As they dig deep into the human genome, pathologists are finding increasingly more precise—and complicated—molecular information for clinicians. The table shown here is a small excerpt from a 611-gene resource (the Cancer Genomics Resource List 2014) that Marina Nikiforova and 15 colleagues spent two years compiling. They tracked next-generation sequencing–based cancer tests and where they were offered; tests for nearly 400 of the mutations were only offered at one or two institutions.

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Activating mutations in ligand-binding domain of kinase.
I remember the first time I saw DNA,” says molecular pathologist Marina Nikiforova, an MD, with a gleam in her eye. “You can see DNA forming,” she says. “You can see it by eye.”

Anyone can: Break the cell membrane with enzymes and pull away proteins to unzip that familiar ladder shape. With the right temperature and a little patience, it’ll appear—white, gauzy wisps swirling in a test tube, the schema for our entire being exposed.

“It’s like long strands, just folding, folding, folding… It was one of the most fascinating, important moments,” Nikiforova says.

Swiss scientist Friedrich Miescher first discovered DNA and RNA in 1869. In 1995, when Nikiforova first saw the human genome, it hadn’t been sequenced yet; technologies that transform that test tube gossamer into useful information were still in their infancy. While it takes about eight hours for a cell to replicate its DNA, to decode 99 percent of the human genome took researchers around the globe nearly 15 years, culminating in 2003.
Patients are already seeing benefits from this initiative. Yet, “personalized medicine” or “precision medicine” has a way to go to before it’s fully realized.

Modern pathology has a huge role to play in bringing it about. And the more pathologists are enamored with the still stubbornly mysterious and maddeningly complex fabric of life, the more we all stand to benefit.

Picture the classic college lecture hall: The brilliant professor broadcasts her knowledge to her students in the least time-intensive manner possible. Some students don’t understand the material, but those individual needs are not within the purview of a 200-seat classroom. They’d better ask a classmate (or Google) for clarifications. This teaching method certainly isn’t guesswork, but it’s probably not the best way to reach individual students, either. That’s medicine in the pre-genetic sequencing era: Here’s a treatment—we have reason to believe it works for most people. Good luck, dear patients.

Now picture those upper-level science courses of undergrad and graduate school, with smaller enrollments, more intimate seminar settings. Boom, that’s precision medicine—the class is grouped into students with similar backgrounds and needs, which means instruction, though not quite one-on-one, is much more specialized. In medical parlance: Here’s a treatment we know targets this subtype of this ailment, and we have several more treatments in the toolshed. Or as Nikiforova puts it, “Now the doctor won’t just pick any drug and give it.” She’ll use evidence-based genetic profiles to get it right on the first try.

Precision medicine isn’t at the tutor-pupil level just yet, and may never be—despite some reporters’ claims that we’ve got made-to-order drugs once the human genome was sequenced, that’s unlikely to happen. (You wanted your own pharmaceutical brand? Sorry.) More likely, molecular pathologists like Nikiforova and other biologists will narrow in on increasingly specific disease markers, and, in response, more targeted drugs will be manufactured as both primary and secondary treatments.

As we approach this more precise era in medicine, we expect the role of professor will be taken up by the treating physician. Yet, clinicians often are unclear about the meaning of the mutations that sequencing discovers. Someone needs to translate. That’s a pathologist’s job. (Already pathologists make or confirm 60 to 70 percent of diagnoses in UPMC hospital patients.)

But heck, some pathologists are overwhelmed by the genetic stew of possibility. The magnitude of this problem is hard to overstate; clinical interpretation and translation are a real challenge beyond sequencing. So getting more precise medicine to patients is going to take some seriously skilled pathologists.

Consider: Every person has his or her own 3 billion base-pair recipe, and those base pairs tend to vary in about 2 million ways—some harmful, some not. Let’s take a moment to look at the ingredient list.

The genome is the whole shebang—all the genetic material in a human. When scientists say we’ve “mapped the human genome,” they mean we’ve determined the sequence of nucleotides in a given sample of DNA. It’s important to emphasize that researchers have mapped a human genome, not the definitive blueprint for humanity. Each individual has variations therein. (For the curious: Anonymous volunteers donated DNA for the Human Genome Project. Nobody involved in the massive effort knows exactly whose samples were used.)

From big to small on the genetic continuum: We’ve got 23 pairs of chromosomes (a total of 46), which help pack 6 feet of DNA into each human cell. That wispy DNA is made up of base pairs—cytosine, guanine, adenine, and thymine—which pair C-G and A-T, over and over billions of times to make us who we are.

