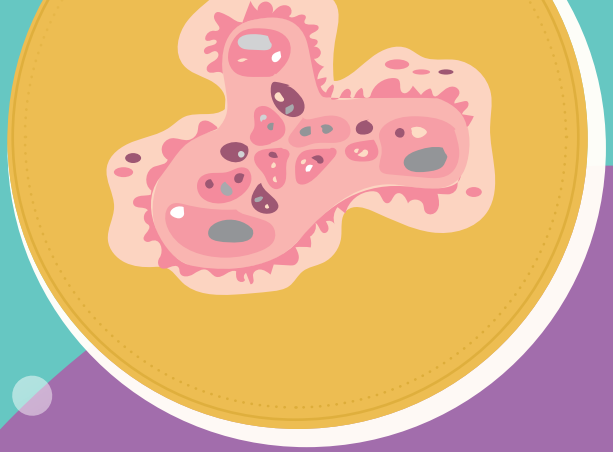
(Bio) Banking on PATHOLOGY'S Future

The field of pathology is ever-changing – but as precision medicine and molecular techniques become integral to the laboratory, pathologists need to embrace their increasingly important role as tissue archivists



Curating Pathology's Future

Biobanks are vital to biomedical research and clinical diagnostics, but we have a great deal of work to do before we can realize their true potential

By Fay Betsou

The word "biobank" first began to take off in print in the mid-2000s. More than 15 years ago, an Internet search would have returned almost nothing; today, there are over a million results. It's a very short existence for a concept that I believe is vital to modern pathology – both in research and in the clinic.

The biobanking initiative first came from the Organization for Economic Cooperation and Development (OECD), which not only started advocating for the importance of biobanks, but also insisted on the need to have an accreditation system. In the years following the proposal, the governments of various countries began funding research infrastructures for biobank operations. One such country was France, where I began my own career in the very first autonomous biobank to get ISO certification in 2005. And by 2008, the French government had developed and begun applying a national certification standard for biobanks. It's approximately equivalent to ISO 9001: a basic quality management system, but nothing more. But professional biobanks – those whose sole purpose is sample collection, processing and management – should be held to a higher standard.

The preanalytical problem

The most important aspects of a biobank are consistency and quality. When researchers come to us and say, "I need 30 lung cancer samples," we ask, "Okay, what kind of lung cancer? What kind of sample?" But most of them are not pathologists; they don't know the different histological types or sample preservation options, so they just ask us for "lung cancer." We have to educate basic and translational scientists to understand what they need in greater detail – because it's difficult to provide a professional service when the clients can't clearly articulate their requirements.

Sample characterization – clinical, pathological, immunohistochemical and preanalytical – is a large part of what we provide. Most of that may seem obvious but, until now, preanalytical characterization has been almost completely neglected despite its importance. We can't just forget to take into account the potential impact of factors such as cold ischemia time, fixative type, or even storage temperature on the downstream results; these are all critical elements that professional biobanks should track – and, fortunately, most of them do. As a result, when asked for samples, the biobank can select them according to their suitability – and the researchers can then specify in their publications where their samples originated


“THE MOST IMPORTANT ASPECTS OF A BIOBANK ARE CONSISTENCY AND QUALITY.”

and how they were handled. Without that information, it's easy to introduce invisible bias into the work – and then researchers are surprised when their findings cannot be reproduced!

Along with the Biospecimen Science Working Group at the International Society for Biological and Environmental Repositories (ISBER), we have developed a tool called SPREC – the Standard PREanalytical Code (1) – an evolving seven-element code that summarizes the nature of the sample and its history. For instance, the seven elements of a tissue sample SPREC are:

- specimen type,
- collection type,
- warm ischemia time,
- cold ischemia time,
- fixation/stabilization type,
- fixation time, and
- storage conditions.

So your specimen might carry the code *TIS-BPS-N-E-NBF-G-P*. That would make it a solid tissue specimen (TIS), collected via biopsy (BPS), with warm ischemia time not applicable (N), cold ischemia time of 30–60 minutes (E), fixed in neutral-buffered formalin (NBF) for 48–72 hours (G) and stored at room temperature in a paraffin block (P). Don't have time to code all your samples by hand? A publicly available tool, the SPRECALC, will automatically generate the codes – and there's even a second tool to convert them into barcodes for labeling.

