In next-gen sequencing, aiming for wider access

Roundtable on regulation, AI, making the most of the sample

May 2022—*Next-generation sequencing—the worries, the wins, and what's new. That's what came up when* CAP TODAY *publisher Bob McGonnagle led an NGS-focused roundtable on March 14. With him were Jeremy Segal, MD, PhD, of the University of Chicago; Pierre Del Moral, PhD, MBA, and Fiona Nohilly of Illumina; Sohaib Qureshi, PhD, of Thermo Fisher Scientific; and Andy Johnson, DPhil, of Janssen. Here's what they had to say.*

CAP TODAY*'s guide to NGS systems begins here.*

Jeremy Segal, in the past year what events have occurred in the field of NGS that may have been surprising, or not surprising because they're part of the natural evolution of this technology in cancer care?
Jeremy Segal, MD, PhD, director, genomic and molecular pathology, and associate professor, University of Chicago: What worries me going forward is what will happen with the FDA regulation of our tests and what it means in terms of requiring FDA-approved instruments. There's a big push to change the way our tests are regulated and overseen and in a way that is frightening to people who run academic hospital laboratories.

A lot of your testing is what we could call laboratory-developed tests, correct?
Dr. Segal: Yes.

Pierre Del Moral, from your perspective in industry is that an issue that worries you?
Pierre Del Moral, PhD, MBA, associate director of clinical marketing oncology testing, Americas, Illumina: As a solution provider we need to be adaptable to what the FDA does and says. The oversight may prevent some flexibility. The space is evolving rapidly, so having more stiffness in the process may not yield to the advances the field is looking for.

Sohaib Qureshi, can I get a quick comment from you?
Sohaib Qureshi, PhD, director of product management, instrumentation, clinical NGS division, Thermo Fisher Scientific: Ambiguity around oversight and changes to current regulations are concerning for both customers and solution providers. We need to make sure we're adapting and providing solutions for laboratory-developed and in vitro diagnostic tests.

Dr. Segal

Jeremy, is this something new or is it a return of something that is part of the environment?
Dr. Segal (University of Chicago): There's an active effort now to pass the VALID legislation [Verifying Accurate Leading-edge IVCT Development], and people are trying to attach it to the next Medical Device User Fee Amendments coming up. If they attach it, it will not be voted down. If it is not attached, we have a chance to be involved in the process.

The tests and the technology change rapidly, and regulators are concerned that everything is like the Wild West. I don't see it that way. What we have done in terms of creating tests and a national infrastructure for tests over the past decade has been remarkable—what has been created, what we do daily, how it has changed the way pharmaceutical companies develop and trial their drugs. Everything about cancer testing, treatment, and management has changed incredibly over 10 years. This amazing transformation of our field over the past decade

is a testament to the power of the LDT pathway to enable new discoveries to be translated efficiently into care. So we don't feel like we're the problem; in fact, we feel like we have been the solution. But the LDT pathway is under constant—and I believe misguided—attack, and what they aim to impose on us will be terribly damaging to our laboratories and more importantly to our future patients. The problem is especially severe in the academic laboratory community—some of our labs have 200 LDTs on their books and no way to transition to a future under VALID.

This legislation, should it pass, could put your efforts in jeopardy and harm patient care, no question in your mind?
Dr. Segal (University of Chicago): The VALID proposal is to grandfather in every LDT that currently exists, but as far as my reading of it, no updates to existing LDTs will be allowed. And all new assays will need to be on FDA-approved instruments, which typically don't exist when new technologies become available. The biggest problem is how this will affect tomorrow's technology. Someday soon another disruptive technology will emerge that will allow us to do all sorts of new things, and we're going to learn—just like with next-gen sequencing—a lot about cancer and genetic disease. People will want to use it to support care, and what happens if there's no mechanism for doing so in a time-sensitive manner? It will take years for an FDA-approved device to be made. Every new product is marketed as an investigational tool first, and then companies have to plan how they will make the transition to manufacturing of FDA-approved devices. It's a multi-year process of trying to strategize. That's multiple years that patients can't wait.

I don't understand the thought process behind pushing for a new system that disallows the rapid integration of new science into medical care. And it's not as if we don't support increased standards—we would be happy with increased stringency. But treating us like national manufacturers and requiring us to go through a multimillion-dollar, full FDA process for each LDT just does not comport with reality.