The ATCG nucleic acid pairings determine how proteins are expressed and which enzymes a cell rallies to trigger our basic biological processes like metabolizing drugs or digesting sugars. Variations within these pairs, called single nucleotide polymorphisms, or SNPs, are common within a population, but sometimes result in nonsense or missense alterations.

With some 20,000 genes and more than 100,000 proteins, there’s a lot of room for error. And that’s just the coding part of the genome. Something like 95 percent of the human genome is noncoding—what scientists used to call “junk” but now suspect contains the power switches to the coding parts. The epigenome, or chemical tags that ride on the backbone of DNA but are separate from the genetic sequence, influences which of these switches get flipped.

So, variations in any part of this biological formula can influence or directly cause disease (or not). There are parts of the genome that are totally unstudied. Even the known parts are extremely complex and vary by individual. Because the genome is so incredibly large, sometimes genomic pathologists focus on just the exome—the 1 percent or so of the human genome that happens to harbor most mutations related to disease. In short, the future of medicine—precision medicine—is a veritable flood of possibility.

Well then, how is this new era evolving?

Today’s pathologists work on a scale from macro to molecular. Anatomic pathologists look at the big stuff, the gross tissues themselves. After the anatomic pathologist slips the slide under a microscope, he narrows down to the micro-anatomy of cells, sometimes on a FISHing expedition, known more technically as “fluorescence in situ hybridization”—a colorful dye-job that helps scientists see cellular structures.

Nancy E. Davidson, an MD, director of the University of Pittsburgh Cancer Institute and UPMC CancerCenter and Distinguished Professor of Medicine at Pitt, wrote in a March Journal of the American Medical Association editorial on breast biopsies that this “critical tissue diagnosis from the anatomic pathologist directly determines patient management. That diagnosis is based on morphology, the relationship between cellular and architectural features.”

Yet that examination itself is subject to interpretation, depending on who’s doing the biopsy, how it’s sampled, and who looks at the tissue. Though this step yields undoubtly important clinical information, the sample demands a closer look.

More recently, pathologists have been able to zoom in further, down to the submicroscopic, the molecules, the DNA and RNA themselves. (RNA transcription is yet another layer in this molecular genetic jumble.) This closer look is expected to be somewhat more objective, notes Davidson.

“Will cytopathology die? No,” says Nikiforova.

“All this [molecular approaches] will be in conjunction with traditional pathology . . . to improve patient management.”

Spurred by the sight of DNA, Nikiforova soon thereafter completed a clinical pathology residency and a fellowship in molecular diagno-
tics. She then took up seven years of postdoctoral study with endocrinologist James Fagin at Cedars-Sinai Medical Center, whom she, along with her husband Yuri Nikiforov, MD/PhD, followed to Cincinnati. The three of them studied and published on mutations and other molecular rearrangements that lead to thyroid cancer.

Her work since has focused on improving diagnostic and prognostic tests for cancer. Today she acts as liaison between oncologists and the UPMC molecular and genomic pathology laboratory, which she directs. In the years since her postdoc, researchers’ knowledge of and ability to gather genetic information has exploded.

Sam Yousem, an MD and the E. Leon Barnes Professor of Anatomic Pathology, is a traditional pathologist who has spent his career investigating lung disease development and diagnoses. He’s got a big microscope on his desk, stacks of manila patient folders, and cartons of glass slides piled around his office. As the vice chair and medical director of anatomic pathology at UPMC, his job is to make clinical and research connections happen—get the pathologist out of the basement and into the clinical spotlight, guiding research where it’s needed most. One way he’s done that is by courting the Nikiforovs.

Nikiforova and Nikiforov study molecular genomics, the tiny bits of humanity. About 10 percent of cases for pathology now cross their desks, and their molecular findings get folded into the anatomic pathologist’s final report.

“They’re both brilliant,” Yousem says. “Yuri is the academic leader. And Marina Nikiforova, with the a on the end, is sort of the operations person, the management person that puts everything into effect. So it’s really the cerebellum just coordinates everything—that’s Marina.”

Yuri Nikiforov, a professor of pathology, as well as the vice chair for molecular pathology and director of the Division of Molecular and Genomic Pathology at Pitt, has slicked-back hair, a relaxed demeanor, and fluency in the alphabet soup of genes and mutations. Nowhere is this more apparent than at the monthly Genomic Tumor Board meetings, instituted and led by the Nikiforovs and pathology fellows. Each month, oncologists, radiologists, pathologists, and other interested parties gather to discuss the genetic profiles of three to four anonymized, real-life patients.