Controlling quality

One major source of error in biobanking is poor annotation. Most clinical and pathological annotations come from medical records that lack standardized language and, on top of that, it's not uncommon for them to be transcribed inaccurately. The other significant error source is the quality of the samples themselves; either the preanalytics aren't accurately documented or quality control tests haven't been run – or both.

Almost all of our existing samples suffer from the first problem. If you went into the average biobank today and tried to annotate its samples with SPREC, 90 percent of the time, you would simply write *TIS-SRG-X-X-NBF-X-P*, because some information was never recorded. Unfortunately, there's no way to fix that; all we can do is ensure that protocols are documented going forward. But we can solve the second problem – even if you don't know how samples were collected or processed, you can still apply quality control tests to them, or to their derivatives, and use that information to stratify them into quality categories. For example, you might extract DNA, perform a multiplex PCR, and see to what extent the genetic material is still amplifiable. Of course, that brings us to a further need: the development and validation of such quality control assays

ISO Technical Committee on Biotechnology

The International Organization for Standardization (ISO) includes a technical committee, TC 276, responsible for developing standards related to biotechnology. The committee has an active working group for biobanks and resources that is currently developing a technical standard for biobanks (DIS 20387), which may eventually be used in accreditation. The standard would make traceability and quality control measures mandatory for any institution that wishes to be compliant.

DIS 20387 is currently in the inquiry stage. What still needs to be done before it becomes a formally published standard? First, national bodies will have 12 weeks to vote and comment on the draft text, including making technical changes. Then, if successful other than technical changes, the text will be updated and submitted as a final draft international standard (FDIS) and voted on again – this time without the option of technical changes. Finally, if approved, the text will be sent to the ISO Central Secretariat for publication as the International Standard.

– but, in my opinion, that is the only solution that can allow us to use with confidence the millions of legacy samples stored in biobanks and pathology labs around the world (2).

Teaching and training

We are constantly involved in spreading the word about biobanking – why it's necessary, who can benefit, how it's done... When I worked at a university hospital in France, we organized training for clinician-researchers; now that I'm in Luxembourg, the work continues. We have developed a university certificate on biobanking that is targeted more at biobankers themselves, but we often see researchers and clinicians signing up because they want to learn more. We organize seminars at hospitals and research institutes to educate the faculty, and they are always very surprised when we explain to them, "You ask us for lung tissue – did you know that there are different histological types? Did you know that a sample with 10 percent tumor content will give you completely different results in your analyses than one with 80 percent tumor content?" It's a revelation to them. Clearly, there's a lot of work to be done!

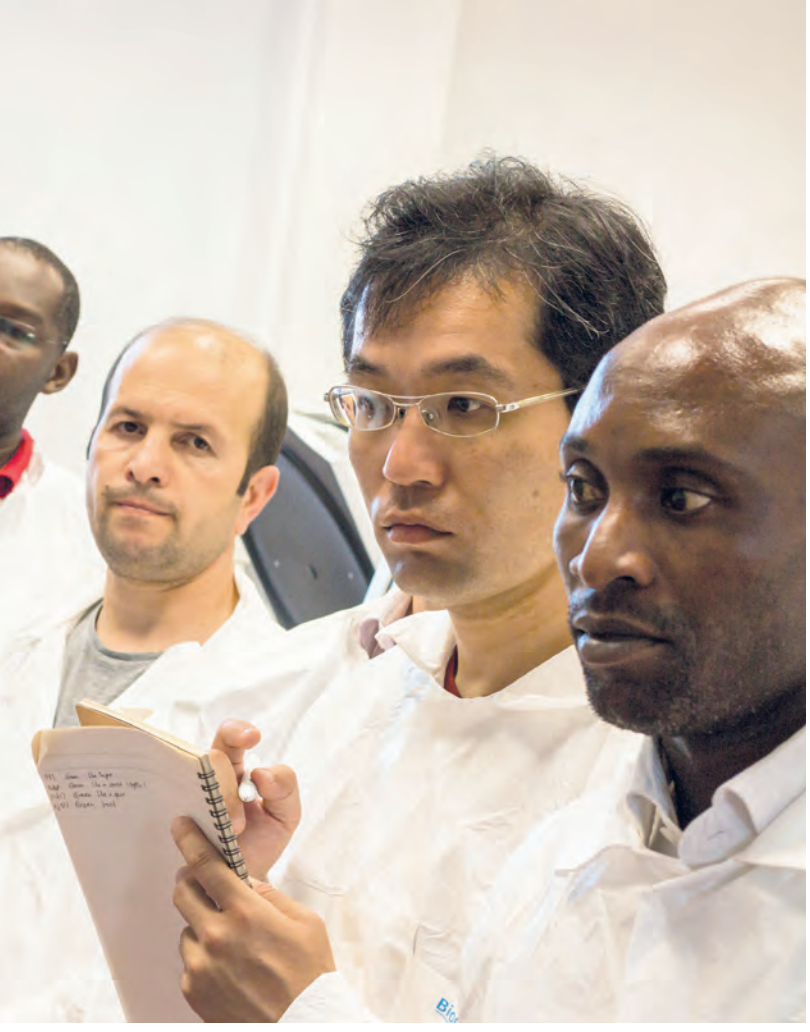
For many years, I have been saying that professional biobankers need to submit abstracts to scientific society meetings. After all, our work is applicable to every area of biomedical science: immunology, cardiology, oncology, infectious diseases, hematology, and the list goes on! So any biobanker can assemble an abstract that addresses a few key questions:

- What are biobanks?
- What kind of work do they do?
- Why are they important?
- How can they help with your field of study?

We don't do nearly enough of this kind of outreach work. In my opinion, we should be at all of the major scientific meetings. We need to make the research community aware of our services and help them to understand why they need us – and we need them.

Enabling access

The biggest obstacle to bringing researchers and biobanks together is the question of supply versus demand. If you are a researcher who needs samples and associated data and you try to request them from a biobank, you will almost never find what you are looking for. Why? Because the needs of each research project are so specific that often, even big biobanks won't have what you need. In fact, this is a subject of much discussion in the biobanking community: what is the best way to operate? Should we operate on stock and try to build a huge library of samples so that we can provide as many different options as possible? Or should we operate on project-based demand? At the moment, most



biobanks follow the first model – but experience shows that it is neither the best nor the most efficient method. Much of the time, researchers don't have a use for what we have in stock, whereas we cannot provide them with what they do need.

I think the best approach is to switch to prospective, project-driven collections – but of course, for this you need professional biobanks with all of the necessary infrastructure in place to begin collecting immediately. If you have to wait a year while you assemble an ethics committee and establish everything you need from an administrative point of view, your clients will go elsewhere – or won't be able to conduct their research at all. Professional biobanks already have the administrative and the quality management systems required. You send them your request; they begin collecting in a consistent and controlled manner; and after only a few months, they deliver exactly what you need.

I don't know of any biobanks that currently work to this model, but it is something we are trying to develop. The first step is networking. You need to be in small, bottom-up networks to provide samples efficiently; if you don't have what a client needs, it's possible that another biobank does, which prevents the need to start from scratch. This kind of functional networking already exists in a few countries – in Spain and the United States, for example, and there's a government initiative to establish something in Germany as well – but it's lacking in most places. Even in those that claim to have such networks, it's often more like a list or catalog of existing biobanks, rather than a true relationship between them.

At IBBL, we believe in "trusted biobank networks" – but it will take time to build them. In the interim, we advise potential users to locate biobanks that are serious and professional to help them get the samples they need.

It's clear that our work as professional biobankers is just beginning – not only in our sample procurement and preservation work itself, but also as documenters, educators and promoters. The future of pathology lies in biobanking, and it's up to us to step forward and make these services the best they can be.

