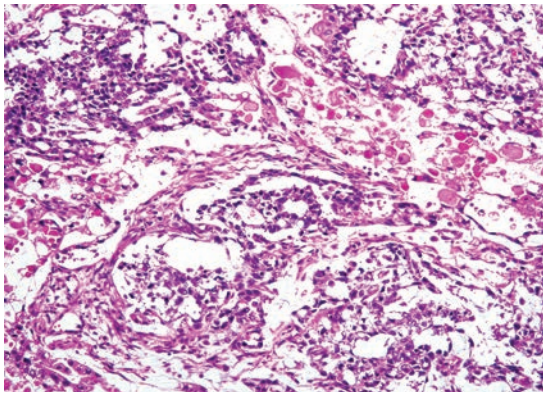
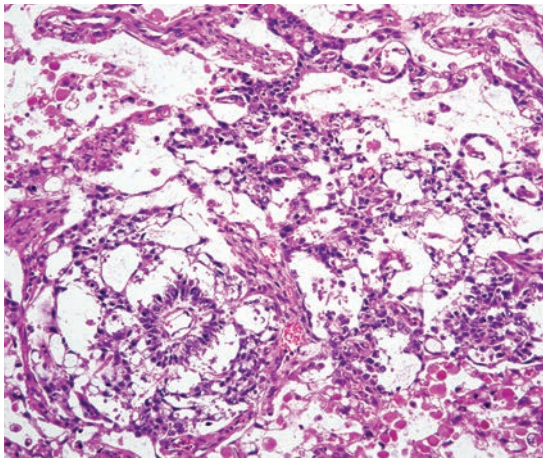
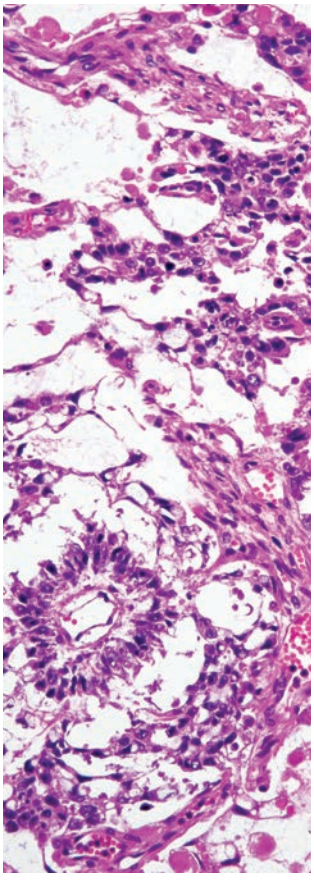
Case of the Month







Submitted by: Da Zhang, The University of Kansas, USA.

Uterine Tumor

The tumor shown here was removed by hysterectomy from a 55-year old woman complaining of metrorrhagia of three weeks' duration. The endometrial biopsy performed prior to surgery was interpreted as adenocarcinoma.

On gross examination of the resected uterus, the tumor was identified as an ill-defined, 5 cm mass protruding into the uterine cavity and superficially invading the myometrium.

What is the most likely diagnosis?

- *A* Villoglandular carcinoma
- *B* Papillary adenocarcinoma
- *C* Serous carcinoma
- *D* Yolk sac carcinoma

To register your guess, please go to http://tp.txp.to/0417/case-of-the-month
We will reveal the answer in next month's issue!

Answer to last month's Case of the Month…

D: Plasmacytoid carcinoma

The tumor is composed of round to polygonal cells arranged into loosely structured nests and cords. Tumor cells resemble plasma cells, but are actually epithelial, as proven by the positive cytokeratin immunostain. These cells may also be weakly positive for CD138, but they differ from those in plasmacytomas, which are strongly positive for CD138 and react with antibodies to immunoglobulins. Plasmacytoid carcinoma of the urinary bladder is a highly malignant tumor that typically invades the muscularis propria and the blood vessels.

Submitted by Ivan Damjanov, The University of Kansas School of Medicine, Kansas City, USA.

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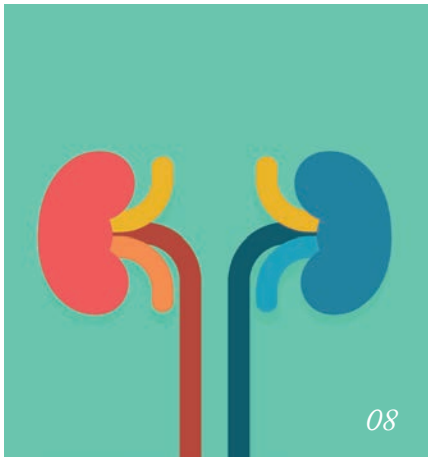

On The Cover

An image depicting the jump from traditional, knowledge-based medical education to an active, competency-based future.

Upfront

In My View

Feature

In Practice

NextGen

Profession

Sitting Down With

the Pathologist

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Start Spreading the News

Change is on the way – and you should be a part of it...

Change. Such a simple word, but one that inspires many strong, mixed emotions... fear, excitement, anxiety, optimism, trepidation... As I write this editorial, I'm in the process of transplanting myself from the UK and into the United States – a significant move! Rest assured, I'm not abandoning The Pathologist; on the contrary, we're expanding – and I've been given the incredible opportunity to set up Texere Publishing's first US office in New York. Right now, I am all too aware of how it feels to battle with extreme and conflicting thoughts, but my overriding emotion is hope. Still, although I'm certainly going to embrace the opportunity and the prospect of professional and personal development, I do know that change can be difficult to handle – even if you admit to yourself that a move away from the status quo is likely to be beneficial.

Take the changes that are happening in pathology. In early April, I was at the first global congress of the Association of Molecular Pathology in Berlin and saw a staggering infographic that plotted the explosion of new molecular diagnostic technologies over the last 10 years. Innovation in our field is coming so thick and fast that the latter part of the line chart was nearly vertical! Such remarkable progress leads to an inevitable need for change – some of which is not so welcomed by the pathology and laboratory medicine community. Why? Given my conversations with many of you, it's because of the challenges that accompany this new technology-driven era: the need for more money (during a time of austerity), higher workloads (with no increase in resources), greater training and education needs (when the number of new pathology recruits is diminishing and when course attendance is a luxury rather than a fundamental aspect of the job), IT system improvements (once again, during a time of austerity)... I guess that most of you can relate.

On the other – very positive – side of the coin are the impacts of change already occurring in the diagnostics space. Diseases are being detected more accurately, more rapidly, and treated in a more targeted fashion than ever before. Better yet, the situation is continually improving. From what I see, the laboratory community is more than accepting of these positive changes – it's often a case of struggling to find clever solutions to the aforementioned challenges. And that's where we can help. It's our job to seek out those enterprising pathologists and institutes who are implementing creative solutions against the odds.

There will always be challenges, but I'm a firm believer in the old adage, "where there's a will, there's a way." So I'm ready to embrace change – and all of the challenges that come with it. Will you join me?

Fedra Pavlou
Editor

Upfront

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

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

Email: edit@thepathologist.com

Exosome Exploration

Digging into biomarkers of immunologic transplant rejection

"There is a critical need for time-sensitive, noninvasive biomarkers to monitor transplant organ rejection or injury," says Prashanth Vallabhajosyula, assistant professor of surgery at the Hospital of the University of Pennsylvania. "In transplant patients, complications associated with transplant organ rejection/injury, and with the required immunosuppressive drugs, are the major causes of morbidity and mortality. Therefore, any biomarker platform that can accurately enable noninvasive monitoring of the transplanted organ would have a direct translational impact." To that end, Vallabhajosyula and a team of researchers (also from the University of Pennsylvania) showed that blood-based transplant exosomes can be a noninvasive alternative to needle biopsy in islet and renal transplants (1).

The investigators transplanted human islets xenogeneically into mice and found that transplanted islets undergoing rejection quickly expressed a lower level of exosome signaling via miRNA. "I was expecting to see changes in transplant tissue-specific exosomes in a time-specific manner, but it surprised me to see the changes occur so early in the acute rejection process – when there was minimal T cell infiltration and no damage of the allograft," says Vallabhajosyula. "We sincerely believe that our proposed exosome platform will enable development of a noninvasive biomarker for monitoring transplanted tissues – and that means earlier detection of rejection, minimized need for frequent tissue biopsy of the transplanted tissue, and titration of immunosuppression based on the status of the transplanted organ."

But that's not all; Vallabhajosyula suggests that transplant tissue exosomes could be manipulated in vitro and then reintroduced into the host... The first glimpse of a new therapy on the horizon? *WA*

Reference

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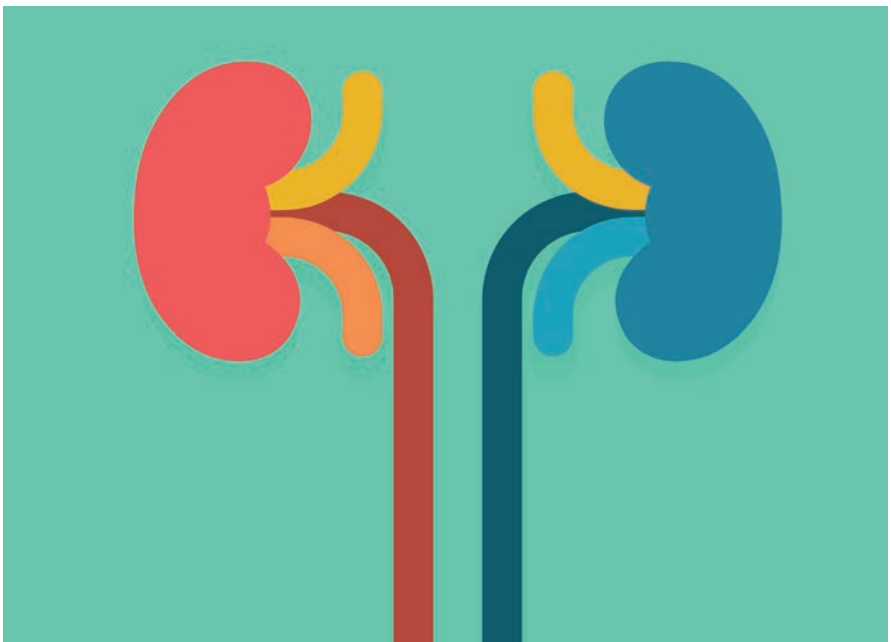


From Neurobiology to Prostate Cancer Pathology

Can the drebrin/EB3 pathway be used to predict the invasiveness of the most common cancer in men?

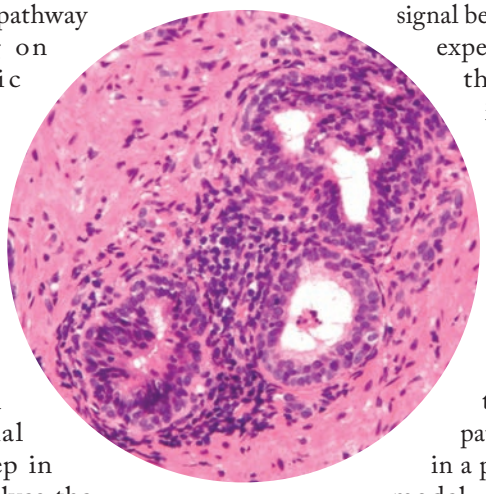
Metastatic prostate cancer is incurable – symptoms can only be managed – and there's currently no way to predict when or if the disease will metastasize (1). To that end, researchers at King's College London dug into the mechanisms behind prostate cancer – using knowledge gained in neurobiology – and discovered that the drebrin/EB3 pathway appears to play a role in prostate cancer's invasiveness (1). Knockdown of either protein's in vitro expression decreased the ability of the cancer cells to invade the prostrate stroma, while over-expression had the opposite effect. To find out if the pathway could be used as a therapeutic target or a biomarker for progression to metastasis, we speak with Philip Gordon-Weeks, lead investigator and professor of developmental neurobiology at King's College London.

What do your findings mean for diagnosis and prognosis?

We haven't cured prostate cancer, but I think we've taken a big step in the right direction. A key clinical issue in prostate cancer is predicting which prostate tumors will become metastatic. Evaluating the drebrin/EB3 pathway might help clinicians stratify patients by distinguishing between benign and malignant prostate cancer. However, I don't think this would be done in isolation – one would want to examine a panel of prognosis predictive biomarkers. The drebrin/EB3 pathway might also be a suitable target for pharmaceutical disruption. In our paper, we described using a drug (BTP2) that targets drebrin to disrupt prostate cancer cell invasion as a proof-of-principle.

How does a professor of developmental biology end up working on prostate cancer?

Well, we actually discovered the drebrin/EB3 pathway while working on the embryonic development of the nervous system. We found that it enabled embryonic neurons to respond to homing signals in the embryo that helped them to build neuronal circuits. One step in this process involves the migration of new born neurons from their birthplace in the embryonic nervous system to the final position they will occupy in the adult. The event has similarities with cancer cell invasion and metastasis – both involve homing signals and re-organization of the cytoskeleton – and so we wondered whether cancer cells might use the same cellular machinery as neurons to do this.



When we started working on prostate cancer, I thought that we would be on a one-way-street – simply applying all the conceptual insights, reagents and tools that we had worked on in our developmental neurobiology studies to investigate prostate cancer cell invasion. But we also made several unexpected discoveries about the drebrin/EB3 pathway in prostate cancer cells that encouraged us to go back and look again at developing neurons – so we were on a two-way-street after all!

What were the major challenges you had to overcome?

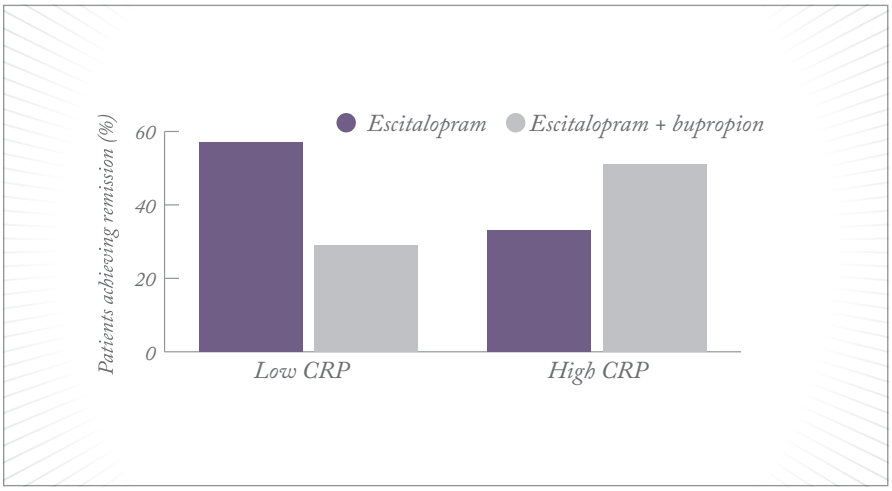
An experimental hurdle for us was trying to mimic the in vivo situation in a tissue culture dish so that we could more easily study cancer cell invasion. This meant setting up 3D cultures with concentration gradients of homing signals. We chose the chemokine CXCL12 as a signal because there is good experimental evidence that it is involved in stimulating prostate cancer cells to invade the prostate stroma and to metastasize to bone (2). We also wanted to test the role of the drebrin/EB3 pathway in metastasis in a pre-clinical in vivo model, but at the time there were none that mimicked bone metastasis.

What's next?

We are about to apply to the UK's Medical Research Council for support to continue our work, including exploiting a newly described pre-clinical mouse model of prostate cancer metastasis to test the role of the drebrin/EB3 pathway. These are conceptually simple experiments, but very powerful. We will edit out the drebrin/EB3 pathway in human prostate cancer cell lines using CRISPR/Cas-9, and orthotopically transplant the cells into the prostate of immunocompromised mice. If the transplanted cells multiply but fail to metastasize then this will directly demonstrate the importance of the drebrin/EB3 pathway in prostate cancer cell metastasis.

The Combination Question

Testing systemic inflammation could help personalize treatment for depression



Remission rates of patients with low vs. high CRP levels treated with escitalopram either alone or in combination with bupropion.

When facing down depression, doctors can find themselves taking shots in the dark. With dozens of different medications available – and only self-reported, nonspecific symptoms to use in diagnosis – how do they decide which patients should receive which antidepressants? Well, current methods are no better than flipping a coin – or so says Madhukar Trivedi, author of a new study from the University of Texas Southwestern Medical Center (1). To address the problem, Trivedi and his colleagues have introduced a new fingerprick blood test that they believe will change the way antidepressants are prescribed.

The researchers randomly gave depression patients escitalopram either alone or in combination with bupropion – and also measured each participant's C-reactive protein (CRP), serum amyloid P component, and alpha-2-macroglobulin (2). They discovered that patients with high baseline CRP levels – indicating systemic inflammation – were more likely to achieve remission with combination therapy, whereas those with low CRP levels (<1 mg/L) saw better results from escitalopram alone.