The VALID Act represents a certain meeting of minds between industry and people who wanted LDTs; it was seen as kind of a middle way a number of years ago. But we'll have to see what happens. It's hard to imagine how the Cancer Moonshot initiative would be successful without laboratory-developed tests.
Dr. Segal (University of Chicago): We have to have a system that allows for safety and doing things the right way. It has to allow for rapid translation of new discoveries into diagnostics. The FDA is more concerned about risk mitigation than it is about making sure patients have the technology available to them.


Dr. Johnson

Andy Johnson, you have important drugs that depend on the detection of genetic markers often in what one might call a low percentage of presenting patients. Doesn't that make next-gen sequencing a critical technology for Janssen as it looks at drugs?
Andy L. Johnson, DPhil, integrated evidence team lead, solid tumor targeted therapy U.S. medical affairs, oncology, Janssen Scientific Affairs: Yes. The approvals in exon 20 insertion mutations and *KRAS* mean the field has reached a threshold where NGS provides the most value to patients in terms of being able to identify the most patients with a driver mutation in the least amount of time, with the least amount of tissue, and hopefully the least amount of cost overall. The value that comes with NGS makes a lot of sense.

Do your portfolio development colleagues understand that many of these drugs are targeted therapies that can't exist in the marketplace without a detection technology like NGS?
Dr. Johnson (Janssen): Yes. It goes into our portfolio and development planning. We need to celebrate when the

right patient gets the right treatment, and that comes when a patient receives a comprehensive analysis on their tumor.

We're expanding our precision medicine and targeted therapies, especially within solid tumors. The development of the testing to find mutations and therapies have to go hand in hand. We can't treat patients if we haven't found the mutation.

Dr. Del Moral

Pierre, I assume that means the demands you face as a company for platforms and software are increasing all the time?
Dr. Del Moral (Illumina): Yes. The democratization of NGS is tremendous. The acceptance curve is evolving rapidly—the ease of use of the technologies, the incorporation of comprehensive genomic profiling that has DNA and RNA content, that can simultaneously assess all variant classes, increase the diagnostic yield, look into complex signatures such as tumor mutational burden and microsatellite instability, look at RNA for detection of splice variants, known and unknown fusion partners, and getting patients the therapy that matches their tumor profile. HEOR [health economics and outcomes research] or real-world data showcases how NGS has improved the outcome for patient testing along with what pharmaceutical companies are doing.

There's been in the past couple of years a tremendous uptake in NGS technology from tissue and liquid biopsy. COVID has also improved the uptake of liquid biopsy testing.

Every group I speak to is talking about serious shortages in staffing in laboratories, including of pathologists, PhDs, programmers, et cetera. Jeremy, are you seeing staffing shortages and difficulties?
Dr. Segal (University of Chicago): It's certainly gotten harder. The number of resumes that come in for the positions we post is fewer than we used to see, and it takes longer to find someone. As an example, we have someone for a research and development position whom we have wanted to bring in for months. But because of her visa issues, she's stuck in Vietnam, and she can't get an appointment with her embassy because of COVID. Fewer people from other countries want to come here to work because of the last administration's policies. COVID-related embassy problems are a factor. The inflow of the talented people we need has certainly seemed to slow down.

Between our labs here we have four bioinformatician positions, and recently we were down to one individual and had three empty positions. We're starting to fill those now but it isn't easy.

Andy, does the staffing shortage make everybody's job a little harder?
Dr. Johnson (Janssen): Because things continue to get more complex in terms of the scientific and clinical questions we're answering, it would have been hard regardless of staffing. Given the changes that have occurred the past few years—what Jeremy said about global movement of people and trying to bring people in—I can see how it would make things harder.

It highlights the need for operational simplicity and efficiency, trying to implement upfront work and reflex testing so less effort is needed from the clinicians, pathologists, and nurses and things can flow through more efficiently, including getting testing data into the EHR so it's at everybody's fingertips and is more searchable. We can develop new technologies from the scientific side, but we need to simplify user interfaces by enlisting the skills of graphic designers to help get the data to the people who need it.

Dr. Segal (University of Chicago): We have something going for us in the molecular next-gen lab. It is seen as

emerging technology, and young people are interested in the field because they see it growing.

We face worry over the coming years on the cytogenetics side. People who are in the field have concerns that it will be replaced by optical geomapping or sequencing technologies or something else. People who are training in laboratory genetics and genomics programs are looking for jobs that have molecular components. Nobody who's coming out of that training wants a cytogenetics-only job. Most of the technologists in cytogenetics are aging and more of the younger techs are going into things like molecular. People are worried about being able to find cytogeneticists; there are so many open positions now, it's concerning.