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References

1. *S Lehmann et al., "Standard preanalytical coding for biospecimens: review and implementation of the Sample PREanalytical Code (SPREC)", Biopreserv Biobank, 10, 366–374 (2012). PMID: 24849886.*
2. *F Betsou et al., "Assays for qualification and quality stratification of clinical biospecimens used in research: a technical report from the ISBER Biospecimen Science Working Group", Biopreserv Biobank, 14, 398–409 (2016). PMID: 27046294.*

The Promise of Precision Pathology

No one is better placed than pathologists to drive the precision medicine of the future – and a new kind of pathology will be crucial

By Michael H.A. Roehrl

Precision healthcare is the future – of that, I have no doubt. But how do we go about successfully developing it for the patients who need it? The key, in my opinion, lies in the comprehensive availability of high-quality human samples for all aspects of research – from basic bench work to clinical trials. And who better to ensure that availability than pathologists? Pathology is the central specialty of personalized precision medicine. It is pathology that provides the skills, infrastructure, and scientific vision we need to lead the way in science-driven biobanking, and it is pathology that can help to ensure optimal research use of human samples. And that's why my pathology colleagues and I have taken on the task of setting up a major new initiative at Memorial Sloan Kettering Cancer Center – the Precision Pathology Biobanking Center (PPBC).

Founded in 2015, the PPBC represents an institution-spanning collaborative research center that is being built around five highly interconnected pillars (see Figure 1): next generation, "future-proof" biobanking; "big data" computer science and database development; a hub for developing and evaluating the next wave of theranostic pathology technologies (like proteomics, metabolomics, and molecular imaging); a hub for pathology to take on a proactive role in the latest generation of specimen-driven clinical trials and drug development; and a platform for pathology to develop strong joint research, development and commercialization partnerships with the private sector. It's easy to see how a thoroughly annotated, high-quality biobank underpins every one of these pillars.

Building a better biobank

When we designed the PPBC's specimen acquisition, preservation, storage, and distribution workflows, the concept of "future-proofing" was front and center: all samples (tissues, bloods, other liquids) are procured at high speed (ideally directly in the operating rooms or interventional radiology suites) and uniformly held in vapor-phase liquid nitrogen, rather than dry ice or -80˚C freezers. Previous research has convincingly shown that some of the most interesting components of the pathophysiome – like RNA, post-translational modifications of proteins, or small metabolites – degrade unpredictably, even at -80˚C, over time spans of months to a few years. In vapor-phase liquid nitrogen (which cools to below -160˚C), on the other hand, they remain stable – thermodynamics is one's friend. The PPBC banks specimens from approximately 7,000 new cancer patients per year, including surgical resections, interventional radiology biopsies, and companion blood and body fluid collections – so we certainly don't want to lose those samples just a few years down the road.

How do we prepare our samples? Lesional and matched normal tissues are flash-frozen in liquid nitrogen without further additives; then, we prepare spatially indexed formalin-fixed and paraffin-embedded (FFPE) blocks that match each sampling location of a corresponding frozen vial. Blood (frequently both pre- and post-treatment) is processed into frozen serum, plasma (double-centrifuged for use as a source of circulating free DNA), and buffy coat (white blood cell) aliquots. Of the more than 30,000 specimen units we create annually, over 1,600 units of frozen samples and 1,000 units of FFPE material are used for immediate research. The rest of the material isn't simply relegated to long-term storage, because we have many innovative projects underway. For instance, a significant and rapidly growing portion of the PPBC's activities (amounting to about 1,700 units of fresh samples) is related to "living" biobanking – the creation of organoid cultures (see Figure 2), mouse xenografts, primary cell lines, and so on.

Our biobank division has developed innovative QA/QI metrics and processes, including RNA integrity monitoring in sentinel samples and participation in international proficiency testing schemes, such as the International Society for Biological and Environmental Repositories' Integrated Biobank of Luxembourg

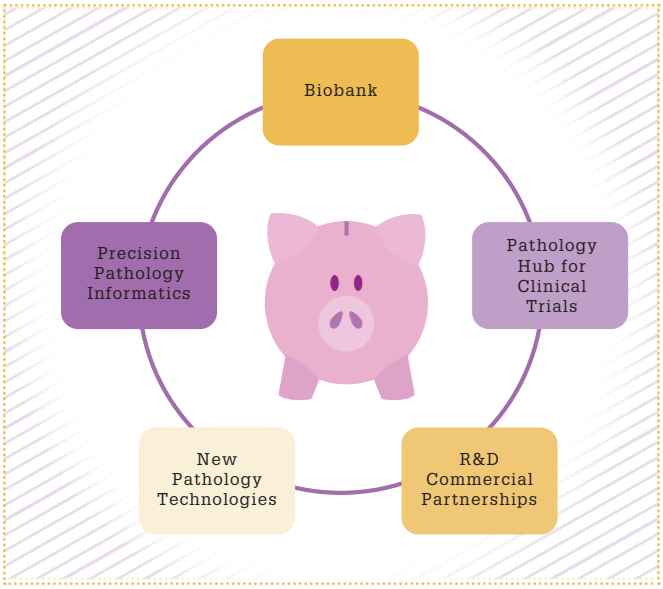

Figure 1. The five pillars of activity around which the Precision Pathology Biobanking Center is designed.