Trivedi believes that the results may extend to a host of antidepressant medications, and hopes to move on to larger studies that will test other drugs and alternative biomarkers. "Both patients and primary care providers are desperately looking for markers that would indicate there is some biology involved in this disease," says Trivedi, whose research offers a glimmer of hope. Is it possible that a simple fingerprick in the doctor's office could help guide patients to the most effective solution for their depression in the not-too-distant future? Trivedi aims to make it so. "Otherwise, we are talking about deciding treatments based on question-and-answer sessions with patients – and that is not sufficient." *MS*

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Li Detector

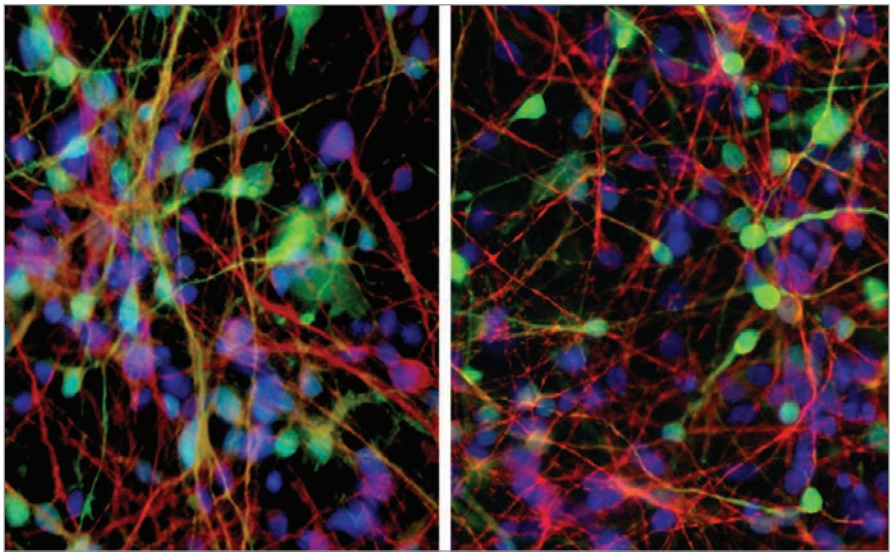
Looking at lymphocyte-derived neurons of bipolar disorder patients could offer insight into lithium therapy response

Patients with bipolar disorder often face years of difficulty before receiving an accurate diagnosis. And that's not even the end of it. Lithium – the best-known and most thoroughly researched treatment for bipolar disorder – is only effective in about 30 percent of patients. The rest? Diagnosis and treatment initiation can mean up to a year of waiting before being switched to a different treatment method.

Believing that a year is too long to wait, especially with such a stressful disorder, senior author Rusty Gage and his team of researchers wanted to find a way to predict those patients most likely to respond to lithium treatment.

The test builds upon previous work that highlighted the hyperexcitability of neurons from patients with bipolar disorder; notably, some neurons were calmed by maintenance in a lithium-containing medium, whereas others were not (1). But obtaining neurons from each patient with the disorder would be impractical in the extreme – so the researchers reprogrammed immune cells from bipolar disorder patients to generate lymphocyte-derived neurons (2). The newly derived cells exhibited the same hyperexcitability, but they also held another secret: two very different electrophysiological patterns – one for lithium responders and another for non-responders.

Hoping that the patterns could predict the potential for response in new patients, the researchers trained a computer program on 450 total neurons, using five patients as a teaching set and the sixth as



Credit: Salk Institute.

Neurons from two patients with bipolar disorder. Left: lithium responder; right: non-responder.

a test of the program's ability to classify a patient. The result? A system that could identify lithium-therapy responsiveness with 92 percent accuracy.

David Panchision oversees a National Institute for Mental Health program that supports the work. Highlighting the fact that most such experiments use cells from only two or three patients, he stated (3), "The fact that Gage's group can replicate the hyperexcitability characteristic in neurons from additional bipolar disorder patients is very important. Findings like these are needed to utilize these cells to develop new drugs to treat mental illnesses." *MS*

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Taking the EV Option

Extracellular vesicles open up new avenues in liquid biopsy-based cancer diagnostics

As the techniques behind liquid biopsy become more advanced, we should be able to diagnose and evaluate an increasing number of cancers using simple blood tests... in theory. Unfortunately, not all forms of the disease are cooperative. Breast cancer, for instance, has long eluded detection in this manner – but it may not remain hidden for much longer.

"Phosphorylation is one of the major regulation mechanisms in many diseases, including breast cancer," says W. Andy Tao, a biochemistry professor at Purdue University. "Phosphorylation and kinases have been the major target for cancer therapy, but unfortunately not for diagnosis." Why? Because the use of phosphorylated molecules as diagnostic biomarkers in blood is confounded by plasma-dwelling phosphatases, which remove the phosphate groups. The solution? Extracellular vesicles (EVs), according to a team of researchers led by Tao (1): "Our findings highlight that isolating EVs from plasma allows us to use blood or other liquid biopsy for potential cancer screening based on the status of protein phosphorylation – something that's not been done before."

The EVs found in blood plasma form a protective shell around the phosphoproteins, preventing dephosphorylation. To measure the cargo inside, the investigators lyzed the EVs and then used trypsin to create phosphopeptides ahead of analysis and identification using liquid chromatography-tandem mass spectrometry. Their study detected 144 phosphoproteins at higher concentration levels in breast cancer cases than in healthy controls.

The findings hold significance not only because of the improved ability to measure phosphoprotein biomarker levels in blood, but also because of the abundance of EVs in plasma, which could allow the detection of thousands of phosphopeptides from a single milliliter of sample.

Next, the team plan to investigate potential biomarkers in other cancers and diseases – a promising avenue, given that their initial investigation revealed over 10,000 unique plasma phosphoproteins. *WA*

Reference

1. *IH Chen et al., "Phosphoproteins in extracellular vesicles as candidate markers for breast cancer", Proc Natl Acad Sci USA, 114, 3175–3180 (2017). PMID: 28270605.*

In My View

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

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

Contact the editors at edit@thepathologist.com

How Do We Prove Our Worth?

Simple: we boost our contribution to the delivery of value-based healthcare

By Michael Misialek, Associate Chair of Pathology at Newton-Wellesley Hospital, Newton and Medical Director of the Vernon Cancer Center, USA

Pathologists stand at the intersection of every medical specialty and, as such, are the perfect ambassadors for the implementation of change. According to Michael Porter (Bishop William Lawrence University Professor at Harvard Business School's Institute for Strategy and Competitiveness), the core purpose of healthcare is value for patients. The value formula is based on the health outcomes that matter to patients and the costs of delivering those outcomes. If we are to adhere to this formula, healthcare delivery must shift from volume to value.

Quality also features in the value formula; in the lab, value is represented by a cumulative sum of accuracy, precision and timeliness of result. Opportunities abound in the clinical laboratory to substantially reduce costs and to improve outcomes – and ultimately increase value for patients. It's important to recognize that we pathologists must contribute to value-based healthcare to prove our worth in the healthcare team.

Here, I offer nine areas where we can boost our value.

1) Reduce process variation. By implementing Lean and Six Sigma principles (1), every pathologist should make sure their lab has an effective quality management program.
2) Eliminate low value services. Identify tests of low value, those that may be harmful or costly and do not provide higher quality of care. Several examples are available from the Choosing Wisely Initiative of the American Board of Internal Medicine. In my own lab, a process was implemented to review all test orders that previously were sent to a reference lab, regardless of expense. This involves a pathologist researching the test, identifying potential alternatives, review of the medical record and/or discussion with the ordering provider, and setting up the test when approved. This has saved tens of thousands of dollars.

"Pathologists can and must facilitate change."

3) Minimize the use of skilled staff for less skilled activities. An excellent example here is the use of automation for a previously manual lab test, such as chemistry testing in the core lab or slide staining in histology. Human error is minimized, accuracy and precision is improved – and all at a lower cost.
4) Move routine services out. An example of special relevance for pathology is the use of centralized

labs or the consolidation of reference labs. Services don't always have to be outsourced, but rather redistributed. Pathologist specialization can be used to improve quality and value by directing cases to the best-trained people (2). Alternative practice models can be particularly effective in offering high-value care (3).

5) Improve utilization. Value can be delivered through test utilization initiatives (4) and lab formularies (5). Pathology is positioned at the intersection of all medical specialties, with the lab generating massive amounts of data. As a consequence, pathologists have the unique opportunity to leverage a hospital's IT system to deliver value and measure results. Indeed, cloud-based computing has been demonstrated to improve utilization and outcomes (6). The clinical laboratory, instead of serving as an ancillary service, then becomes a partner in the healthcare team, contributing to the delivery of high value for patients.
6) Rationalize redundant administrative units. Pathologists should create process maps throughout the lab. Through an understanding of the entire care cycle of a patient, test ordering can be made more efficient. One can identify "invisible cost centers" associated with defects in a value stream, meaning that waste can be eliminated. Doing so will often involve units outside of one's department; for example, our lab streamlined a complex preauthorization process for costly genetic testing by working with the lab, oncology, neurology, gastroenterology and a genetic counselor. Working with other labs and payers may sometimes be necessary.
7) Reduce cycle times. Something simple for pathologists to consider is the discontinuation of routine repeat testing of critical values (7). Valuable time can be saved, which eliminates any delay in reporting critical values to clinicians.
8) Add services that lower total cost. Here, one must be able to measure actual costs of patient care. Consider bringing in-house tests that were previously sent out to a reference lab; for example, vitamin D, celiac, tick panels. Recently, our histology department implemented testing for HER2 in breast cancer cases, and it will reduce the need for reference lab testing by 60 percent.
9) Increase cost awareness. Pathologists can play an important role in clinician education on test costs (8) – an area where there is a clear need for improvement. One of the mistakes of healthcare today is that it is too broad in focus. Instead, focus should be on the individual provider. Pathologists can help by monitoring utilization rates among clinicians and providing a "report card" on patterns. Porter states that many cost reduction opportunities will actually improve outcomes.

In many cases, it's clear that pathologists must partner with clinical colleagues to measure outcomes that matter for patients. Of course, I do acknowledge that change can be difficult. One example is the new HPV and Pap smear guidelines; despite recommendations on utilization and positive data on outcomes, many practitioners have been slow to implement change (9). Nevertheless, pathologists can and must facilitate change and help educate our clinical colleagues.

As the transition from volume to value continues, pathologists need to be proactive and effective team members; it will be crucial to the success of new care models (for example, Accountable Care Organizations, Patient Centered Medical Homes). We pathologists must recognize our value, and our clinical colleagues must also become more aware of the high value of having pathologists as a part of the care team. But it's our responsibility to make it happen.

"As the transition from volume to value continues, pathologists need to be proactive and effective team members."

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As Education Changes, We Must Too

We need to get involved with integrated teaching in medical school to make it relevant for the pathologists of the future

By Emyr Benbow, Senior Lecturer in Pathology, the University of Manchester, UK

Medical undergraduate courses have evolved from tedious exercises in intensive rote learning into well-planned conversions of raw school leavers, or graduates from other programs, into doctors well-prepared for practice. In some areas, the evolution has occurred very rapidly, but elsewhere progress has been glacial – or even non-existent. To some degree, a lack of resources has hampered progress, but that is often not the issue. Ironically, advanced medical education ideas are often enthusiastically embraced by countries that are struggling with poverty in the developing world, while the same ideas are resisted with equal enthusiasm and impressive tenacity in countries with vast wealth! It's time for a change of mindset. Here's why.

Doctors work by applying appropriate sets of knowledge items – often called "scripts" – even if they are unaware that they are doing so. For instance, a doctor dealing with a patient with acute abdominal pain will have a mental script based on his or her knowledge of abdominal anatomy, the pathophysiology of inflammation and ischemia, human psychology, and much more – including, especially, previous experience of such cases – to aid initial diagnostic formulation. They will use other "scripts" to determine investigation and management. In essence, doctors call upon their training in many different disciplines to reach a final diagnosis. Given that a doctor's approach to determining a diagnosis integrates knowledge of multiple disciplines, surely to teach these disciplines individually during their education is inefficient and not reflective of real life.

In a bid to address this, various integrated teaching courses have been developed. Many of these use some form of case-based or problem-based curriculum – in fact, there are so many forms that a taxonomy of methods exists! What they all share in common is that learning is driven (usually) by fictional cases about clinical problems, presented within authentic scenarios, and selected to reflect the breadth of a curriculum. Because an authentic scenario typically crosses many traditional disciplines, no single discipline dominates. These scenarios, in most problem-based curricula, are used to encourage students to develop and fulfil their own learning agendas; tutors guide rather than teach.

Teaching within an integrated curriculum has major challenges though, especially for disciplines not seen as central by practitioners of the larger specialties such as medicine and surgery. Where there are no individual courses in pathology, there is a very real risk that such teaching can disappear from the curriculum altogether, and laboratory doctors will be in danger of having no opportunity to meet students.

The latter is a big problem, because research shows that the most potent factor in students' choice of specialty is identification with a positive and inspiring role model – and if you don't get to teach or tutor students, you can never become a role model able to recruit them as your potential successors. Many pathologists in the UK, including very senior members of the Royal College of Pathologists, responded with hostility to the pioneers who developed such integrative teaching courses.

Recognizing that a return to formal "-ologies" was simply not going to happen, some of us engaged with the new direction in tutoring instead of reacting against it; nothing else was likely to address the looming problem. Becoming involved with the development and design of integrated courses has allowed us to introduce relevant elements of histopathology, microbiology, hematology, and so on. It also allowed deletion of copious elements, often of basic sciences, that had no realistic application in the near future. For instance, I was able to ensure that the biochemistry being learned by students was relevant to understanding how bodies worked, and how to investigate and repair them when they don't work; the products of arcane research were ruthlessly eliminated.

As well as designing cues to learning about pathology and what it achieves, there are opportunities for pathologists to tutor and lecture: because no single case is limited to a single discipline, there is no requirement for a tutor to be a specialist in the material being learned. However, some understanding of the general principles is an advantage – and the breadth of knowledge required by a good pathologist makes for a good tutor. If you're interested in getting involved in similar teaching programs, you should go for it. After all, by judiciously demonstrating what a fascinating life a pathologist leads, you may just plant the seed in the mind of some perceptive young person that you are practicing medicine in a way they would like to emulate. One of my colleagues routinely asks candidates wishing to become histopathologists to explain their choice, and I'm gratified to learn that many cite my teaching, and my obvious enthusiasm for my discipline.

Clinical Metabolomics: Will it Deliver?

Blindly searching for biomarkers in the metabolome has failed to deliver on early promises – it's time for a new direction

By Martin Giera, Head of the Metabolomics Group at Leiden University Medical Center, Netherlands

Numerous research articles have proposed, addressed and promoted metabolomics as one of the key tools for biomarker discovery and personalized medicine. Personally, I am not blessed with a lot of patience, but even those who are might be starting to wonder, "After more than a decade of metabolomics-driven research, can anyone actually name a single resulting biomarker routinely used in the clinic?" I have to admit that, besides trimethylaminoxide and some markers related to gene defects (for example, 7-dehydrocholesterol), nothing comes to mind.

"I don't believe that lack of effort is the problem; I think we just took the wrong path."

But why? Have we not used the most advanced analytical and computational approaches available? Have we not invested enough money, manpower and dedication? I don't believe that lack of effort is the problem; I think we just took the wrong path.

In the beginning, when metabolomics was first used in case–control studies, it all seemed pretty straightforward. Many believed that with the right equipment and the right bioinformatics approach, we would easily identify some discriminators between all the molecules we can monitor. But the human body contains more than five liters of blood, we eat more than 500 g of (highly diverse) foods and drinks every day and, to make this picture even more complicated, our molecular fundament depends on genes, sex, weight, race and lifestyle. On top of all these variables, the metabolome is further influenced by circadian rhythm, hormones (mood), menstruation and medication. Say you are looking for a cancer marker – how are we going to find this one molecule, possibly secreted by a few million cancer cells somewhere in your brain or lungs, hidden in a constantly changing five-liter bucket of blood? Frankly, I am not convinced there is a high chance of success.

I don't want to paint too dark a view here, but simply illustrate that metabolomics biomarker discovery is a very complex endeavor. It's possible that our vision was blurred to the difficulties by the high hopes we had. Nevertheless, I am convinced metabolomics will make its way into the clinic, and hopefully fill the pipelines of clinical chemistry with new molecular tools. In life, you have to fall and get up many times before you learn to walk, and it's time for clinical metabolomics to take two seminal steps forward.

The first step is to change our mindset – away from traditional biomarker discovery studies and towards understanding the systems effects of metabolites, as outlined in a recent article from Gary Siuzdak's lab (1). The second step is to define the framework of human metabolism. In other words, what are the actual (true) concentrations of metabolites, what is the range these metabolites are to be expected in vivo, and how are these concentrations affected by circadian rhythm, food intake, tissue distribution and many other factors?

"I don't want to paint too dark a view here, but simply illustrate that metabolomics biomarker discovery is a very complex endeavor."

Such steps are increasingly being taken in several recently established phenome centers. In my view, these are exactly the right steps, in the right direction, at the right time (if not a little too late...). Clinical metabolomics has learned from its past failures and too few successes, and is ready to start taking strides into the future.

Reference

1. *Johnson et al., "Metabolomics: beyond biomarkers and towards mechanisms", Nat Rev Mol Cell Biol, 17, 451–459 (2016).*

The Competency Changeover

Medical education is no longer just about what you know – it's about how you turn that knowledge into action

Medical education is evolving. The didactic, lecture-based form of schooling that filled the heads of young doctors with information for later recall is now shifting to an action-oriented discipline. Trainees are no longer asked simply to list the characteristics of a disease, or the steps in diagnosing it, or the options for treating it. Instead, they're asked what they would do with that knowledge – a new type of teaching known as "competency-based medical education," or CBME.

Why is the nature of medical education changing so fundamentally after a century of the established methods? How are trainees responding to this radical shift in the way they're being taught? And what, ultimately, are the benefits to the most important person in the healthcare system – the patient? Expert medical educators share their experiences with CBME – its promise, its pitfalls, and its potential to turn the future of medical school on its head…

Skill Switch

How pathology training in Canada is transitioning to a modern, competency-based model – and why the whole medical profession should follow suit

By Marcio Gomes

Knowing how to do something doesn't necessarily translate into the ability to actually do it. And yet, for the last century, medical education has been organized around two things: structure and content. It was a classic formula that reigned unchallenged – until the 1970s, when the concepts surrounding "competency-based education" first came to light. And once on the scene, the idea of teaching people by competencies rather than knowledge rapidly gained traction.