There's a shared perception that the quality of cancer diagnostics and care is bifurcating. There's always been a difference between academic and community practices, but that gap seems to be widening. Jeremy, do you share that perception?
Dr. Segal (University of Chicago): That's probably true. At our center we are doing complete genomic profiling for 100 percent of our lung cancer patients. But from the community, you hear the numbers are 40 percent, and you wonder how that can be true. Five years ago it might have been a similar percentage for both places. So many patients aren't getting it and the reasons are many-fold, and it's going to take more work to learn about the issues and probably increased effort at education.


Dr. Qureshi

Sohaib, what's the view from Thermo Fisher?
Dr. Qureshi (Thermo Fisher): I agree with Jeremy that there's probably a lot of differentiation in the use of NGS in academic medical centers versus more regional, community care settings. The academic medical centers that are setting themselves apart have been there from the beginning, so they have the expertise in-house, the resources, and the funding. As you go into communities with smaller hospitals, there are challenges with resources, access to trained personnel, and availability of novel technologies that can improve patient outcomes. Our mission in NGS has been to make NGS accessible to a broad spectrum of labs with solutions tailored for clinical labs. The adoption of NGS in community care settings is increasing rapidly as labs get access to technology that works in their settings.

Pierre, can you speak to the same question?
Dr. Del Moral (Illumina): The gap is narrowing because there are already send-out options for oncology testing. There are service labs that provide the opportunity for their patients to be tested for comprehensive genomic profiling, for example. We also see hospital networks implementing that type of testing in-house and community oncology moving toward that.

The reason for the discrepancy is often access. It could be access to testing in-house. For example, as a solution provider we're working with automation vendors to automate our solutions and provide the most flexibility to implement NGS testing in-house. Access could also mean on the payer side—who's going to pay for that test? I also think of access to clinicians, where we're investing a lot in clinical decision support and software. How can a general oncologist interpret the data without the formal genomic training and be able to say, I'm going to provide the best therapy to my patient. This access piece is going to be important in narrowing the gap.

Nohilly

Fiona, as you look at the customer base, do you see movement between in-house solutions for NGS versus the use of send-out laboratories? Or is that at a steady state?
Fiona Nohilly, staff product marketing manager, Americas regional marketing, Illumina: That can vary a lot depending on the physicians or oncologists at the particular hospitals. For example, there may be a sponsor, someone who's advocating for bringing in new technology because they've had exposure to it in their fellowship or residency. So when they go into a community-based hospital, they have the knowledge to advocate for it.

Whether they opt to send out or bring it in-house, there are many variables, and we want to support all options and situations. We have developed analysis tools for all the way from the sequencing FASTQ file to aligning against a reference, variant calling, and interpretation. We're investing in the interpretation side to help those who may not have the exposure or experience to be able to get the results in a report they can read within seconds.

Jeremy, can you comment on the movement to set up a center internally within a system or hospital versus the send-out alternative, which so many must rely on?
Dr. Segal (University of Chicago): I may be biased, but I think the best thing is to have it done in-house. By doing it in-house we make it a multidisciplinary team effort to diagnose the patient's tumor, look at the genetics, and correlate it together. Many times questions arise about what we should test or prioritize, and we'll loop in the oncologists and get feedback from them in real time.

Everyone thinks of NGS as a technology for determining treatment for the patient, but just as important, it's useful for figuring out the diagnosis. There are a lot of cases in which we don't know the diagnosis or are surprised by the next-gen sequencing results. Being integrated with our anatomic pathology group means we discuss as a group. If they have a case they don't know what to do with, they'll order immunohistochemistry testing, but they may also order our test and we can talk about the results. We make better diagnoses for patients that way, and having our in-house service is a positive for everybody.

Maybe you could get the equivalent if you sent out the testing and had everybody reviewing it and integrating the results together, but that's not what tends to happen. It tends to be that the oncologist sends the test out, the result goes back to the oncologist, and the pathologists may not have an opportunity to see it and correlate their findings. It needs to be a team-based effort.

We have talked about the importance of a democratization of NGS on the vendors' side and doing work on the analytics to help make that happen. Are you satisfied, Fiona, with the progress you're making in simplifying and democratizing NGS?
Fiona Nohilly (Illumina): We are looking at two types of customers. We have folks who are new to NGS or to Illumina technology who need simple solutions. So we have efforts to develop technologies as end-to-end solutions.