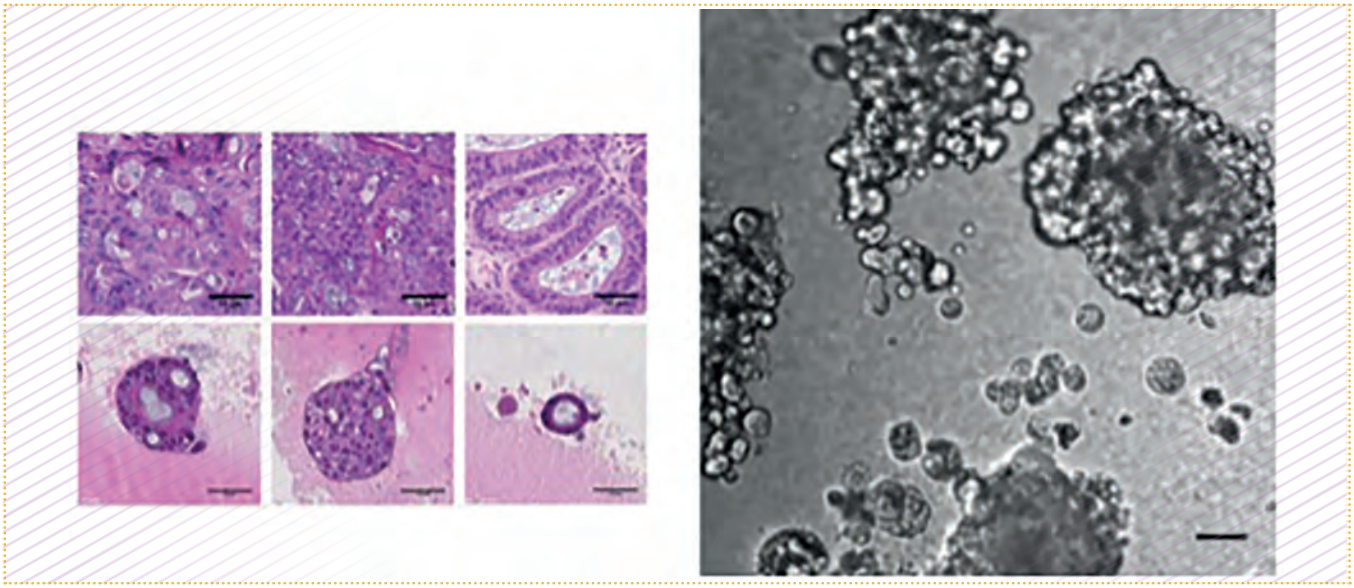

Figure 2. Examples of a "living biobank" (organoids of pancreatic adenocarcinoma). Living biobanks are an area of rapid growth, but need further innovations in biospecimen handling and preservation.

program (see "Curating Pathology's Future" on page 17). Most importantly, we made a strategic decision early on to embed our research biobanking activities intimately into existing clinical workflows. One good example is our rapid tissue acquisition setup, which takes samples from the point of acquisition to liquid nitrogen storage in less than 15 minutes. We accomplish that by pairing up licensed pathology assistants (PAs) with biobank technicians according to daily schedules and making sure that the clinical PAs assigned to biobank service on any given day aren't distracted by clinical responsibilities on those days, letting them dedicate their time and effort fully to research biobanking.

Informatics impact

A physical repository of biospecimens is only as good as the level of annotation and knowledge that can be associated with each and every specimen in the bank. Recognizing that data federation (the aggregation of disparate data sources), research databases, and smart "big data" query tools remain a major challenge, the PPBC has started to put significant effort into developing innovative data informatics and computer science tools (see Figure 3). We feel strongly that pathology as a discipline will increasingly evolve into the medical specialty of dynamic data management and big data integration to drive patient care – theranostics – rather than the status quo of "just" providing a static diagnosis.