> "With competency-based education, we don't just ensure that the learner has the information for a given task, but also that they can demonstrate the competencies required to complete it."

With competency-based education, we don't just ensure that the learner has the information for a given task, but also that they can demonstrate the competencies required to complete it. So it goes beyond pure knowledge by encompassing skills and attitude as well, and it demands that learners show social accountability. The public needs to know that professionals in training can do their jobs safely and effectively. That wasn't happening with traditional medical education and it fueled a sea change. Now, pathology is also moving toward a competency-based model of medical education (CBME).

Knowledge versus competency

To understand competency, it helps to relate the concepts to childhood activities with which we're all familiar. If you want to teach a six-year-old how to ride a bicycle, you won't begin with a lecture on the parts of the bike, the laws of physics, and the rules of the road. Instead, you'll teach what's actually involved in the process of riding the bike and perhaps add a firm push... The child will watch other people do it, then start copying and practicing. And practicing. Later, you can start adding extra knowledge (with a focus on safety) as appropriate. But no one needs to know how a bicycle is put together to ride one!

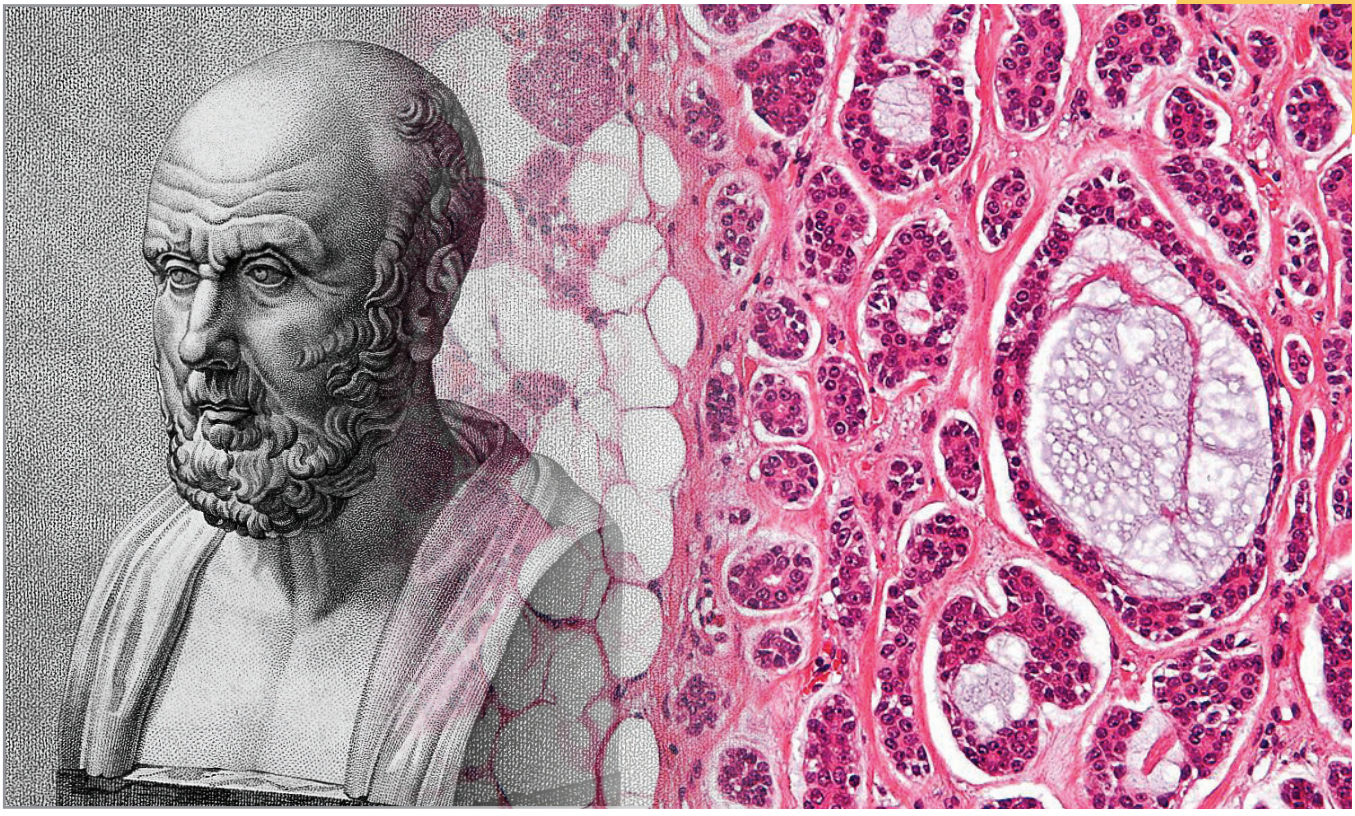
We've developed multiple educational frameworks for teaching competencies, but all of them deal with the overarching roles of a physician. Obviously, the central role is that of medical expert, but there are other ones – called intrinsic roles – that each physician needs to fulfill:

- Health advocate
- Collaborator
- Communicator
- Leader
- Scholar
- Professional

These six roles are integral to any physician's day-to-day work – but because they're quite broad, it's difficult to teach or assess them as competencies. A better way is to use a proxy – entrustable professional activities. These are the tasks that form the core of any given specialty, so that each physician in that specialty should be able to perform them competently. It's far easier to assess trainees by observing them while they perform those tasks than to try to pin down the nebulous overarching concept of a competency.

In pathology, examples of entrustable professional activities include performing intraoperative consultations, gross examinations, and autopsies; preparing complete and accurate pathology reports; communicating results effectively with clinicians; and participating in multidisciplinary cancer conferences or tumor boards. Every competent pathologist must be able to do these things – so if you want to infer that trainees have the necessary competencies, you can do so by watching them perform each of these tasks.

Why is this so important? Because until recently, the way we taught medicine hadn't changed for ages; we were still trying to teach every student everything we knew about medicine. In reality, most doctors don't need all of that knowledge to practice competently. That's a point that becomes especially true when you consider how far medicine has advanced over those



years; the amount of knowledge we have now is completely overwhelming. We need to focus on teaching students the things they really need to be competent, safety-conscious physicians. Trying to fill their brains with everything from medicinal leeches to molecular pathology is a Sisyphean task!

How to train toward competency

If you want to know whether or not a six-year-old can ride a bike, you have several options. You could write a multiple-choice exam or you could ask open-ended questions such as, "What would you do if a car crossed your path?" and "How do you stop a bicycle?" But far better than either of those options is simply to observe and correct along the way, providing effective feedback on the things the child is doing right and wrong.

Now, let's say that I want to teach something a little more complicated – pathology, for example. Until recently, we taught the entire pathology curriculum to every medical student, but only about one in 100 students is going to choose that specialty. Do they need to know all of that to become clinicians? Isn't it more important that I teach them how to interpret the pathology results they'll receive as non-specialists? We need to look at the curriculum and see what these students really need to do to be competent as non-pathologists. Of course, the concepts of pathology are really important – but do they need extensive microscopy training, for instance, or is it more important for them to understand and integrate the concepts into their practice?

Clinicians need to know how to choose pathology tests and interpret their results. What is the best type of biopsy to increase yield in different clinical scenarios? What is the current role of molecular pathology? How do we interpret immunohistochemistry results? How do we use those things in the differential diagnosis of cancers, or for predictive biomarker testing? This is the pathology 21st-century physicians are going to need – especially those who work with pathologists rather than as pathologists. I might give my students a stack of pathology reports and ask, "What are you going to do with this patient based on these reports?" That's far more valuable for most of them than handing them slides and asking them to provide a diagnosis. I want my students to ask, how will this affect treatment? Prognosis? Management? What does this pathology mean for my patient?

Since we've begun using CBME, I've noticed that newly trained pathologists are much more aware of system failures

and communication/collaboration issues than their older colleagues. Now, when I talk to my residents, they are completely familiar with the different competencies required for a pathologist – so when we discuss a topic like quality assurance (QA), they understand that it requires a number of extra steps to increase patient safety, they know how to check the "nuts and bolts," and they do it automatically because they view it as a necessary part of being a practicing pathologist. In the past, QA was seen as the province of lab management – but it's far better for the people who are actually doing the work to incorporate QA. It plays into the intrinsic roles we discussed – leader, health advocate, collaborator, communicator – and I think it goes a long way toward minimizing errors and failures.

A training transition

In the beginning, students were a little hesitant to get on board with such a radically different system. It didn't take long for them to grasp its importance, though. We practice a lot – for instance, after tumor boards, I debrief with my residents. "What did you see? What did and didn't you like about the discussion?" Their answers aren't just from the perspective of the medical expert anymore; now, they talk about professionalism, communication, and collaboration between doctors. They understand that the patient is at the center of care, and that it's more important for medical team members to work well with one another than for individual physicians to remain in the ivory tower of their own expertise. It's very motivating for them to see pathologists getting out from behind the microscope and providing direct patient care.

Now that we've been CBME-focused for several years, incoming trainees can look to older ones for guidance and role modeling. But many of them are already familiar with the system – medical schools not just in Canada, but internationally, are now using competency frameworks similar to those in our postgraduate program. The framework from Canada's Royal College of Physicians and Surgeons is used in more than 30 jurisdictions around the world, so it's clear that CBME is here to stay. And with good reason; we have preliminary results indicating that trainees might learn more effectively with the new model.

Assessment plays a huge role in CBME – most of which is formative (observing and offering specific feedback on how to improve). Over multiple cycles of observation and feedback, the learner acquires the competencies, and it's easy to trace the sources of any difficulties and ensure that there are no obstacles to progress. After completing the formative assessment cycle, you also perform a summative assessment to evaluate the learner – but it's not a pass/fail scenario; instead, you get an overall idea of their performance and understanding. Finally, you decide if additional training is needed or if the student is ready to practice the activity independently.

It's a bit like giving a series of "micro-licenses" for individual competencies. When they've collected all of those micro-licenses, the training is finished. The process allows faster learners to progress at their own pace without creating difficulties for those who need more time. It also allows educators to accommodate variability in a learning group without punishing students at either extreme.

You might be concerned that, with students progressing at different speeds, there is potential for stigma. And though that may be true, I think the advantage of respecting learners' individual needs far outweighs the risk. In Canada, we have a large number of international medical graduates, which creates different backgrounds at the beginning of residency. Some may be more advanced than Canadian graduates, whereas others may not have reached quite the same stage of development. But the inequities don't stop there. One student might encounter health problems during training; another family problems; another might have a child. You can't treat residents as a homogeneous population, and I think CBME allows you to respect them as individuals. The milestones of progress are no longer the years of training; instead, they are the stages of competency – and that allows for much more adaptability.

> "It's more important for medical team members to work well with one another than for individual physicians to remain in the ivory tower of their own expertise."

Will this lead to a structural change in the way medical schools are run? For sure. But for now, we're implementing CBME in waves of a sort. The first wave was to help people understand exactly what CBME is and introduce them to the frameworks. Now, we're starting to change training programs from knowledge-based to competency-based models. That takes time, because we need more teaching hours; we need faculty development; we need changes to examination procedures; we need transition periods for trainees moving from education into practice. We're working on bringing in all of those changes, but a major overhaul like this can't be done in a day.

Beyond the schoolhouse walls

The CBME concept isn't limited to trainees and offers benefits at every level. For instance, the Canadian Association of Pathologists is restructuring its national conference to incorporate those same principles. We're bringing in more interactive sessions, more workshops, more parallel learning tracks to accommodate different needs and interests, and a series of interdisciplinary sessions to access the expertise of non-pathologists. We're also introducing an overarching theme that is important to all pathologists, regardless of scope or specialty. For 2017, the theme is "wellness" – how to develop strategies for a sustainable career in pathology. So many of us are overworked and under-resourced – how can we address those problems without compromising patient care?

In the next few years, we're planning to introduce a leadership summit at the conference, and to begin providing performance assessments. The Royal College mandates assessments for recertification, so we're going to offer opportunities for practicing pathologists to complete them on-site. We're also expanding on the availability of interprofessional education, which we hope will help pathologists better understand the notion of collective competency and collaborative practice. Collaborative practice is a real cornerstone of CBME – and we must remember that even if an individual is competent, the team as a whole might not be, and that still ultimately leads to poor patient care. To guarantee that every patient receives the best possible care, we need to teach pathologists how to work within a team – not in isolation. And we need to ensure that professionals from all areas are equally competent, equally involved, and equally respected as members of the health care team.

As you can tell, we have many ideas for improvements, and the concept of competencies runs through them all! In my opinion, knowledge is easily acquired; translating that knowledge into action is the difficult part. You can always look up information (though you certainly can't retain it all in your head permanently); knowing how to apply it in context is a skill that can only be acquired through time and training. Why is this so important? Because we want to make pathologists leaders in the field. I often feel like we simply wait for things to happen, and I'd like that to change. I'd like to see us become role models for other specialties. I'd like to see us play a part in the evolution of medical training – and of medicine as a whole. Transitioning to a new model of education is the first step along the path to leadership, and I'm looking forward to the rest of the journey.

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Case Study: The Ontario Molecular Pathology Research Network

By Marcio Gomes

The Ontario government did a study to understand why the process of implementing molecular pathology in research and in the clinic was lagging so far behind other locations. What did they find out? That one of the main bottlenecks is the pathologists themselves – they simply weren't up to speed with the necessary new competencies.

What can be done?

They devised a project, the Ontario Molecular Pathology Research Network (OMPRN), to improve the quality of molecular pathology competencies in the province. Among other things, the OMPRN partnered with the Canadian Association of Pathologists (CAP-ACP) to bring molecular education to the Canadian pathology community.

When they first sent me what they wanted to teach, a quick read revealed that it was all knowledge-based. I had to have a pleasant, but challenging, conversation with the project's leaders, to say, "We have to bring this material to pathologists as entrustable professional activities. They need to understand the activities you want them to be able to perform, not just the knowledge you want them to have."

They aren't educators, so they come to the table with the things they want pathologists to know. I'm an educator, so I look at that and ask, "What are they going to do with that knowledge?" Information is so democratic these days – everything is on the Internet and you can search for anything you need, so it's no longer important to have it all in your head. It's important to know how you can translate that knowledge into action.

What did you do?

I turned their objectives into a CAP-ACP workshop by translating everything into competency-based language. To do that, I had to determine their ultimate goals, create the appropriate entrustable professional activities, condense them into the available time, and then develop a curriculum that would allow us to deliver them. It's a completely different approach to educational design!

The Slide and the Sequence

For a comprehensive education in pathology, we can't afford to overlook either one

By Stephen Yip

The practice of pathology has changed a great deal over the last two or three years. It's no longer enough to provide a glass-based "analog" diagnosis – a histological description of a cancer for the oncologist to decipher before making a clinical decision. Today, you have to focus much more on molecular and genomic findings that can inform the oncologist's decisions. Pathologists are slowly getting on board with the additional responsibility – but it can be hard to convince those who are already happy with their workflow to change it. The answer? We need to make a concerted effort to integrate genomic pathology into training right from the start. It's crucial; if we don't, other specialties (medical or non-medical) will step in to take over. And the way I see it, genomics is – and should remain – an integral part of pathology. Many alterations to the genome drive the appearance or behavior of disease, and that's what "pathology" means to me: the description and identification of disease mechanisms. I want the next generation of pathologists to learn how to integrate genomic pathology into the clinical workflow, and how to use it to obtain a more integrated and comprehensive diagnosis.

Transitional teaching

Pathology education is falling behind. Either there isn't enough of it or it isn't focused on the right areas anymore – so how do we get the most out of it?

In my opinion, we start by updated the training to reflect the modern landscape of pathology. We've been teaching our students that it's enough to look at glass slides and memorize them as if they're curating a database of picture memories. But they'll need much more. The images our students are memorizing are driven by underlying genomic and epigenomic changes, and I don't think we currently place enough emphasis on that aspect. Clinical skills are clearly vital, but we give them so much of our attention that medical schools (some more than others) are failing to integrate genomic medicine into the curriculum at all. Our postgraduate trainees are underserved by this lack of attention, and it's my mission to convince every school to change. Ten years from now, people will come into the doctor's office for real-time sequence analysis from a fingerprick or cheek swab, and the doctor will need to figure out what that genetic data means – so it's vital to start educating at the ground level.

Most medical students have studied undergraduate biology, so they know about DNA and RNA. But there are so many clinical skills to learn in medical school that they forget the basic science – and that's what we need to reignite in pathology! Our number-one focus should be to ensure that our trainees not only have a solid grasp of those fundamentals, but know how to actually apply them to medicine. There are already science questions on the pathology board exams, but that's not good enough; residents study for their exams, but don't bother to retain the knowledge into the future, even though they need to understand its practicality in their day-to-day work.

Fortunately, the number-two concern is easier to change right now. Genomics plays a role in many diseases but, right now, most of the research is in cancer – a big part of clinical pathology – which gives us a great opportunity to integrate molecular teaching into our regular curriculum. For example, a resident who sees a lot of lung cancer should be taught to understand the molecular changes that take place in lung cancer, and how some of them may serve as biomarkers. Target each resident's subspecialty and you're guaranteed to keep their interest!

> "Our number-one focus should be to ensure that our trainees not only have a solid grasp of the fundamentals, but know how to actually apply them to medicine."