We also have customers who are more familiar with our sequencing technology, and they are looking for data analysis platforms that enable them to analyze massive amounts of data, and we're working on developing solutions for that too. We're supporting those customers with our newest informatics platform, Illumina Connected Analytics [ICA]. We launched ICA this year fully at scale to customers. It's been several years in the making, and we acquired companies that helped make the user interface more user-friendly and integrated data compression into our DRAGEN [Dynamic Read Analysis for GENomics] secondary analysis platform. ICA is a scalable and secure cloud-based platform that health care systems like SickKids in Canada have implemented for their COVID-related sequencing projects.

ICA will be the future of our analysis platforms in the cloud, and we're working on new cloud partners. It's on AWS now, but it will be scaled out to other cloud partners.

Let us hear the Thermo perspective.
Dr. Qureshi (Thermo Fisher): Since we last spoke, we have launched several products to further our mission of making NGS more accessible to labs with all levels of expertise. We launched the Genexus Purification system, which, combined with the Genexus Integrated Sequencer, allows labs to go from preprocessed samples to NGS report in as little as 24 hours, with two touch points and 10 minutes of hands-on time. Both instruments have built-in technology to minimize errors, which is crucial in clinical settings. We also launched Oncomine Reporter, which enables labs to create reports linking biomarkers to relevant evidence, greatly simplifying bioinformatic analysis.

The last piece is integration into electronic health records. I speak to many in the field who say their EHR is out of date. So there's work to do on the solution provider's end, but also in hospitals and other care settings.

Jeremy, do you want to speak to the EHR at the University of Chicago?
Dr. Segal (University of Chicago): We're in the process of upgrading to Epic Beaker for our whole laboratory. They claim it can do everything but we'll have to see what the reality is when we install it.

That's an important question because in no one's wildest imagination was the EHR supposed to be able to display complex results like NGS.
Dr. Segal (University of Chicago): Our internally developed NGS lab information system covers our upfront workload, what sample needs what, the pooling, et cetera, and it also includes our variant interpretation platform. The variant interpretation part of the system will have to be retained because I don't believe Beaker can replace it. Hopefully Beaker will do a good job with the upfront accessioning and laboratory process tracking.

Pierre, how is artificial intelligence affecting your life and work now?
Dr. Del Moral (Illumina): AI is a bigger component of everyday life. When we're thinking beyond traditional care in oncology, multiomics, and of additional signatures that could be involved, artificial intelligence is taking a greater part in data aggregation and insight, which our Connected Analytics platform provides. Pharmaceutical companies, within their clinical trial design, are looking and asking for more of that capability.

Sohaib, can you speak to that from the Thermo point of view?
Dr. Qureshi: I don't think you can live a day without some interaction with AI, whether you know it or you don't. That mindset will need to be applied to NGS. There's a lot of development at Thermo Fisher Scientific in terms of leveraging the power of NGS. It's not just for data aggregation; it helps improve the overall sequencing quality. There are a lot of new entrants in the field and they are going to be applying AI technology to improve their platform from an aggregation perspective and with overall data quality.

Jeremy, can you give us your take on artificial intelligence today?
Dr. Segal (University of Chicago): It has a ton of potential for the future. It's already being used to some degree; for example, many of the algorithms for refinement of basic sequencing quality are AI-based. But you could also imagine AI-based detection systems for a variety of genomic applications, such as assessments for homologous recombination or other biomarkers like methylation pattern recognition.

I do have concerns about how we should go about validating something like that. With our normal pipeline, we like to think we know what it's doing under the hood. However, I must say I don't really understand all the steps of the Burrows-Wheeler Alignment algorithm. But at least it's a program that someone sat down and wrote. The AI algorithms, on the other hand, they weren't written but instead evolved, and you don't really know what they are looking at or doing. This raises concerns about how we get comfortable to put our medical seal of approval on the results of AI-based algorithms.

I think we'll ultimately find that AI-based algorithms are better for many aspects of sequencing analytics than our current methods, but we will have to figure out how to develop the highest level of confidence in them, and that may only come through exhaustive testing.

You worry a lot in pathology, and pharmaceutical companies do too, about getting adequate samples to work up cancer patients from the beginning through NGS analysis, which includes large gene panels, because of the limitations of materials. Where are we in terms of understanding best practices for procuring tissues, sampling, doling it out? Jeremy, what are you seeing at the University of Chicago?

Dr. Segal (University of Chicago): We do a lot to maximize what we can get from any sample. Most of the samples from lung cancer patients are small, so we do a few things. We do a lot of endobronchial-guided ultrasound procedures, and we've found value in focusing on fine-needle aspirate material rather than biopsy material.