Translated to biobanking, it means we need to build tools that cross-reference physical samples in real time to all other data we may have on a patient (clinical status, therapeutic status, imaging results, clinical trial participation, molecular features of the disease, and any other relevant information). We attempt to build a longitudinal representation of every patient, from diagnosis through stages of treatment and recurrence to long-term follow-up. We map each physical sample onto a common timeline along with all other observational or interventional medical events. For example, we could ask, "How many frozen research samples containing cancerous tissue does the bank hold from patients born after 1960 with a diagnosis of KRAS-mutated colon cancer (see Figure 4)?" As convoluted as that sounds, we can readily build much more complex Boolean queries on the fly and still have results within seconds. And it's not just to show off the power of our data organization. Queries like that one have already become instrumental tools for feasibility arguments in grant submissions and hypothesis generation for numerous biomarker studies – and we foresee even greater possibilities for them in the future.

Technology marches on

A pathology-controlled biobank is a major scientific asset for our discipline. We are currently at the beginning of a wave of disruptive technologies that I predict will become essential in our diagnostic and theranostic toolsets. With next generation sequencing reaching technological maturity in clinical laboratories, we already see new technologies (such as mass spectrometry-based deep proteomics, functional assessment of pathway activities, metabolomics, highly multiplexed immunofluorescence, ex vivo living models of drug response,