At the University of British Columbia, our first-year residents go to a week-long "molecular boot camp" where they review the basics of molecular biology. What's an intron? What are the different types of mutations? How do they translate to diseases? Next, we say, "As pathologists, it's important for us to spot these changes to inform our diagnoses." How do you optimize a tissue sample to test for mutations? What are the pros and cons of a particular molecular assay? In 2017, for the first time, we're also incorporating four hours of molecular genomics into the

orientation – both the basics and its practical application to cancer diagnosis and treatment.

A curriculum in competencies

There is a strong emphasis on molecular pathology in hospitals devoted to its practice, training, and implementation in clinical care. Every Monday morning at the Massachusetts General Hospital, there are molecular pathology rounds to discuss the latest discoveries in the field. It's pretty impressive that, even in a clinically oriented specialty, residents are encouraged to do that! There is also a requirement for residents to complete four weeks of molecular rotations – things like inherited disease or cancer genetics – so that, even before entering daily practice, they have a well-rounded exposure to molecular pathology.

I think many teaching programs realize the importance of molecular biology, but I also think that American hospitals are ahead of Canadian ones in that respect. In the United States, molecular genetics is a specialty certified by the American Board of Medical Genetics and Genomics – but in Canada, we have no similar program. We're making strides now; I'm working with the Ontario Molecular Pathology Research Network to develop a teaching curriculum for genomic pathology. Initially, we want to implement it in all of Ontario's medical training programs – ultimately, we'd like to submit the goals and objectives to the Royal College so that it can be applied to pathology training programs throughout Canada.

Hopefully, our efforts will tie in with the larger changes happening in the medical education system – namely, the transition to "competency by design" (see "Skill Switch" on page 18). What kinds of competencies would we want to see in genomic pathology? A first-year resident might be expected to understand the fundamental science and be able to process tissue optimally for

genomic testing. The next year might involve more tissue-specific goals; for example, identifying the necessary tests for different types of diseases and responding appropriately to the results. With increased training and experience, the goals and objectives become more challenging – just like in any other branch of medical training.

How can trainees prepare?

Every resident is different. Some want to work in hospitals, others in private practice, still others in research. But no matter what your aims, genomics will be a big part of your work. A decade ago, when a clinician asked, "What does this diagnosis mean?" the response might be: "This is a carcinoid tumor and here's what each of these features indicates." Ten years from now, as well as giving the histological diagnosis, you'll probably have to say, "The sequencing has revealed a mutation, and here's the implication for the patient." So every pathologist – no matter what career path he or she takes – will need to understand genomic medicine, use it safely and effectively alongside glass-based histological description, and relay it proficiently to a clinician.

> "Every pathologist will need to understand genomic medicine, use it safely and effectively alongside glass-based histological description, and relay it proficiently to a clinician."

At the same time, aspiring pathologists must not overlook the ongoing importance of glass slides. Sometimes I get asked, "If you can sequence a tumor, why don't you just sequence everything and forget about glass altogether?" But that takes time and resources. An experienced pathologist can look through a microscope and – in five seconds – make a very tight differential or even a diagnosis based on the tumor's appearance. Not to mention the fact that glass slides are transferable between hospitals; not every site has the capacity for genomic testing, but everyone has a microscope. Glass slides remain the common currency among pathologists, so this is not about replacing them; it's about making the most out of tissue- and glass-based pathology by integrating additional information. At the moment, that might be imaging or immunohistochemistry, but in the next five to 10 years, it will be biomarkers and other genomic findings.

And let's not forget that we will still need to make decisions about which samples need to be sequenced. Some might argue, if sequencing is becoming so much cheaper, why not just sequence everything and develop a huge database for each case? I don't think that's practical in a clinical pathology lab; the scale of data storage, computation and interpretation required is still beyond what we can efficiently handle. So we need to triage our samples. It's something we already do – for instance, we use immunohistochemistry (along with a host of other factors) as a triage tool for additional FISH testing, because the test is too costly to perform on every sample. Glass will continue to be extremely important for the foreseeable future, because we're already familiar with the visual features of tumors and we're now linking that knowledge to our understanding of the underlying genomic aberrations.

In short, trainees shouldn't toss their microscopes just yet. Instead, they should focus on connecting the disparate pieces of information they gain from both slides and sequences.

What's coming next?

Usually, when we talk about investing in new technologies, it's about buying the equipment, hiring staff and changing laboratory infrastructure. People often overlook the fact that we need to invest in educating the next generation of pathologists. We need to teach our residents to take ownership of genomics and its integration into the study of disease as a whole. We need to prepare residents for the role of the future pathologist.

There is a sort of dichotomy: educators need to move as quickly as possible to include genomics in their teaching curricula – but to go slowly when they're actually teaching it. Trainees must have the fundamentals in place before studying more complex aspects of genomics, or else they'll just end up with an overdose of information with no background knowledge to process it. It also takes time to instill what I would argue is an essential passion for genomic pathology. Young pathologists need to see that genomic pathology is the future of their field – then they'll realize how exciting it really is and how affective it is. Pathology is really pushing the forefront of medicine. And, in my opinion, there has never been a more thrilling time to be a pathologist!

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The Cornerstone of Competency

Milestones provide a way to assess learners' progress toward professional performance

By Alexandra Wolanskyj

Competencies aren't like facts where you either know them or don't; instead, you go through a series of milestones that cover the spectrum from novice to expert. There's a continuum of observable behaviors by which a learner demonstrates that they have actually acquired the knowledge. That's the real difference between this type of learning and previous kinds – now, it's not enough just to say that you know something; you have to actually be able to show it.

My background is in graduate medical education. I spent the first decade of my career as program director of a hematology/oncology fellowship, including developing and implementing an assessment system for our fellows. We used the word "competency" – but only as a descriptive term for our objectives. If students were able to list the information we wanted them to know, then we considered them to have acquired proficiency. Clearly, that's not the way we use the word nowadays!

It was the need for public accountability that prompted a revolution in American medical education. It was no longer sufficient to simply conclude that residents had capability based solely on their capacity to reel off medical knowledge; they needed to actually demonstrate it. As a result, there was a transition to a more milestone-based assessment method that takes learners from basic skills all the way to professional performance. Medical education is a continuum that runs from medical school to postgraduate training and residency fellowship and finally to practice. Our teaching and evaluation needs to follow the same continuum – so it seemed to me that, if we were implementing competency-based medical education (CBME) for our residents, it made sense to start at the beginning and introduce it in undergraduate medical education as well.

A generation apart

The current generation of medical students is perfectly suited for this new style of evaluation. The idea for our recent study – "Milestones and millennials: A perfect pairing – competency-based medical education and the learning preferences of Generation Y" (1) – came to me when I was developing milestones for our hematology fellowship. I presented them at a meeting where I also gave a talk on intergenerational learning – and it just became obvious how beautifully CBME and the current generation of learners intersected.

We use a milestone system that takes learners through different steps, and each subsequent step requires greater levels of observed competency. At the "novice" level, you may not be able to adequately discuss the goals of a particular intervention or the side effects of a treatment. At the next level, you might discuss it intermittently – inconsistently, or with errors or gaps in your knowledge. When you reach the "adequate" level, wherein you can apply your knowledge consistently and correctly, you're considered ready for independent practice. But you're not quite finished; there's still the "aspirational" level, where you not only apply your knowledge properly, but can also teach other individuals to do the same. That's how the continuum of milestone-based assessment works, and you can apply that to anything – interpreting a blood smear, looking at tissue under the microscope, distinguishing similar diseases... Milestones really are the cornerstone of CBME, because they allow us to effectively evaluate an aspiring doctor's ability to do the job.

> "Medical education is a continuum that runs from medical school to postgraduate training and residency fellowship and finally to practice."

There's no question that millennials are Internet natives, and their attitudes and approaches reflect that. They're facile with technology, but they also tend to have fairly short attention spans; if you don't grab their focus within a few minutes, you've lost them. And that can be easy to do if you aren't teaching to their needs. I think that's where technology can be very helpful – it allows learners to control what content they receive and how fast they receive it. That way, they can skip over sections they know well and spend more time on those they don't. They can also network virtually to learn new material using tools like

Google Docs and Twitter conversations. It's very important for physicians to learn how to work in teams and, in that respect, millennials – to whom online collaboration comes naturally – are ahead of the game.

I mentioned earlier that one of the driving forces behind the transition to CBME is public accountability. That's another area where millennials are one step ahead. As a group, they feel very passionately about making a difference in the world. Their desire to have an impact spurs them to become the best version of themselves that they can be – and, as a result, they respond extremely well to defined goals and directed feedback that helps them get there.

Mentoring millennials

Most millennials have been raised by "helicopter parents" who spend a lot of time running interference. They're used to having an adult who is on their side, constantly looking out for them. Mentoring works very well with this generation of learners because they're already accustomed to that kind of relationship – you don't have to be their best friend, but you do have to demonstrate that you care about their outcomes to help them achieve their highest potential. I've found that it works best in an informal setting; if you build a relaxed rapport with them, they'll be more open to receiving feedback and incorporating it into their work. It can be tricky to do that while still maintaining firm boundaries – assignment due dates, skills demonstrations, and so on – but if you tie those boundaries into motivations ("This will help you become a more professional and therefore a more successful pathologist"), it can be easier to encourage mentees without losing the friendly relationship. The mentor learns a new way of working with colleagues, whereas the mentee grows and develops professionally. I consider it a win-win!

But what about when the learner graduates from the relationship and becomes a leader in their own right? When mentoring is done well, one of the skills you teach your mentee is how to be a mentor themselves. The profession of medicine is one where the mentor-mentee relationship continues throughout your career. I have been in medicine for 25 years, and although I have mentored many students, residents and

even junior faculty, I also continue to be mentored myself. It's not an all-or-nothing, one-or-the-other situation; most medical professionals are both, and there's a lot to be learned on both sides of the equation.

Faculty development is an extremely important – and sometimes overlooked – aspect of mentoring. We choose our faculty members and advisors carefully, but then we invest in them through annual development programs on mentorship. We even have a required half-day workshop on millennials: how they learn best, what tools educators can use, what resources are available, and how to handle hypothetical scenarios. We don't just take it for granted that our faculty will know how to provide the best possible education for every student; instead, we equip them with the knowledge they need, and we cultivate their existing passion for teaching well.

Tips for teachers

Unfortunately, I think that millennials are often unfairly maligned. I think their attitudes – how they approach the educational process, how they receive feedback, how they work together – really set them up to thrive in a CBME context. I'd like to see educators around the world embrace this and realize that it's actually quite easy to engage these learners if you genuinely invest in them. Give them specific milestones, help them understand how reaching those milestones will help them achieve their own objectives, and don't make the mistake of setting your expectations low. These learners want to be challenged. They want to be held to high expectations. They want to work hard, do well, and accomplish extraordinary things. They genuinely want to make the world a better place – that's why they choose careers in medicine; that's why they pursue specialties like pathology – and it's your job as an educator to help them do it.

Alexandra Wolanskyj is Associate Professor of Medicine at the Mayo Clinic, Rochester, USA.

Reference

1. *JR Desy et al., "Milestones and millennials: A perfect pairing – competency-based medical education and the learning preferences of Generation Y", Mayo Clin Proc, 92, 243–250 (2017). PMID: 28160874.*

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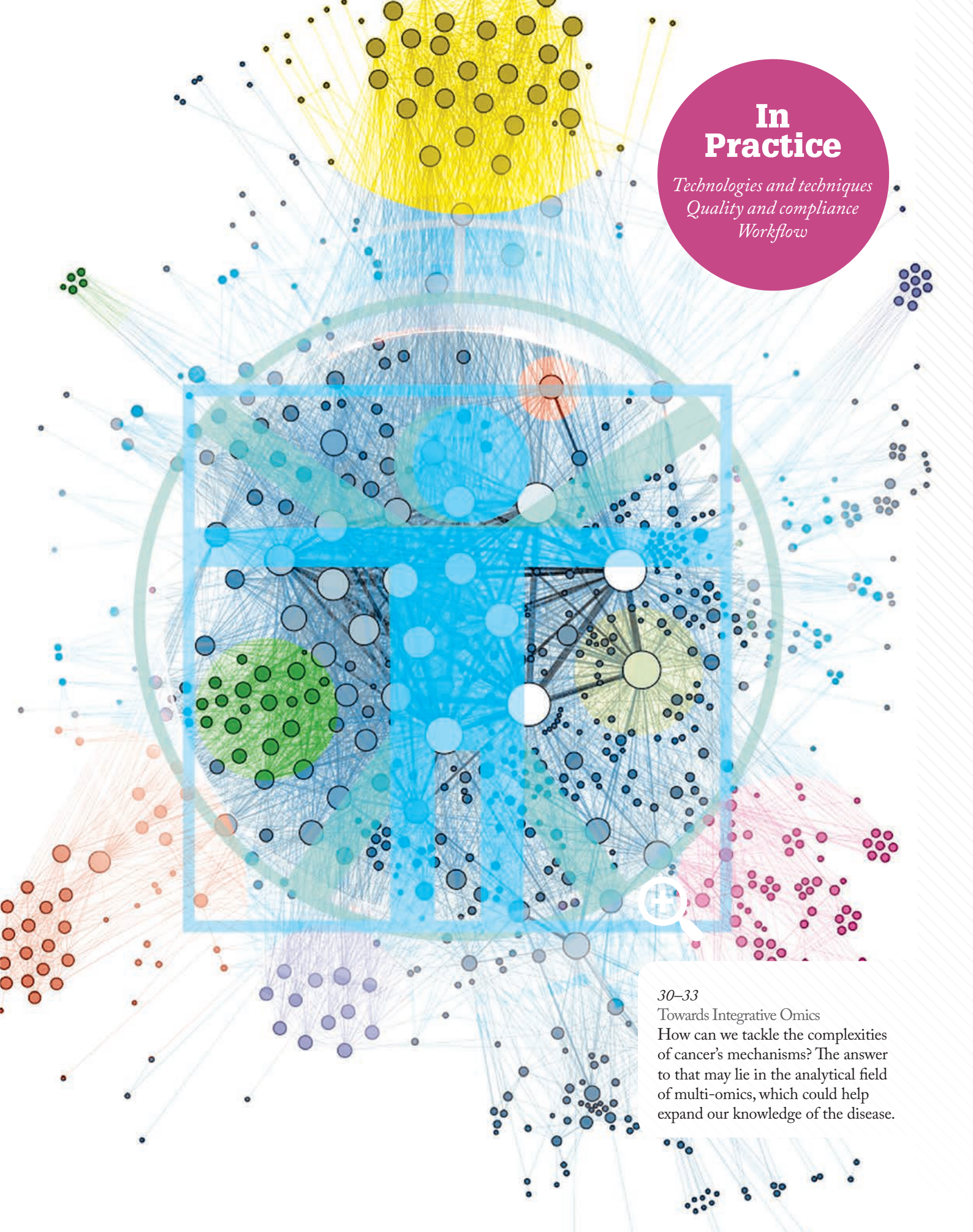

In Practice

Technologies and techniques
Quality and compliance
Workflow

Toward Integrative Omics

Cancer is incredibly complex, posing enormous challenges beyond the biological field. Taking a multi-omic approach can help us make sense of this diverse set of diseases – and, ultimately, allow us to better understand ourselves as human beings.

By Amanda Hummon

I started researching colorectal cancer for multiple reasons, but a significant part of my interest was triggered by grief; a member of my immediate family died as a result of metastatic colorectal cancer, despite having the access to the best medical care. I wanted to understand more of what had happened and why.

At a Glance

- *No one field of –omics is sufficient to understand cancer; we need to look at not just the genome or the transcriptome, but the metabolome, proteome, lipidome and others too*
- *Next generation sequencing drives genomic advances, but recent improvements in mass spectrometry are driving proteomics*
- *To derive the greatest benefit from new technologies, we must use them in conjunction with smart data analysis tools like database searching algorithms*
- *Other "–omes" are poised at the edge of advancement and will grow exponentially as our ability to analyze the data improves*

Reading about colorectal cancer, it was apparent that while the genomics and transcriptomics of the disease had been well studied, the proteomic changes that accompany the disease were not as well understood. I believe the prejudice is related to the tools that were/are available to tackle the problem. After realizing how much remained to be done in the field of cancer proteomics, I decided to devote my career to studying the molecular changes that underwrite colorectal cancer. The more I work in this field, the more I recognize how truly deep understanding – from genotype to phenotype – is the only way we can tackle cancer.

The caterpillar and the butterfly

Multi-omics approaches attempt to make sense of the genome, the transcriptome, the proteome, and the metabolome all together. If you look back to the articles that were written around 2000 (and the publication of the human genome) you will find a glimpse of what we could achieve with this information. With a greater understanding of our genes, transcripts, proteins, and metabolites, we can better understand how the 'blueprint' corresponds to reality.

The classic example that I give to my students is the caterpillar and the butterfly. Both have the same genome, but the phenotype of the two animals is shockingly different. That striking physical difference is the result of the transcriptome, the proteome and the metabolome at work.

Multi-omics has of course been gaining traction for the last decade. The major developments that have brought it to the forefront are: i) the completion of the Human Genome Project and ii) the development of high-throughput methods to analyze the transcriptome (first microarrays, later next gen sequencing) and the proteome (mass spectrometry). Multi-omics studies are now everywhere. I would bet that for any major disease there are several manuscripts characterizing the genome, transcriptome, proteome, and/or metabolome of healthy versus diseased tissues. Similarly, it is now routine for the chemical characterization of any organism to start with the sequencing of the genome. When I last checked (April 2016), the NCBI genome archives held over 75,000 genome sequences, and many of those species will have also been analyzed for transcriptomic and proteomic contents.