“They’re fascinating events,” Nikiforov says, citing back-to-back meetings with an interesting coincidence. One month, a physician offered his case study of a brain cancer patient whose treatments were failing. The patient’s mutation profile happened to show a rare marker that usually appears in pancreatic cancer. A pathologist noticed it and was able to offer an atypical but clinically appropriate course of treatment. The next month, with the exact same two docs, the roles were reversed—a pancreatic cancer case study benefitted from the brain specialist.

Yousem says the Belarusian duo has worked with every clinical group around to maximize molecular testing in oncologic subspecialties.

“I can honestly say—Scout’s honor and everything—we’re one of the top three or four programs in molecular testing in solid tumors in the country... For solid tumors, people look to Pittsburgh.”

Pathology is more than just tumors, obvi-
of those points increases the risk of breast and ovarian cancers by double-digit percentages. Patients found to have a BRCA mutation may elect preventive surgeries or have more frequent exams to catch abnormal tissue earlier.

But the concept of mutation itself isn’t as cut and dried as it sounds. There are many ways a gene can mutate: It can fuse with a nearby gene or swap positions. It can multiply and drop an important element or gain an extra nucleotide. Pathologists and oncologists are increasingly turning to analysis of these sequence variations to track disease, as well as trace its progression.

Researchers are learning that gene expression itself exists on a continuum of degrees, with some debate on the middle-of-the-road expressions. This is a deeper, more ambiguous part of making medicine more precise.

A recent study headed by Michalopoulos, with Jianhua Luo, an MD/PhD professor of pathology, and Joel B. Nelson, the Frederic N. Schwentker Professor and chair of urology, identified eight fusions—those smushed mutations—known to signal aggressive, recurrent prostate cancer. Their findings, published in the American Journal of Pathology last September, will be used to appropriately gauge treatment after prostate cancer is identified. To get there, the team had to sequence 289 RNA samples some 1,300 times. (And, in another study, to be published, a group at Pitt has identified gene fusions in advanced breast cancer, including an estrogen-receptor related fusion that blocks antihormone action.)

One of the most successful cancer drugs to date, Gleevec, also targets a fusion site, notes Jeremy Berg, PhD director of Pitt’s Institute for Personalized Medicine. Known generally as imatinib, the drug has been heralded as a cancer breakthrough for its success treating chronic myelogenous leukemia and some gastrointestinal tumors—nearly doubling five-year survival rates for the former to 59 percent.

Imatinib targets the activity of the product of chromosome 9’s ABL1 gene and its cell division processes. When fused with the BCR gene, activity of the overexpressed protein goes haywire; imatinib selectively interferes with that process, stopping irregular protein and enzyme production.

Berg, who’s also associate senior vice chancellor for science strategy and planning in the health sciences, as well as Pittsburgh Foundation Professor of Personalized Medicine and of computational and systems biology, wonders whether scientists should be looking for more fusion targets (rather than “point mutations,” or single alterations).

He also stresses that genomic findings “need to be correlated with outcomes. . . . It’s also important not to say that with these molecular tools, all these ambiguities are going to go away, because they’ll initially get more ambiguous.”

The deeper we dig, the more we find—which means more knowledge and also more to synthesize.

Michalopoulos, the Maud L. Menten Professor of Pathology, says that despite advances in technology and bioinformatics, the field is facing serious knowledge gaps, for which he half-seriously offers a solution: “There should be a national draft for biologists—everybody should do two years of biology research. It’s just so many years of work to be done.” Right now, researchers are devoting their entire lives to studying one or two genes. In Michalopoulos’s view, that pace is too slow.

If you talk to young pathologists about modern pathology’s methods, Nikiforova says “they will tell you, This is unprecedented; this is the most exciting part of pathology. They all want to learn and to know and to be part of it. For older generations of pathologists, it’s very difficult to learn about this and to get engaged in the process.”

Pitt pathology, for its part, has anticipated and antecedent the larger field’s trends. Yousem, who was the former vice chair of the pathology department, saw the writing on the wall a dozen years ago and insisted that his colleagues get focused. “I gave everybody two years’ notice and said, We’re going subspecialty. . . . We were probably the second hospital in the country after...
This is the Rolls-Royce of sequencing technology, called "deep" sequencing, processing 96 patient samples—nearly 5,000 genes—in about 10 days. And just last October, molecular and genomic pathologists at the University of Pittsburgh and the University of Pittsburgh Medical Center, to grow molecular testing at Pittsburgh, recruited Sanja Dacic, MD/PhD professor of pathology and director of the FISH and Developmental Biology Laboratory, to grow molecular testing at Pitt. And just last October, molecular and genomic pathologists at the University of Pittsburgh and the University of Pittsburgh Medical Center, to grow molecular testing at Pittsburgh, recruited Sanja Dacic, MD/PhD professor of pathology and director of the FISH and Developmental Biology Laboratory, to grow molecular testing at Pitt. And just last October, molecular and genomic pathologists at the University of Pittsburgh and the University of Pittsburgh Medical Center, to grow molecular testing at Pittsburgh, recruited Sanja Dacic, MD/PhD professor of pathology and director of the FISH and Developmental Biology Laboratory, to grow molecular testing at Pitt.