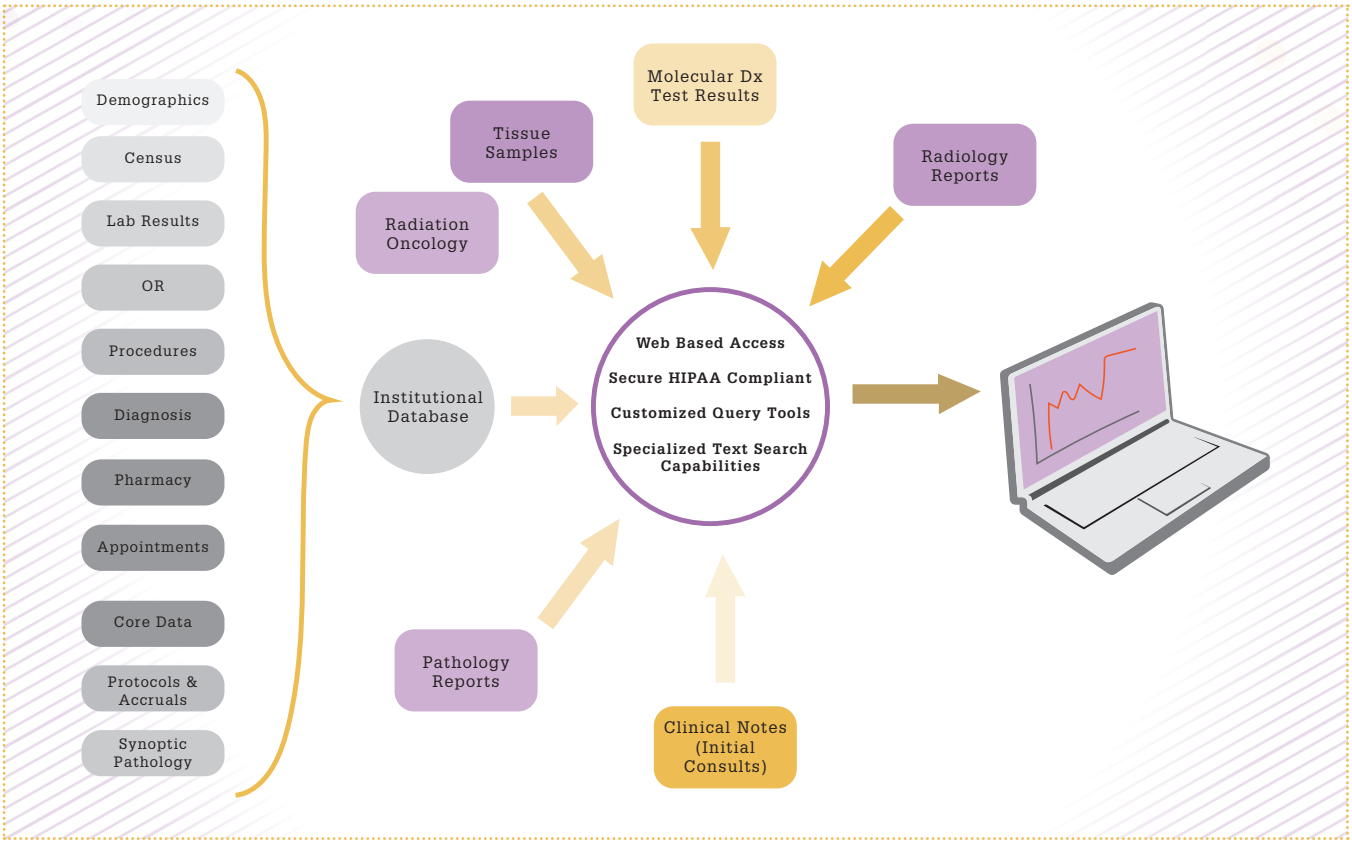

Figure 3. The organizational framework we use for research data at Memorial Sloan Kettering Cancer Center.

and more) that promise to change the way we will assess and monitor disease.

By tightly integrating biobanking into the PPBC's overall mission, the real-life evaluation and clinical assessment of new technologies becomes a natural fit. At the moment, we are assessing high-resolution Orbitrap liquid chromatography-mass spectrometry (LC-MS) as a highly quantitative, highly multiplexed tool that can precisely measure several thousand proteins in tissue in parallel. If it works the way we hope, it will be able to complement – if not replace – conventional immunohistochemistry. And not only does mass spectrometry require no antibodies, but it can also directly detect mutations at protein level and post-translational activation states, such as phosphorylation. So why are we the perfect testing ground for such innovations? Most new technologies require living and biobanked samples of the highest quality. Conventional FFPE-based clinical archives are either suboptimal or altogether unusable for these applications. Cutting-edge, forward-looking and science-driven biobanking is clearly the way forward.

Trying out trials

Pathology has not historically been a driver discipline in clinical trials or drug development, with its role often limited to providing slide review for patient enrollment or sending FFPE material to third-party trial sponsors. In the era of what I like to call "specimen-centered, molecularly driven" clinical trials (for instance, basket trials like NCI-MATCH), pathology's role is rapidly changing and our discipline is becoming a central player. This development has significant ramifications for pathology training and education, as well as for our understanding of pathology as an increasingly clinical discipline.

The PPBC has a division that provides a dedicated platform for pathology's representation at every stage of a new clinical trial; it includes design, protocol writing, budgeting, direct discussions with sponsoring pharmaceutical companies, specimen acquisition, companion diagnostic development, and any other aspect you can imagine. To provide just one example, we've created a dedicated Phase I biobank for patients on first-in-man clinical trials that provides an unmatched resource for research. We believe pathology belongs at the forefront of new medical science, and we're pulling out all the stops to make sure it gets there.

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Figure 4. An ad hoc database query using data federation between various databases (in this case research biobank, cancer registry, molecular diagnostics, and anatomic pathology).

PPBC R&D partnerships

The combination of comprehensive biobanking and new technologies provides a natural, externally visible infrastructure that now allows the PPBC – and pathology as a discipline – to engage directly with the biotechnology and pharma sector. We are enabling pathologists and commercial entities to carry out joint projects, such as co-development of new companion diagnostics, evaluation of biomarkers, or the use of new instrumentation. Such projects frequently hold opportunities for intellectual property generation. And there are even more tangible benefits; research biobanking is often difficult to support through traditional funding mechanisms, so funding raised through research and commercialization can represent a major contribution to its long-term sustainability.

We're at an exciting junction in pathology's growth as a medical specialty, and I'd say it's becoming clear that pathology-driven biobanking is both central to our core expertise and, even more importantly, a powerful enabler for many of the most promising growth areas of our discipline: precision healthcare, clinical trials and drug development, theranostics, and functional assessment and monitoring of disease. I'm eager to expand biobanking's role in pathology, and eager to see where this new platform can take our discipline next.

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