In cancer, chemical analysis is highly complex because you are dealing with very different types of molecules that appear at different points in time and in space. For example, a specific transcript or protein may only be needed at certain points in the lifecycle of the organism. If it is only synthesized in a few copies for a short window of time, it can be extremely difficult to measure. Again, I refer to the example of the caterpillar and the butterfly.

Another enormous challenge is the incredible dynamic range of the molecules. Some molecules are produced abundantly at all times, making it hard to see around them. Albumin is the classic example; it makes up more that half the protein content of human blood. What that effectively means is that researchers trying to analyze human blood for other trace level proteins must first deplete albumin before they can conduct any other analyses to see the lower abundant "more interesting" stuff. Separation is the key.

Understanding colorectal cancer

When I was a postdoctoral researcher at the National Cancer Institute, almost every member of my lab had lost a family member to cancer. Most of the students who walk into my office tell me they are there because they want to contribute to cancer research. It is a complex problem that affects so many people. From a molecular perspective, it is both fascinating and incredibly motivating. I am hopeful that with greater understanding, we can do a much better job of treating colorectal and other cancers.

Colorectal cancer is a good research

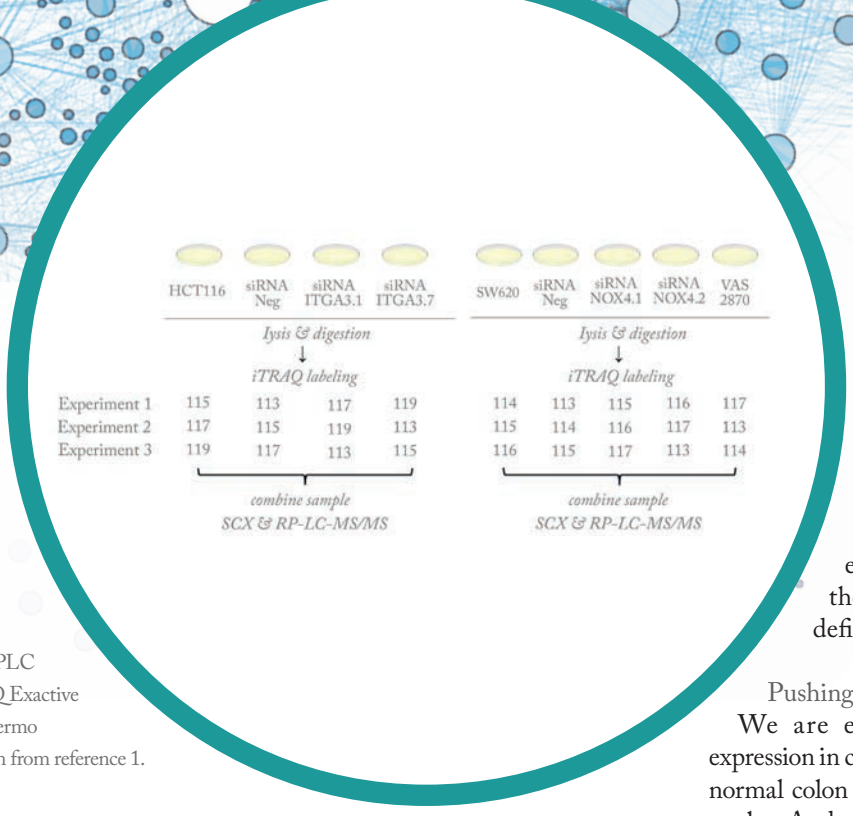

Figure 1. Workflow of experimental design. In brief: i) HCT116 cells were silenced with siITGA3 siRNAs ii) *NOX4* was inhibited in SW620 cells with siRNAs or VAS2870. Samples were analyzed with a nanoAcquity UPLC (Waters) coupled to a Q Exactive mass spectrometer (Thermo Scientific). Figure taken from reference 1.

target for several reasons. First, it follows a sequential path of genomic instability – more so than other soft epithelial cancers. In colorectal cancer, there is a common pattern of mutations and genomic instability that is observed in approximately two thirds of all colorectal cancer patients. We and others have hypothesized that this similar pattern of genomic instability would result in conserved patterns of proteomic changes – and we are still investigating this phenomenon. Second, though it is one of the most common types of cancer, colorectal cancer is not as well studied as some other cancers. I wonder if the functions of the organs involved result in people being less interested in this disease – unless they have a personal connection. Finally, like many other cancers, colorectal cancer is linked to obesity, meaning that it has the potential to be an increasing health burden in the future.

Driven by mutations

Like many soft epithelial cancers, colorectal cancer starts with a few driver mutations – that is to say, a few mutations that push the cancer along. In fact, there are five critical genes – *PI3K*, *APC*, *TP53*, *TGFB*, *KRAS* – that are part of several pathways and have been causally linked to changes in the genome.

Colorectal cancer cells frequently show gross changes in the genome – amplifications and deletions of entire chromosomes are common. And it has been shown that these genomic changes directly correlate with changes to the transcriptome. However, the correlation with the proteome is much less clear. In some of our recent work, we have demonstrated that the amplifications in the genome, while resulting in upregulation of transcripts, do not necessarily result in higher corresponding protein abundance.

Our current understanding of the disease reflects the tools that we employ to detect cancer chemically. For example, to examine changes in the genome, either spectral karyotyping or comparative genomic hybridization are effective analysis strategies. Changes to the transcripts can be assessed by many different high throughput strategies. Microarray analysis is commonly used to survey the expression levels of thousands of transcripts, but are increasingly being replaced by more global next-generation sequencing methods, such as exome and RNA sequencing.

Fundamentally, cancer is a single term used to describe a huge range of diseases. And though the chemical component is very important and dictates the behavior, it's the phenotype we care the most about at the end of the day. The final definition of whether something is cancer or not is defined by how the cell behaves. Does it grow, proliferate, spread, and metastasize? Although chemicals enable these processes, it is the processes themselves that define the cancer.

Pushing proteomic knowledge

We are examining how protein expression in colorectal cancer differs from normal colon tissue from many different angles. And we are also considering how these expression patterns change over time – as the disease progresses. We use 3D cell cultures to examine spatial differences in expression patterns in tumor mimics.

"From a molecular perspective, [cancer] is both fascinating and incredibly motivating."

Our hypothesis is that the genomic changes that are so evident and pervasive in the colorectal cancer genome also play out in the colorectal cancer proteome. As proteins are the action molecules in the cell, they are the best chance we have to develop rational strategies to turn off a cancer-associated signaling pathway.

As we are looking at protein expression, we primarily use mass spectrometry – applying different platforms based on the specific question we are asking. For example, when we are examining the global

differences in protein expression level between a normal and a cancer sample, we use quantitative labels and nLC-MS/MS to perform a quantitative comparison (1) – see Figures 1 and 2. When we are examining the differences in spatial distribution in a 3D sample, we employ either imaging mass spectrometry or serial trypsinization to harvest sequential concentric rings of cells for nLC-MS/MS.

We are also interested in gaining a better understanding of how to make treatments more effective. To that end, we've developed a powerful imaging approach to visualize drug penetration in tumor mimics, which allows us to see how and where a drug is metabolized. Our approach has been adopted by a couple of European pharmaceutical companies and I hope it will also be implemented by US companies. I believe it could help get more therapies on the market quicker.

We have another project in the lab where we are examining the molecular changes that occur with fasting, also known as caloric restriction. That work has led to some tantalizing evidence that fasting can improve the efficacy of chemotherapies. Now, we are trying to figure out why that is and how it could be implemented clinically.

We've also had some extremely rewarding results in the transcriptomics space (see sidebar, "The Power of Transcriptomics and Collaboration"

Finally, we are striving to gain a better understanding of why metastasis occurs. The vast majority of cancer deaths result from cells spreading throughout the body. The critical step in the process is the ability of the cells to insert themselves into the secondary location. We are working with Pinar Zorlutuna, a bioengineer, to model a tumor in proximity to a potential secondary site. We have designed the system so that we can manipulate both the chemical and physical stresses. We then evaluate whether the cell succeeds in metastasizing and also evaluate the chemical environment that facilitates or hinders metastasis. I would be delighted if we can decipher a combination of physical and chemical properties that promote – or better yet reject – a metastatic cell. Such information would be incredibly valuable. Thus, five years from now, I hope that we will be applying this knowledge to make potential secondary sites less hospitable for a metastasis.

Cancer is an incredibly complex disease. You can't effectively treat a disease if you don't understand it. Our current methods to treat it – radiation, chemotherapy and surgery – are blunt measures. Those in the field share the same hope that with better understanding of the pathways, we can improve diagnosis and therapy.

Enabled by technology

It's clear that advances in next generation sequencing are driving genomics. But for those of use placing an emphasis on proteomics, the most important technical advances are improvements in mass spectrometers. About ten years ago, the Orbitrap mass analyzer hit the market, making high-resolution instrumentation

The power of transcriptomics and collaboration

One of our most striking results comes from a transcriptomics study. We have been working with Steven Buechler, a statistician here at Notre Dame. Steve performs bioinformatics analyses and, a few years ago, he noticed a striking trend in some of the published colorectal cancer microarray studies. In his analyses, he showed that the gene expression patterns were extremely distinct on the right versus the left side of the colon. The colon is a large organ and initially develops in different parts of the embryo, resulting in differential gene expression patterns. The right side of the colon includes the ascending and transverse segments, while the left colon includes the descending colon to the rectum. The two sides of the colon are very distinct; polyp formation differs significantly between the right and the left.

Though it was known that the gene expression patterns between the right and left sides of colon cancer were distinct, the result had never been used clinically. Steve's lab – in collaboration with mine – discovered that the gene expressions on the two sides were also prognostic of relapse. We identified a panel of five genes on each side of the colon that can be used to predict whether a patient will have a relapse in the next five years. We've been working together over the last few years to validate the expression of these genes in numerous samples (both cell lines and primary tissues) and we hope to translate this information into a clinically actionable test. We are in the process of publishing these results and patenting the tests, so I can't say more at this moment, but I am extremely excited about this work. It's a project that could make a real difference to people's lives.

We couldn't do the project without Steve Buechler; he brings the statistical expertise and we have the bench-top know-how. The project only works when we both work together. In fact, many of the current projects in the labs are only made possible through collaboration with other research groups.

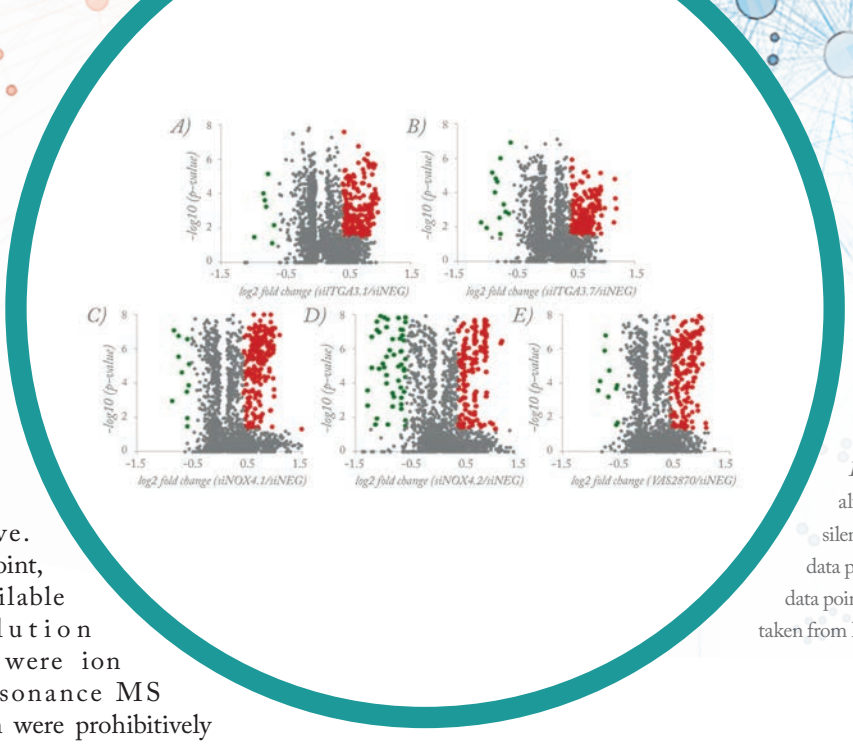

Figure 2. Volcano plots displaying protein expression changes that are statistically significant. (A, B) Proteins changed in expression with *ITGA3* silencing. (C–E) Protein altered in expression with *NOX4* gene silencing or chemical inhibition. Green data points = downregulated proteins; red data points = upregulated proteins. Figure taken from Reference 1.

less expensive. Prior to that point, the only available high-resolution instruments were ion cyclotron resonance MS systems, which were prohibitively expensive for most labs. And though Orbitrap technology is expensive, it is relatively more affordable and has enabled global proteomic analyses in a way that wasn't possible a few years ago.

To truly enable advances in the field, mass spectrometry must be paired with really smart data analysis. So I'd like to acknowledge the incredible importance of database searching algorithms, such as MASCOT and SEQUEST. Using such search tools, we can rapidly identify thousands of peptides, and thus proteins, from a complex mixture.

The development of these tools was really seminal for the field of proteomics – a fact that becomes more evident when you consider the current state of metabolomics. In metabolomics research, the separations and mass spectrometric analyses are similar to proteomics research. However, the databases and the search algorithms are not yet mature. The current standard practice to confirm identification is to test your compound of interest against a known standard, which is expensive, labor intensive and low throughput. As a result, while the field is growing, there isn't a widespread consensus on how to identify features. I anticipate that within the next few years, someone will develop an approach that enables rapid confirmation of mass spectrometric metabolite datasets, which would be transformative for the field, gain great traction and have a huge scientific impact.

Our omics-driven future

Going back to multi-omics, there is an excellent article from Shelia Jasanoff, in which she compares the human genome to the US Constitution: "Like the Constitution of the United States, the human genome turned out to be a sparse document, containing fewer genes than expected. This means that, as with the Constitution, the genome's meanings will evolve over time, as scientists, lawmakers, and [the public] make sense of the fixed elements of the sequence in relation to the variables and unknowns in the surrounding environment." She also addresses some of the criticism that has been leveled at the Human Genome Project and the fact that it has not resulted in fast medical breakthroughs: "A decade is not nearly enough time to measure the impact of a scientific revolution [...] It is too soon to tell whether cures for genetic disease were oversold [...] What matters is that we found a powerful new way to represent human identity, and the moral implications of that re-representation are just beginning to unfold."

I like these analogies. I think the problems we are trying to answer are incredibly complex and it is unrealistic to think that huge sweeping medical changes will result immediately. That being said, there are already some medical changes occurring. Just in the last couple years, it has become possible for pregnant women to learn about the genomic status of their fetus through circulating fetal (cf) DNA sampled from a blood draw. That is an enormous advance and I anticipate that within a few years a range of tests will be available on cfDNA and other valuable samples. Similarly, another area of research that I think is on the cusp on making a breakthrough scientifically is the analysis of circulating tumor cells.

Tumors shed cells into the bloodstream and researchers are making great strides in their ability to enrich for these cells and perform omics analyses on them. Success in this arena would have a huge impact on cancer diagnoses in the next few years. Both of these developments fall under the umbrella of personalized diagnostics. And I anticipate that we will see many more of these important developments in the near future.

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1. *KM Bauer et al, "Proteomic and functional investigation of the colon cancer relapse-associated genes NOX4 and ITGA3", J Proteome Res. 13(11), 4910–8 (2014). DOI: 10.1021/pr500557n.*
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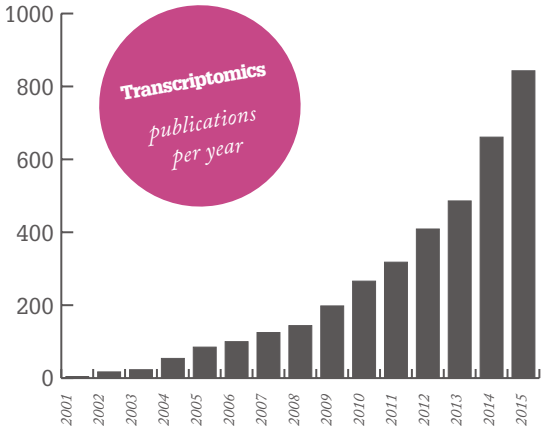
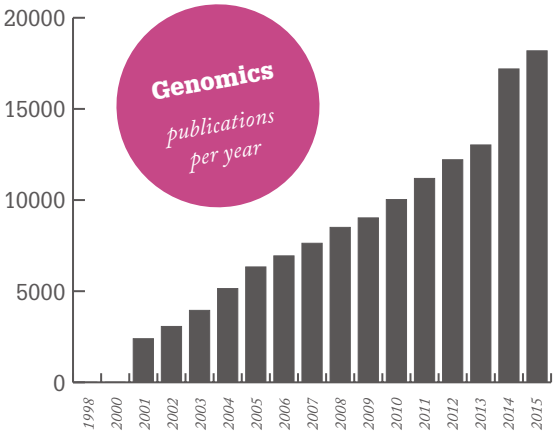
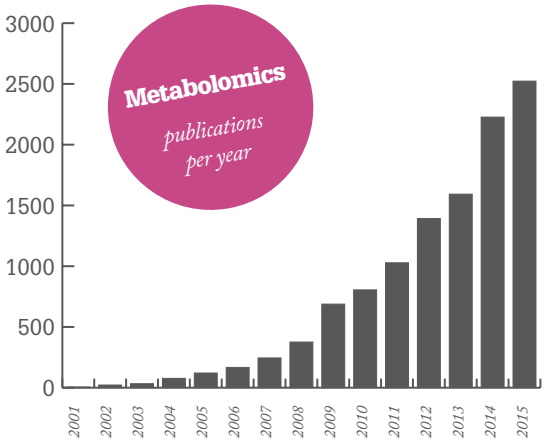
Omics in the Literature

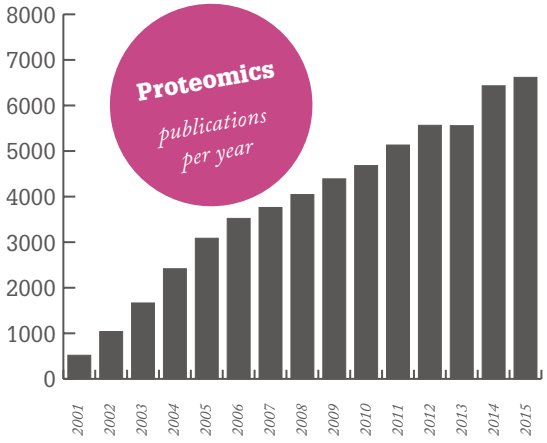
What does analysis of the last 15 years of literature on the different omics tell us about the growing importance of the field, and the move towards a more integrated approach?