In the early days of our laboratory, our rapid on-site evaluation team would review smears for basic diagnostic adequacy. Then the focus of the procedure would shift to biopsies or additional FNA passes for cell blocks, and we would focus on these specimen types for our testing. But we found these specimens to be quite variable, and if they were inadequate we would be back to square one. So we shifted our focus to doing repeated FNA passes and having the rapid on-site evaluation team assess them for molecular adequacy. The smears work great, and now we use those smears for more than half of all our lung cancer testing. The great thing is we get a determination of molecular adequacy up front, so we know we'll be able to do the testing successfully before they finish the procedure with the patient.

We've changed the way some of our other procedures are done to try to maximize tissue. We've set up reflex systems, especially for lung, so as soon as the tissue hits cytology or thoracic pathology, they can put the orders through for our tests. That means when they order recuts for immunohistochemistry, they also order recuts for us at the same time. So we only have the specimen going on the microtome once instead of multiple times, and that saves a lot of tissue.

On the laboratory side, if we have a tiny FFPE specimen, we might stage the extraction. For example, we might take half the slides and do a DNA extraction to see if it's enough to run our DNA base panel. If it is, we'll spend the rest of the slides and get RNA for our fusion panel. If it's not, we have to decide and maybe get the oncologist to weigh in—how much do you prefer this test over another test? This helps maximize our testing throughput and helps focus our testing on the biomarkers most important to our oncology team.

Pierre, what are your thoughts on this? In the early days of NGS there was a lot of concern about adequacy of sample.

Dr. Del Moral (Illumina): There always is. What Jeremy said is right, and it showcases the need for in-house testing, because the oncologist-pathologist relationship is crucial to perform sample shepherding and maximize the sample. We've seen with some of our customers that reflex comprehensive genomic profiling had significantly lowered the rate of nonbiomarker informed care. That was enabled by the oncologist and the pathologist discussing it, by automating some of those processes, and by maximizing the tissue and having the ability to test for all variants and signatures using a single panel.

The quality of the NGS reads is also important. We've implemented DRAGEN pipelines for tissue and liquid biopsy. We participated in an FDA challenge assessing bioinformatics pipeline accuracy. When NGS reads from Illumina were combined with the DRAGEN pipeline, it yielded the industry's most accurate results. So one side is the oncologist-pathologist relationship and the other side is how accurately the technology can translate tissue information into genetic information.

Sohaib, please speak to that same question.

Dr. Qureshi (Thermo Fisher): As Jeremy noted, biopsy samples can be quite small, particularly for lung cancer. This can manifest in limited surface area or tumor content, both of which can limit access to sufficient nucleic acid for analysis. This creates a challenge not only for sequential or reflex testing but also for parallel testing.

Sample stewardship has become an important consideration for clinical labs as the demand for biomarker testing and genomic profiling has increased with approved targeted therapies. We have been able to help labs with sample stewardship by providing technology that requires minimal sample input. A key feature of the AmpliSeq

chemistry, which is at the core of our NGS technology, allows for minimal DNA and/or RNA to be interrogated using short targeted sequences, which affords greater depth of coverage and high accuracy. With as little as 10 ng of nucleic acid, labs can generate genomic profiling results for both DNA and RNA analysis. This significantly increases success rate, hence reducing quantity not sufficient, or QNS, results and the need for rebiopsy.

Andy, in today's discussion we have a health care provider and platform and software people. Does it help clarify what the dilemmas are for Janssen, which offers targeted therapies?
Dr. Johnson (Janssen): It does. In the end, it all comes down to one patient's tumor. Regardless of whether that patient is treated at an academic center or a small community practice, our goal is that they receive comprehensive biomarker testing and the appropriate therapy. I like Jeremy's approach, which I frame as: Begin with the end in mind. With reflex testing in place, the diagnostic team knows what "perfect" looks like but can pivot, for instance, to liquid biopsy because there was insufficient tissue.

It's not just the technology though; it's the overall operational approach, the thinking from everybody involved to go from beginning to end. We want to get patients the appropriate therapy. We have good data from the real world and clinical trials to show that getting targeted therapy often provides the best outcome for patients when they have a driver mutation in lung cancer. That's the goal.

Jeremy, as our health care provider here, do you have a final thought about our conversation?
Dr. Segal (University of Chicago): Keep the focus on the patients and try to figure out at every step what they need and what is the right thing to do for them. That will help us make the right decisions, whether it's about setting up tests in the lab, or how to interpret tests, or how to regulate tests. We have to think about their needs first.