Were it not such an expensive machine, one might run a palm over its cover like that of a sleek sports car. Whereas the lab's older MiSeq machine—a real workhorse—can pump out more basic next-generation sequencing results in about seven days, the HiSeq can do so-called "deep" sequencing, processing 96 patient samples—nearly 5,000 genes—in about 10 days. This is the Rolls-Royce of sequencing technology; machines like these have helped pathologists worldwide identify at least 140 genes mutated in cancer, with more expected.

Here's how it works. A lab tech injects chopped-up DNA base pairs into special gel on a plate or in thin tubes that are then zapped by electrodes. The different nucleotides are tagged with dye and lurch forward in response to the electrodes—how far forward they go signals their composition to the machine. The sequencer scans what happens and assembles the data into a readable genetic sequence (ACTGCGGAT...). This process happens over and over to validate the results—redundancy is a pathologist's friend, and that repetition is what makes the sequencing so deep and precise. Because the human genome is so large, pathologists can only sequence segments of DNA at a time. Once they have all the sequenced pieces, there's a bit of guesswork involved in putting it back together again.

On a tour of the lab, Nikiforova explains that the relatively zippy Rolls-Royce is for research only. "We didn't convert it to clinical yet because we're still working on validation," she says. "In clinical lab you have to be very strict.

She would know: Pitt-based pathologists are deeply focused on developing clinical tests that are reproducible, validated, and accurate, including Nikiforova's much-praised ThyroSeq NGS panel, which detects cancer in fine needle aspiration biopsies of the thyroid. This targeted next-generation sequencing approach allows many patients with cytologically indeterminate thyroid nodules to avoid surgery, which is the standard of care.

This February, at the Association for Molecular Pathology meeting, Nikiforova presented on a Pitt-developed sequencing panel for brain tumors, which quantifies them from benign to aggressive. "Nobody's doing this worldwide," she says—that is, mapping mutations, fusions, and anything tumor-specific that can help in both diagnosis and prognosis. Her collection of brain tumor markers isn't quite ready for the clinic, but Nikiforova expects it will be soon. "It's a need within the community," she says, "but nobody [else] has designed it yet. And we do it in our lab, using our own resources, and our minds, and our energy."

That last bit is the real key. Precision medicine might sound like a story of technology, and that is to some extent. But the clinician is people—knowledgeable pathologists like the Nikiforovs and Yousem and Michalopoulos who keep current on a tsunami of research, who can combine gross pathology with sequencer outputs and apply the results to real-world decision-making.

Again, some of that process is automated, as it should be. Once a sample goes through the MiSeq, the machine pops out a multicolored report highlighting disadvantageous mutations while marking neutral mutations with a smiley face. The reporting software, developed by fellow-turned-assistant professor Somak Roy, an MD, matches mutation results to ongoing clinical trials that the patient might enroll in, as well as potential drug therapies that might benefit that individual—usually several of each, which a trained pathologist must help the clinician decipher. (Roy is also assistant director of the molecular and genomic pathology lab.)

What's really impressive is the way Pitt pathologists are taking the tiny pictures that have emerged from genomic testing and piecing them back together again—that paradox of the tiny-turned-big. Nikiforova and 15 other researchers assigned by body-site specialty formed a working group from more than a dozen institutions throughout the United States. They cross-referenced known genes, mutations, and panels; compiled them; and sussed out patterns ripe for exploit. Their Cancer Genomics Resource List 2014 was published in the Archives of Pathology & Laboratory Medicine in December.

The working group found 611 genes for which next-generation sequencing is offered; of those, "tests for 393 genes were only offered by one or two institutions." That means there are lots of homegrown testing panels that could be shared across labs and institutions. There's also, as always in this field, a lot more to learn. The idea behind the resource list was to help academic and hospital laboratories, as well as for-profit ventures, develop tests themselves and consolidate knowledge.

Beyond that, Nikiforova wants to focus even further, on patients whose tumor mutations don't blip on the existing panels. She ticks off her ambitions in an avalanche. She's still amazed by the mitutiae of life:

"We're developing sequencing of not only 50 genes, but sequencing of more than 5,000 genes at the same time. And [those]