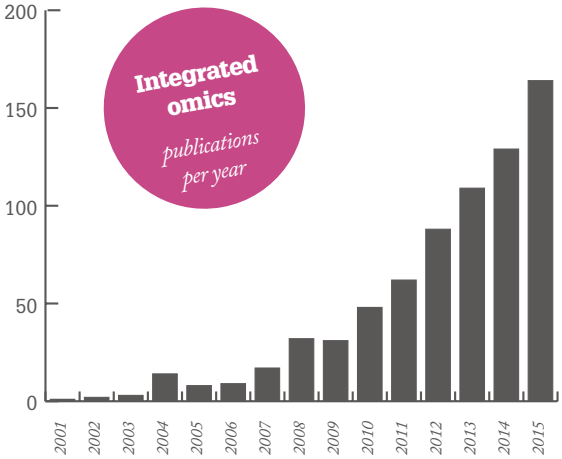
PubMed was searched for "transcriptomics," "genomics," "metabolomics," "proteomics" and "integrated omics" with a date filter of 2001 to 2015. The data were then analyzed in Microsoft Excel 2013.







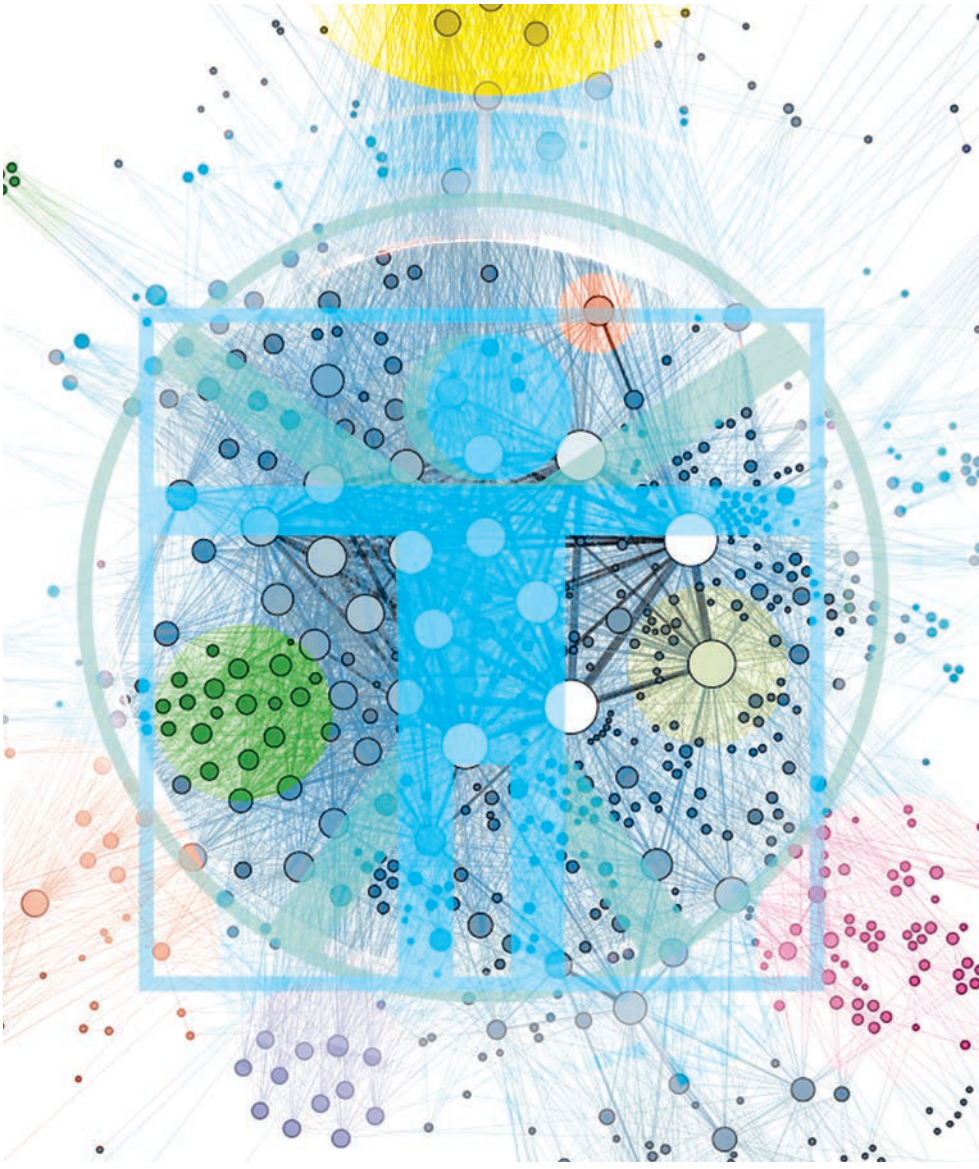




The "One Pot" Approach

Obtaining multiple -omics data sets from a single population of cells

Tom Metz, Integrative Omics Scientist and Metabolomics Technical Lead at Pacific Northwest National Laboratory (Washington, USA), selects six papers that exemplify the power of multi-omics.



When people select one technology over another it's usually because they "grew up with it," which is dependent either on where they did their PhD work or which kind of -omics they happened to apply first to their area of biology. So many molecules are detected with -omics technologies that the false positive rate is likely higher than we expect given today's tools and metrics. When basing subsequent hypotheses and publishing results on single-omic studies, there is bound to be misinformation put forth. Being able to perform additional -omics experiments will help constrain that to some extent. For example, if someone performs a transcriptomic study and has complementary proteomic data (or other -omics data), they will be able to check if what they thought might be going on at the transcript level had propagated through to the protein or the metabolite level.

At a Glance

- *Because they detect so many molecules, single –omic studies are at risk of errors and false-positive results*
- *Using multiple –omics on a single data set can provide checks and balances for each individual study*
- *To see the big picture, you need all of the pieces – not just the genes, but the proteins, post-translational modifications, and more*
- *Protocols that allow researchers to prepare multiple materials with a single technique, like MPLEx (metabolite, protein and lipid extraction) can help*

Pieces of the puzzle

For one thing, transcriptomics doesn't give you any information at all about post-translational modification of proteins, such as phosphorylation and signaling; the only way to capture such information is to do the appropriate proteomics analyses. If resources are not a limitation, then I would suggest that a multi-omics approach should be taken (when reasonable). Clearly, it depends on what questions are being asked, but if the questions are open-ended, then the more data you have, the better.

Here and there, multi-omics is taking

the place of a single -omics. You can tell how seriously we take it at PNNL – we have an integrative -omics group! The Department of Energy Office of Biological and Environmental Research has funded many large programs to study both isolated microorganisms and microbial communities. The goal of these programs is to achieve a systems-level understanding of these organisms and communities such that they could be engineered or otherwise manipulated for the benefit of society, such as for improved biofuels production or carbon sequestration. And the National Institute of Allergy and Infectious Diseases (NIAID) has sponsored the Systems Biology for Infectious Diseases Research Program since 2008. We have been part of that program since its inception and (like others who were funded) have been using transcriptomics, proteomics, metabolomics, and lipidomics to study pathogenic bacteria and viruses. I would say there have been enough papers using multi-omics approaches for me to describe it as in vogue...

Omic efficiency

In essence, with a "one-pot" sample extraction, you're going to save time and effort – one of the big drivers for me and my collaborators in the NIAID systems biology project. It's also likely that it reduces overall experimental variability, because you're no longer trying to integrate data that came from separate cells – you've now got protein, metabolite and lipid data from the same cells. If you were to include a step to extract the genetic material, as others have done, then you could also combine the DNA/RNA data sets.

With microbial communities, for example those bacteria that reside in soils and particularly in association with plant root nodules, the scientific community in general is realizing that we need to get beyond 16S sequencing to discover which organisms are there, and instead focus on what those organisms are doing metabolically. That's not only what metabolites they might be producing and releasing into their respective environments, but also what proteins they're producing and how the microbiota are interacting with each other and their environments, including any hosts. This is also the case for the gut microbiome. Could metabolites and proteins that might be released in the lumen of the gut act as signaling or hormone molecules and affect the health of the host? There have been many very cool studies showing that certain populations of microbiota are associated with certain diseases. Now, we need to mechanistically understand why particular phenotypes are associated with those populations of microbiota – and that means looking at the genes that are being expressed, and the proteins and metabolites that are being produced. It's a very complex problem – but multi-omics is best suited to unraveling all of these questions.

Papers pushing multi-omics

1. ES Nakayasu et al., "MPLEx: a robust and universal protocol for single-sample integrative proteomic, metabolomic, and lipidomic analyses", mSystems, 1, 1-14 (2016).
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7. W Weckworth et al., "Process for the integrated extraction, identification and quantification of metabolites, proteins and RNA to reveal their co-regulation in biochemical networks", Proteomics, 4, 78-83 (2004).

"With a 'one-pot' sample extraction, you're going to save time and effort. It's also likely that it reduces overall experimental variability."

Being an experimentalist at heart, I appreciate those multi-omics papers that focus on methods for "one-pot" preparations of samples to enable extraction of all the molecules necessary for multi-omics analyses. Because my primary area of research is metabolomics and lipidomics, I've chosen the papers that go beyond genes

and proteins to cover metabolomics, lipidomics and other small molecule data. Transcriptomics and proteomics are more mature, whereas metabolomics and lipidomics are still developing and evolving to become more robust. I like it when investigators try to bring in additional data on other small molecules. Why? Even though it's riskier (because the platforms are less mature), I don't think it is appreciated just how valuable such information can be.

"We're always limited by perhaps the most important -omics of all: economics!"

Waste not, want not

For a long time, we've used a protocol to prepare metabolites and lipids from samples by using a mixture of organic solvents. A liquid-liquid bilayer of polar metabolites and non-polar metabolites (which are the lipids) forms, and then precipitated proteins are located in the middle after centrifugation. In metabolomics studies, we would discard the protein because we weren't necessarily doing proteomics. I figured that was wasteful and wondered if we could use the precipitated protein for proteomic analyses. I wanted to make sure that we could get just as good data with the precipitated protein as we would with a traditional proteomics preparation. And so we went through a pretty extensive literature search to see who had done it before, in what context, and with what biological samples. We found that there had been initial demonstrations of using precipitated proteins, but no one had really assessed the reproducibility or quality of the proteomics data. It had also never been applied very broadly – so we thought we'd give it a shot. Of course, we could have been even broader in the sample types and conditions we investigated, but again we're always limited by perhaps the most important -omics of all: economics! The funding only goes so far... In any case, the result of our efforts was MPLEx (metabolite, protein, and lipid extraction, see Figure 1).

We found that precipitated proteins, for the most part, give proteomic data that is comparable with the data obtained from working up proteins using traditional methods. And it turns out that the coefficients of variation (CV), which is a measure of reproducibility, are just as good as the standard approach – sometimes better. We found statistical differences in certain cases, but that was almost assuredly due to the nature of the proteins in those particular sample types. We're certainly very comfortable using the approach to gather multi-omics data. Certainly, our MPLEx method is not the only way to gather all the molecular types needed for multi-omics – and I welcome other investigators to rise to the challenge by adopting or adapting other protocols.

The next step is to also add the genomics data. The authors in the ISME paper – H Roume et al. – included a pre-step where they could isolate genetic material (DNA and RNA). And the paper by W Weckworth does the same for one of the plant samples. I see no reason why one cannot get all the molecular types necessary for a complete multi-omics study from DNA all the way down to metabolites. I believe that the main limitations are the resources and technical skills that the investigator has at their disposal.

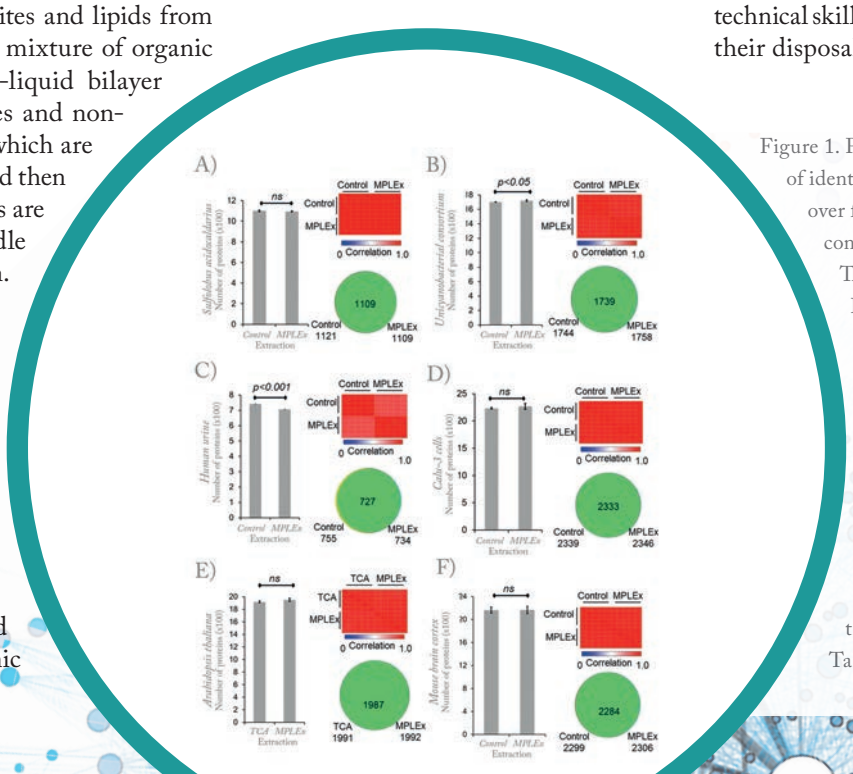


Figure 1. Proteomic coverage, number of identified proteins, and correlation over five replicates for MPLEx and control for different samples. A) The archaeon *S. acidocaldarius*. B) Unicyanobacterial consortium. C) Human urine. D) Human lung epithelial cell line Calu-3. E) *A. thaliana* plant leaves. F) Mouse brain cortex. Abbreviations: MPLEx: metabolite, protein, and lipid extraction; Control: no-extraction control; TCA: trichloroacetic acid extraction. Taken from paper 1.

NextGen

Research advances
New technologies
Future practice

Credit: Ghent University.

Buried Treasure

Noncoding DNA makes up the majority of our genomes, much of it being conserved and transcribed. Though the functions of small regulatory RNA molecules are well known, what about so-called long non-coding RNA? Pioneering scientists are digging up secrets hidden in the labyrinthine depths of our chromosomes…

By William Aryitey

Genomic explorations have offered us a great deal of insight into the intricacies of life. But for all the knowledge we've gained, there is still much we don't understand when we step outside the central dogma of "DNA to RNA to protein." Take non-protein-coding transcripts of over 200 nucleotides as a prime example. New functions and classes of long non-coding RNAs (lncRNAs) – previously thought to be "junk" genetic elements – are rapidly emerging.

And with a recent Nature paper, the field may just have raised its profile again. Researchers have discovered that the *SAMMSON* lncRNA is expressed with exquisite specificity in human melanoma cells. And its presence is no mystery; *SAMMSON* is necessary for the survival of those cells, making it a prime therapeutic target (1). *SAMMSON* stands for Survival Associated Mitochondrial Melanoma Specific ONcogenic, but it's also a reference to the eponymous biblical figure. Just as Samson's power relied on his hair, melanoma cells' survival relies on *SAMMSON*.

"It's a beautiful example of a hypothesis proving true, which is always very rewarding as a scientist. But the most exciting thing is definitely the huge potential, not only for our finding – *SAMMSON* for melanoma – but the mere idea that there could be hundreds of other lncRNAs out there with equally specific expression profiles, meaning that they could serve as diagnostic and therapeutic targets for other diseases," says Jo Vandesompele, study co-author and Professor in the Functional Cancer Genomics and Applied Bioinformatics (FCGAB) lab at Ghent University.

Pieter Mestdagh, co-lead researcher and Professor in the FCGAB lab, agrees. "As a cancer research lab, lncRNAs are a very exciting aspect for us to focus on. We are continuously looking for novel ways to diagnose and treat cancer, and we believe that the field of lncRNAs could be a game-changer."

At a Glance

- *A rapidly emerging field of genetics exists just outside the central dogma of "DNA to RNA to protein"*
- *Long non-coding RNAs have a wide variety of functions – including, in lncRNA SAMMSON's case, holding the key to melanoma cell survival*
- *Because SAMMSON expression is both specific to and essential in melanoma, the lncRNA can serve as a diagnostic and therapeutic tool*
- *And it's not the only one; with over 60,000 known lncRNAs in human tissues, the potential for disease relevance is huge*

One man's trash…

The FCGAB lab uses high-throughput technology and advanced bioinformatics to hone in on RNAs linked to cancer. Vandesompele explains, "Our lab has done a lot of work this past decade on non-coding RNA. We started off looking at miRNA before moving into the exciting new field of lncRNA. Our ultimate goal is always the identification of therapeutic targets, and the very specific expression profiles of lncRNAs open up a number of opportunities in terms of therapeutic and diagnostic applications."

> *"It's evident now that the most important function of RNA is not to code into proteins, but to act as 'glue' that facilitates all kinds of biochemical processes."*

In the past, lncRNAs were largely dismissed as genetic noise. Research into their potential functions only began in earnest about a decade ago – but since then, their relevance in cell homeostasis and disease mechanisms has become very clear. And though the field is still in its infancy, enthusiasm is growing quickly as the new functions of lncRNAs are unraveled. "I think it was very unfair to call it junk simply because we didn't know its function," says Vandesompele. "In school, we all learned the central dogma of biology: DNA is transcribed into RNA, and RNA into protein. But actually, it turns out that a minority of RNA does that. It's evident now that the most important function of RNA is not to code into proteins, but to act as 'glue' that facilitates all kinds of biochemical processes. It's a completely underappreciated functionality of human

cells, and that's part of the reason we were drawn to it."

Mestdagh says he has been excited to witness the explosion of studies in the field. "When we started the study that led to the paper in Nature, there were only around 1,700 lncRNAs listed in public databases. We were able to include them all in a single study. There are over 60,000 known to be expressed in various human cell types."

In their original study, the team were not looking specifically for potential melanoma drugs. They set out to investigate the differential expression of lncRNAs across different cancer types by profiling their expression in a panel of cancer cells. Looking at the expression profiles, one association stood out. "When we started profiling the expression of these lncRNAs in cancer cells, we noticed that some of the lncRNAs were specifically expressed in only one cancer type," says Mestdagh. "It was really a matter of letting the data speak for itself, and the most specific gene in the cohort was *SAMMSON*."

The strength of *SAMMSON*

After profiling numerous normal and cancerous tissues, the researchers concluded that *SAMMSON* expression is highly specific to melanoma cells. Realizing that the lncRNA could have diagnostic or therapeutic possibilities, they decided to focus their ongoing investigation solely on *SAMMSON*. "It was Pieter who looked at the data and said 'Wow, that could be indicative of a major survival function for melanoma cells – let's try to silence that gene to find out how crucial it really is," says Vandesompele.

The team contacted Jean-Christophe Marine (co-lead researcher on the paper, and head of the VIB Laboratory for Molecular Cancer Biology at KU Leuven) to help confirm their hypothesis that *SAMMSON* was an oncogene. Mestdagh adds, "We are not melanoma experts, so we worked with the group at KU Leuven because they had prior experience with melanoma and had model systems in place to start studying it."

When the results of the VIB lab's analysis came back, Vandesompele and Mestdagh were surprised by how completely dependent on *SAMMSON* the cancer was. "Silencing of *SAMMSON* caused melanoma cells to die very rapidly

Spinning Out

The #datasaveslives social media campaign promotes the positive impact that data is having on health. Projects recently highlighted by the campaign include:

As well as working on long noncoding RNAs at Ghent University, Jo Vandesompele and Pieter Mestdagh are also involved in university spin-out Biogazelle, co-founded by Vandesompele and colleague Jan Hellemans in 2007. Mestdagh is a consultant/senior scientist at the biotech, which investigates the coding and non-coding regions of the genome. Biogazelle uses the technology developed at the Ghent lab, but at a larger scale. The company offers RNA biomarker discovery and development services and biostatistical qPCR data analysis software to pharmaceutical customers. Biogazelle also has its own therapeutic program, focusing on blocking cancer-promoting lncRNAs with nucleic acid-based drugs.

Vandesompele is also the co-founder of another Ghent University spin-off company, pxlence, which provides a catalogue of almost a million PCR assays for targeted resequencing of exons and protein-coding genes.

and very efficiently. We hypothesized that *SAMMSON* would have an important role, but we didn't realize its effect on the cells would be so strong," says Mestdagh. The same result was seen in various melanoma cell cultures, including those resistant to an existing therapy, dabrafenib.

To study the effects in more detail, the researchers used GapmeRs (antisense oligonucleotides that inhibit lncRNA function) to knock down *SAMMSON*, which allowed them to investigate the pathways with which the nucleic acid was involved in melanoma. They pinpointed a key function in mitochondria, and eventually concluded that silencing *SAMMSON* causes apoptosis in part by disrupting p32-mitochondrial functions vital for the organelle's homeostasis. The result is toxic over-accumulation of mitochondrial precursors in the cytosol, which eventually triggers cell cycle checkpoints or induces cell death, depending on the status of the cell.

Mighty in mice

The researchers next analyzed the therapeutic potential of *SAMMSON* knockdown in vivo, using patient-derived xenografts of melanocytes in mice. They found that treatment with GapmeR3 to block *SAMMSON* expression decreased proliferation and increased apoptosis of tumor cells, and the results were enhanced when GapmeR3 was combined with *BRAF* inhibitor dabrafenib. Notably, combination treatment with GapmeR3 and dabrafenib didn't cause any severe adverse effects or weight loss in the mice, unlike combinations of dabrafenib with a MEK inhibitor, trametinib.

The results suggest that *SAMMSON* knockdown could have a synergistic effect with existing cancer drugs – an important finding given that combination therapies are increasingly becoming the norm for cancer treatment.

"We're definitely not claiming that *SAMMSON*-targeted therapy would be a single magic bullet. I think it's clear that treating a devastating disease like malignant skin cancer requires combination therapy. But the addition of anti-*SAMMSON* treatments to other molecular targeting drugs could be a major step forward," Mestdagh says.

The team is actively pursuing the therapeutic potential of anti-*SAMMSON* therapy. "We set up a collaboration with a major pharmaceutical company that has a lot of expertise in antisense technology, to explore the toxicity of antisense oligonucleotides directed to *SAMMSON*. These studies will be initiated in mice very soon, with the goal of bringing us one step closer to the clinical space."

The researchers have also been pursuing an alternative avenue to silence *SAMMSON*. Small molecule drugs are still the therapy of choice for most pharmaceutical companies, and have a well-trodden route to the clinic. With that in mind, the FCGAB lab initiated a collaboration with Matthew Disney at the Scripps Research Institute in Florida to identify small molecule compounds that actively bind to the transcript and disrupt its function. "If successful, it could be the first small molecule targeting a lncRNA to treat cancer," says Vandesompele.

SAMMSON's abundant expression in melanoma cells and absence in normal cells could also make it a perfect candidate for diagnostic or prognostic tools. To that end, the team is currently evaluating whether *SAMMSON* is circulating in the bloodstream – and, if so, to what extent it could be used as a diagnostic or predictive marker.

Further research into *SAMMSON* expression has revealed that it is found in melanomas of the eye as well. Uveal melanoma is the most common form of non-skin melanoma, and the most common eye cancer of adult Caucasians (with about 2,000 cases per year in North America). Compared with melanomas in the skin, which can be treated with *BRAF* and MEK inhibitors, uveal melanomas are much more difficult to address.

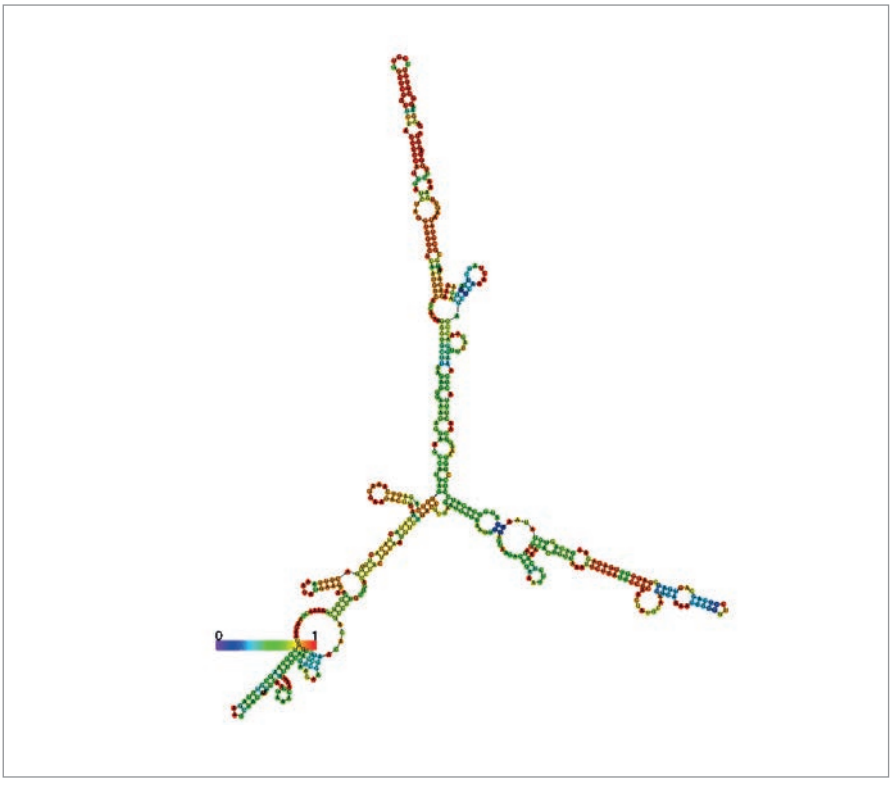
Mestdagh explains, "Metastatic uveal melanoma has virtually no effective treatment, with median patient survival times of less than one year. It's a rare but

deadly disease, and we hope we can make a difference. Similar to skin melanoma, uveal melanoma cells appear to be addicted to *SAMMSON* expression. Inhibiting *SAMMSON* expression in those cells induces their death to a similar extent as observed for skin melanoma cells. We still need to carry out a lot of experiments to prove that we can kill uveal melanoma cells in vivo, but in vitro results of *SAMMSON* inhibition have proven promising."

Unanswered questions

Though *SAMMSON* looks promising as a diagnostic or therapeutic target, much is still unknown, providing a rich seam of future research for the FCGAB team to mine. "We do see occasional expression of *SAMMSON* in non-melanoma cancer cells. It's neither highly nor consistently expressed in these cells, but we're following up with further studies to see whether it has similar roles in these rare cases where it is expressed. Then of course, the question becomes: why is it sometimes expressed and other times not? We have so much more to do, and so many research questions regarding *SAMMSON* still to investigate," says Vandesompele.

Despite the field's relative youth, the researchers may be able to gather these answers sooner rather than later, thanks to a growing toolset of genetic engineering techniques. "By setting up high-throughput lncRNA perturbation screens using techniques like CRISPR interference, we should be able to prioritize functional lncRNAs that can then be studied in more detail to unravel the underlying mechanisms. We are setting up the right platform to enable high-throughput lncRNA perturbation and, by doing so, hope to get a better view of the most relevant lncRNAs related to the phenotypes we're most interested in. And currently, we are performing a large-scale study where we're applying various sequencing methods, such as polyA+ RNA-sequencing, total and small RNA-sequencing on around 300 human cell and tissue types to generate a comprehensive map of the human transcriptome."



SAMMSON transcript

They're also attempting to unravel the relationship between *SAMMSON* and known oncogene *MITF*, its near neighbor on the chromosome. *MITF* and the protein it encodes have a clearly established role in melanoma, so its close proximity seems unlikely to be a coincidence. However, the two genes do not appear to regulate each other, which has left the team puzzled. "It calls for further research to find out if it's really a coincidence or if there is something that we are missing."

But the team's interest doesn't end with *SAMMSON*. They are currently exploring alternative abilities of long-overlooked lncRNAs. Mestdagh says, "Others have shown that lncRNAs can indeed serve as diagnostic or predictive biomarkers in selected cancer types. We're exploring this on a pan-cancer scale. The tissue-restricted expression profile of several of these lncRNAs is extremely appealing for biomarker research, and we're trying to exploit this specificity to evaluate their diagnostic potential in circulation."

"I hope this will inspire colleagues and other researchers to really dig into the lncRNA domain," he continues, "because there are so many genes that still need to be studied and so many functions that still need to be uncovered. If we can find other examples like SAMMSON that are crucial for cell survival, metastasis, or any process in cancer progression, it could lead to great results. We really need a large community of researchers interested in lncRNAs, because there's a lot more work to be done."

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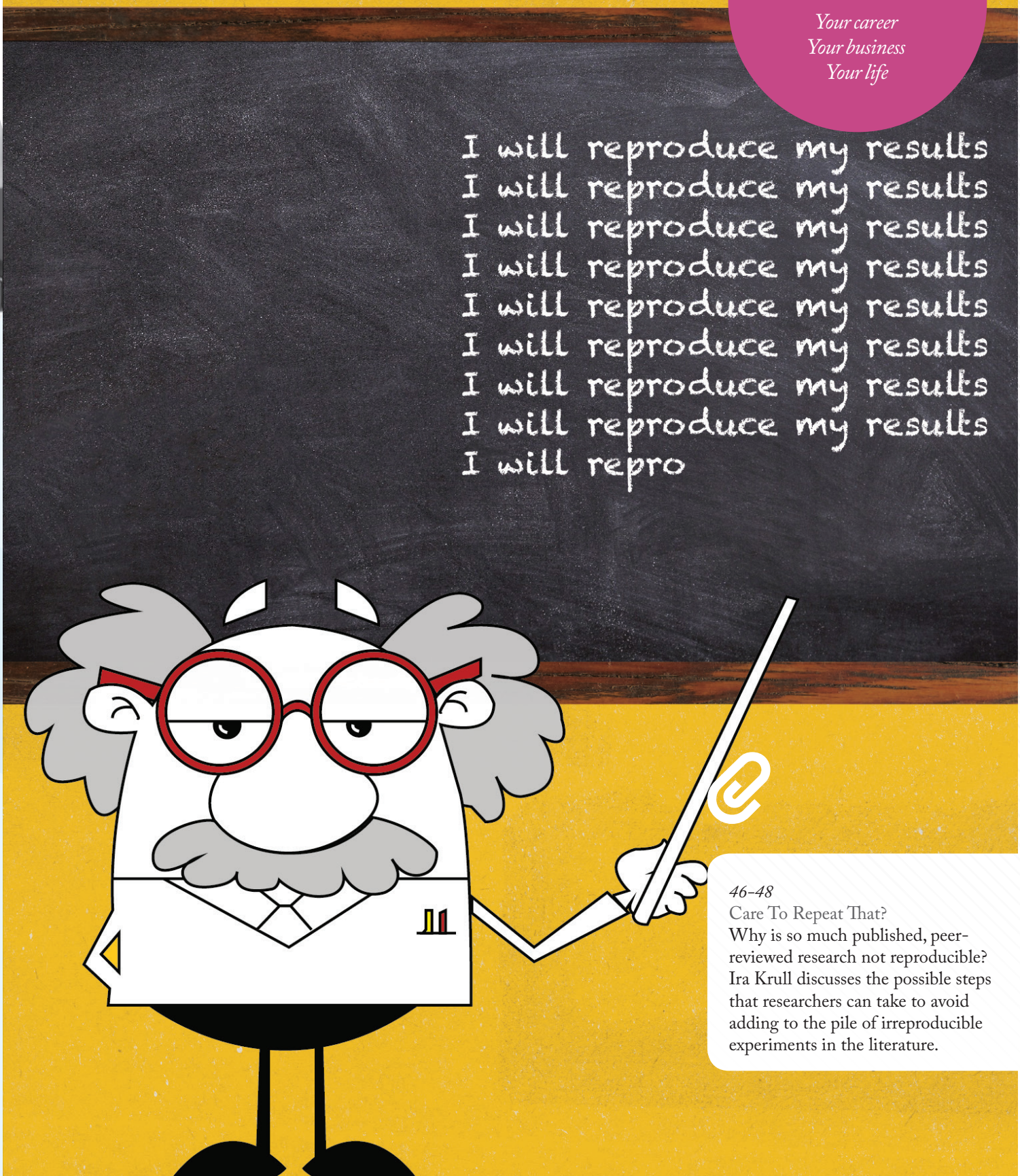


Care To Repeat That?

Today's scientific literature appears to contain an inordinate number of irreproducible papers. Why? And what should laboratory researchers – the bastions of reproducibility – do about it?

By Ira Krull

Over the years, my colleagues and I have amassed a large number of columns, review articles and even books, all dealing with various aspects of analytical method validation (AMV). If one types these three words into a Google search, around 4.33 million results will pop up. It appears to be a very popular area of analytical chemistry. But what has this to do with much of today's scientific literature (especially in the biological and medical sciences) appearing to be of questionable reproducibility (1–11)? Where is this apparent lack of generic reproducibility coming from and how can it be rectified in the future? These are worrying, pressing and, as of yet, not fully addressed questions. And I have many, many more for you...

Much has been written on these subjects but there seems to be some confluence of AMV, reproducibility/repeatability, and publishing poor science in general. Why? And where do the scientific journals (of all types) come into the picture, if at all? Should the burden of responsibility be on authors, journal reviewers, funding agencies, editors, peer review processes, graduate students, postdocs, or elsewhere?

Reviewing reviews

As a reviewer of (mainly) analytical papers for several decades, I receive too many papers that contain little to no true AMV, and no serious discussions of the topic – most of the data are single points with no evidence of any repeatability or reproducibility (n=1). There is, of course, rarely any statistical treatment of said data because there is simply not enough. How is it possible that such manuscripts even reach a reviewer (via the editors)? Why would anyone submit such a manuscript for serious consideration by a reputable journal? Why do some reviewers accept such data, allowing the paper to be published, requesting only minor revisions but no added data or studies?

Inherent heterogeneity or inherent laziness?

Antibody-based publications appear to demonstrate the very least reproducibility of all papers I've seen. Antibodies, being proteins, often vary from source to source, as a function of how they were expressed and purified – perhaps this is the source of some irreproducibility in such articles, but I believe most of the blame lies squarely at the door of researchers themselves.

As a practicing academic with an active research group for decades, I was always amazed by how few academic colleagues demanded that their researchers, graduate students, postdocs, visiting scientists, and undergraduates learn as much about AMV and the demonstration of repeatability and reproducibility as possible – and demonstrate it in all of their studies. It was (is) as if they never considered such behavior an important part of doing quality research or publishing high-quality papers.

Even if the antibodies themselves are not reproducible, good method validation would prove the fact – in addition to indicating the reproducibility of the overall research. If such studies are not pursued or demanded by editors or reviewers, then more and more papers will eventually and inevitably be shown to be irreproducible – which is exactly where we currently find ourselves. Is it possible that biologists are never taught anything about AMV? If so, is it also possible that research advisors and mentors do not require their students to know about this field or push them to work harder towards credible publications in the open, scientific literature? More remarkable is the fact that even PhD theses specifically focused on analytical chemistry often do not contain evidence of true method validation, repeatability or reproducibility.

All of the above leaves me with a big question mark over the reproducibility of the vast majority of papers appearing in analytical journals. Should we discount everything with little to no AMV? In any case, we need to find and fix the underlying problem.

Time to change

I think it's fair to say that the problem lies with our own efforts, and not "in the stars." But how do we correct the problem? How do we ensure a future

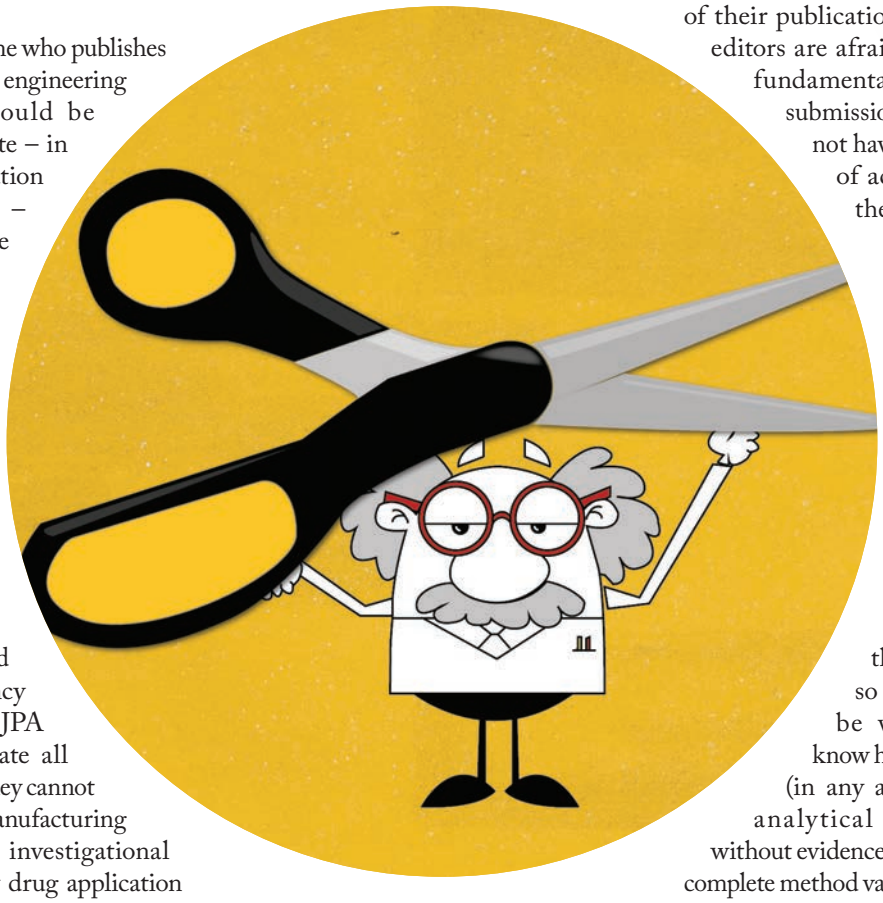
At a Glance

- *Many of the experiments published in today's scientific literature are irreproducible – but why, and who should be held responsible?*
- *In industry, scientists must validate every method before filing marketing paperwork; academics should be held to the same standard*
- *The peer review process needs improved guidelines, and those involved in the process must uphold the highest standards of scientific rigor*
- *Every researcher can make a difference – don't be afraid to call out irreproducible experiments and stand up for better science*

where science will not be discredited by the suggestion that most of its publications are just not reproducible or useful? I think we can all agree that if even the originators of a piece of research cannot reproduce their findings, future researchers will also struggle... and that means everyone is just spinning wheels, wasting time, energy, hope, money and the future of science.

Suffice to say, everyone who publishes any type of science (or engineering for that matter) should be required to demonstrate – in the very first publication using such methods – complete AMV. There is no excuse not to. The field has now been perfected; it is used throughout the pharma/biopharma industries, it is a major part of ICH and all regulatory guidelines around the world for such products. Indeed, scientists in any industry that is regulated by a government agency (whether FDA, EMA, JPA or others) must validate all analytical methods or they cannot submit a chemistry, manufacturing and control (CMC), investigational new drug (IND), new drug application (NDA) or any other request to file and market pharma/biopharma products. However, complete AMV has never really been accepted, respected or adopted by the major group of scientists who publish scientific articles – the group commonly known as academics.

While industry scientists toil over replicate experiment after replicate experiment, academics bear no such cross. They simply need to convince the journal editors, peer reviewers, and funding agencies that their work is analytically valid and reproducible. The burden of the cross has been passed on, in our current system, to the journal editors and peer reviewers who determine if a given manuscript is ready to be published or not. And if these gatekeepers of all scientific literature also fail to practice, understand or utilize the principles of true AMV, then their reviews will be useless or worse.



Better gatekeepers or better gates

We clearly need gatekeepers who understand the science being presented, as well as the method validation requirements that must be met before any manuscript can be accepted for publication. Editors must also take responsibility in all of this mess by requiring, before any kind of peer review, that all manuscripts demonstrate full and complete method validation data, to the standard required of pharma/biopharma submittals to the FDA/ICH and most other regulatory agencies. Why should journals be any different than the regulatory agencies in what they expect of their publications? Perhaps journal editors are afraid to demand such a fundamental requirement of all submissions because they may not have a sufficient number of acceptable papers for the next issue...

There could be a more conspiratorial flaw in the peer review process. If all reviewers simply accept manuscripts without any real validation data, their own submissions are consequently less likely to require such data. Let's hope that the entire system is not so rotten. But it would be very interesting to know how many publications (in any area of science) with analytical data are accepted without evidence of true and (perhaps) complete method validation data. It would certainly account for the apparent lack of reproducibility in so many different areas of science today.

I've asked many questions. And now you are most likely thinking: "Okay, Ira – you've made your point – but how do we rectify the problem?" Rectification comes with due diligence from everyone involved, and in having QA/QC procedures for this assurance. Journals must establish required guidelines for all future submissions. To a large extent, both

Nature and Science now have such guidelines in place – better late than never (12). Such guidelines have been designed to ensure that everything needed to reproduce the work involved is present and that sufficient AMV studies are also indicated and verified. However, if the authors are not made to abide by these guidelines, then we cannot move on from the present impasse. Hence, editors and peer reviewers must enforce the guidelines; if the prerequisite AMV material is not contained within the text of the manuscript, then the paper should be rejected outright or accepted pending further revisions, to fully meet the guidelines. If the authors then fail to provide the information required to meet the guidelines, the manuscript must be rejected. "Guidelines" is perhaps the wrong word to use for academics, as it may imply some degree of freedom – "mandatory rules" may be better. In any case, it should clearly be the responsibility of the editors and (especially) the reviewers to ensure suitable and adequate AMV for all accepted manuscripts.

We can do better

We find ourselves at an unprecedented point in the history of publishing scientific articles, and of science itself: the majority of papers in certain areas cannot be easily reproduced. We have arrived at this terrible juncture because we have been far too lax in what was – and is – required to publish in reputable journals, especially regarding AMV. And though journals may guard the gates, academic institutions and the academics within them have a big role to play. I believe mandatory undergraduate and graduate courses in AMV would make a difference – and at the very least, mentors and advisers should coach best practice in AMV and expect no less. Funding agencies should not take a back seat either, but deny future funding to those researchers who refuse to perform or report AMV in their papers.

"We find ourselves at an unprecedented point in the history of publishing scientific articles, and of science itself."

I look forward to a future where peer reviewers begin to assume responsibility for rejecting manuscripts because of a general lack of AMV; where students no longer gain an advanced degree without knowing a great deal about AMV or how to apply it in the real world; where scientists and their students take AMV seriously, and thereby avoid publishing irreproducible papers that result from work that was never demonstrated to be reproducible in the first place.

Finally, we pathologists and laboratory medicine professionals should be setting the very best example. If we aren't taking AMV seriously, how can we expect scientists in other disciplines to do the same? Don't be afraid to offer guidance when you're involved in a collaborative project that is going "off the rails" – other members of the team may not be as well versed in the need for AMV. And don't be afraid to stand up and decry research or publications that fail to meet even the basic requirements for reproducibility. The whole of science is at stake.

Ira S Krull is Professor Emeritus, Department of Chemistry and Chemical Biology, Northeastern University, Boston, USA.

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Collaborating for the Clinical Win

Sitting Down With... Ron Heeren, Director of Maastricht MultiModal Molecular Imaging Institute (M4I), Distinguished Professor and Limburg Chair at Maastricht University, The Netherlands.

Is there a common theme to your career?

Change and passion. I always told myself that every 10 years I would do something different. I was trained as a physicist, became a professor in chemistry, and now I am working in a molecular imaging institute housed in a medical department. I stay enthused about what I am doing by making it worthwhile – and changing my environment and research topics helps keep my passion for science alive.

I'm very lucky to have been able to set up this wonderful institute within the University of Maastricht. A collaborator of ours said to me recently, "I feel like a kid in a candy store!" And that's exactly the environment that we wanted to create – it encourages people to do great science and great molecular imaging. Here, I get to define the questions I'd like to ask and the best tools for answering those questions, and so explore the world around me. How much better does it get?

"Diversity in passions and interests is crucial for the success of any team."

Tell us about your current research...

Our major focus is using molecular imaging based on mass spectrometry to assess the molecular content of tissues, so we can provide clinicians and medical researchers with feedback on the cellular phenotype. Say a surgeon operates on a patient and removes a tumor; it's sent to a pathologist, who takes a section for hematoxylin and eosin staining and inspection, and we take an adjacent section for mass spec imaging analysis. Half an hour later, we aim to provide the surgeon and pathologist with our findings and see how they match. In a second project – intraoperative diagnostics – we are analyzing the smoke from laser surgery to give surgeons the information they need in real time. Most of our research is biomedical, but we also study new biomaterials, regenerative medicine, drug distribution and metabolism, forensics, and even historical paintings.

And recent findings?

In a study on cholangiocarcinoma, we identified several peptides, proteins and lipids that distinguish transient neoplastic tissue (on its way to becoming a tumor) from full-blown tumor cells and healthy tissue. In other words, we can assess a single piece of tissue and define different cellular tissue phenotypes, which helps us to assess how clean a tumor's surgical margin is; has the surgeon removed enough? Is there any cancerous or precancerous tissue remaining?

How close are these tools to the clinic?

We've developed technology and methods for a number of diseases. The next step is validation – we need to work with large patient cohorts to make sure that the markers we have found in 10 organs are stable and robust in 100 or 1,000 organs.

To establish a validated clinical diagnostic test, several major clinical studies and a lot of administrative and legal paperwork are needed. It's difficult to predict how long it will take, but I hope that within five years some of these tests will be routinely available. For now, they are still in the research phase.

What's the secret to successful collaboration?

Crucial to the success of this type of multidisciplinary research is a willingness to give something up to ultimately gain a lot more. Sometimes we generate great results, but rather than presenting them ourselves, we ask the surgeons on the team to present them at surgery conferences. We give up a little visibility in our own community but, in the long term, we have a much bigger impact in the clinical field – where it really matters.

How important is communication in your career?

Absolutely crucial. Without good communication, it is impossible to start new projects or to motivate other researchers to move in the same direction. To my mind, communication is nothing more than showing how passionate you are about what you're doing, how much fun you're having while doing it, and how good the results are. It's also important for me to showcase and emphasize the importance of the work of my (younger) colleagues, without whom I would be dead in the water.

What does the perfect team look like?

I believe in heterogeneous teams. My current group has a very international flavor and is half female – and that makes it a culturally diverse group. Diversity in passions and interests is also crucial for the success of any team; we have specialists in everything from clinical science to bioinformatics. But they all share one thing: a passion for research. The enthusiasm of young researchers really helps with building such an institute. We aim to come up with new and fresh ideas, and that characterizes my staff: young, willing, enthusiastic, eager, and impassioned by their research.

What are your career highlights, so far?

From a research perspective, the best thing has been seeing how the ideas we had 10 years ago are being realized in the clinical environment. I've always believed our work would make a difference for patients, and seeing that start to happen is wonderful. Talking about it at TEDxMaastricht recently was an absolute highlight for me, personally. Molecular structure plays a much bigger role in disease than we previously thought, and being able to visualize that with the technology we've developed is fantastic.

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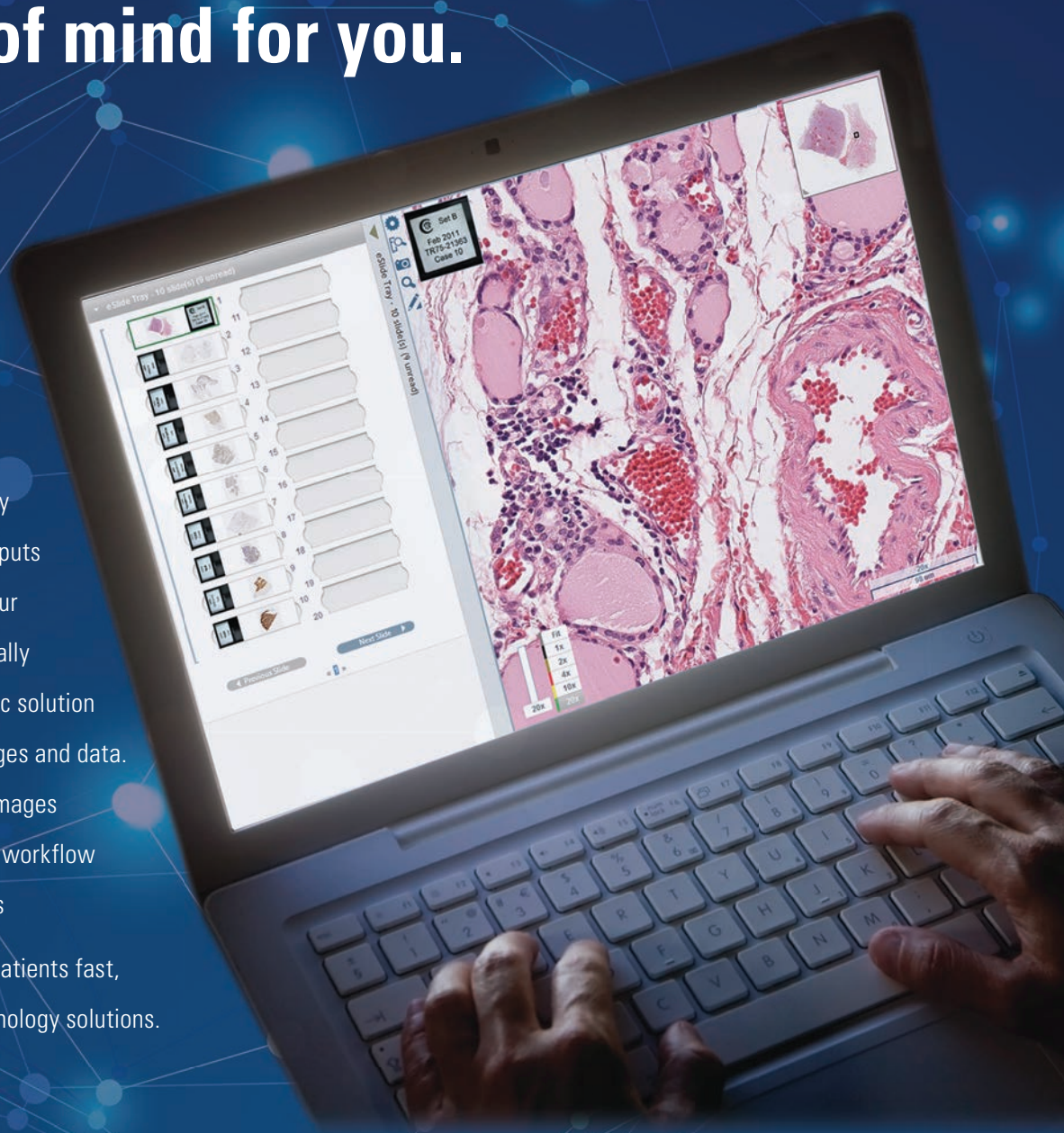

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