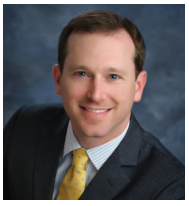
DISCUSSION

EVIDENCE-BASED VS PRECISION MEDICINE

A Discussion With Molecular Pathologist, Dr Gabriel Bien-Willner: Do We Need to Realign Evidence-Based Versus Precision Medicine?



Gabriel Bien-Willner, MD, PhD, FCAP, is board certified in Anatomic Pathology and Molecular. Genetic Pathology.

During this year's American Society of Clinical Oncology (ASCO) Annual Meeting, I decided to attend a pre-meeting session on a critical timely topic. The half-day session, Genetics and Genomics for the Practicing Clinician, included multiple panelists, each of whom presented real-world cases to form the basis of discussions on both tumor and inherited genetic variations, the molecular testing that is now available to clinicians, and when such tests should be administered.

The entire session was insightful, but what remains with me most was when a gentleman in the audience stood up and posed a question to the panelists concerning precision medicine, during which he made the following provocative statement: "I would argue that evidence-based medicine is incompatible with precision medicine and, as currently practiced, is not effective for cancer care."

As one whose blog uses the tag line "It's all about the evidence," I was immediately intrigued. Following the session, I walked over and asked whether he would be willing to be interviewed for my blog to discuss his thoughts concerning precision-based versus evidence-based medicine in this genomic era—he agreed.

An Introduction

Gabriel Bien-Willner, MD, PhD, FCAP, is board certified in Anatomic Pathology and Molecular Genetic Pathology. Gabe, a classically trained human geneticist and molecular pathologist with deep expertise in next-generation sequencing (NGS), has a long history of providing knowledgeable, critical insight into the molecular basis of disease in cancer patients.

Currently the executive director for medical affairs at Molecular Health, Gabe began his role with the company as medical director, when he was responsible for the daily clinical activities of Molecular Health's CLIA-certified NGS laboratory following the launch of the company's cancer genome panel.

Today, the company is focused on developing innovative software solutions for precision medicine for both clinicians and laboratories. Molecular Health has centered its products around the "Dataome" (MH Dataome) technology platform, and its current products are based on analysis of data from a tumor's genetic composition in addition to numerous types of biomedical data, enabling physicians to create a report including qualified and individualized treatment recommendations for the patient and allowing oncologists to make more fully informed treatment and medication decisions. Per Molecular Health's website, based on genomic and molecular evidence, the Dataome provides clinical and molecular data interpretation that can anticipate patient-cohort drug response, predict drug side effects and toxicity, and identify personalized treatment options for patients.

As we began our interview, Gabe stressed that he was speaking as an individual who was expressing his personal opinions and views and was not representing those of Molecular Health in any way.

Challenges for Oncologists in the Genomic Era

We began our discussion by reflecting on how unprepared many clinicians feel when it comes to explaining genomic test results to their patients. "We're in a kind of precari-

ous position," Gabe explained, "where they are given a very complex genetic test result, and now, the way our medical system is today, they're expected to be able to explain the results to the patient, but they themselves don't understand it." He shared an example from last year's ASCO Annual Meeting: a breast cancer oncologist was the moderator for a precision-based medicine biomarker session, and she opened the session by saying, "I'm a breast oncologist; I don't really understand any of this. But, here we go." Gabe's reaction? "I just thought '*Wow! Okay, this is where we are today*!' Everyone knows the value of this, but how could we possibly be using these data correctly? There's this missing link between the information and making use of that information: the interpretation of that data is left to people who don't really understand it."

To address this serious disconnect, Gabe would like to see practice changed, where there is a "learned intermediary." As he explained, "Just like the doctor is between the treatment and you, I think there needs to be this other, new type of molecular physician who should stand between the genetic data and the oncologist, so that what they receive is something that they are capable of digesting. Computational tools to simplify the workflow and knowledge gaps will be a big part of this process, but I personally feel today that you cannot expect all oncologists to now be experts in genomics. I think that if that is the expectation, we're setting ourselves up to fail with this endeavor into precision medicine."

Making a Career Choice

Gabe had always been deeply interested in genetics: "I found it fascinating, the 'building blocks of our lives' and, early on, I was particularly fascinated with the prospect of gene therapy—where at the time, in the mid-90s, it was thought that that was going to cure every disease. Of course, it didn't turn out that way." As he was considering medical schools, he decided to attend Baylor College of Medicine in Houston, Texas, because he believed that they had one of the best genetics departments in the world. Gabe completed both his medical training and his PhD in genetics and genomics at Baylor.

His initial work in genomics was with his mentor, James R. Lupski, MD, PhD, a member of the National Academy of Sciences, when they were presented with a patient who had been diagnosed with campomelic dysplasia. Gabe noted that campomelic dysplasia is "a very rare skeletal dysplasia that is typically caused by mutations in a gene called *Sox9*, and it ultimately became my thesis. Patients with mutations in the *Sox9* gene develop skeletal dysplasia, because the gene is a transcription factor, meaning that it functions to turn on and off other genes and pathways in bone development. But," he continued, "it is also important in sex development, so boys who have mutations in *Sox9* can have sex reversal: they have XY [chromosomes], but their phenotype is female." It was determined that this patient actually did not have any mutations of the *Sox9* gene, so the patient was brought to their lab because they did work on chromosomal abnormalities and chromosomal rearrangements.

As Gabe explained, "It turned out that this patient had a translocation, meaning that the chromosomes are broken and then rearranged with other chromosomes about a million base pairs away from the *Sox9* gene. So, I hypothesized that there were probably several enhancers or DNA elements that allow the proper activation of this transcription factor, *Sox9*, whose expression had to be maintained at a very steady level to function properly. And so what was happening was that there were DNA elements upstream and downstream of this translocation breakpoint, and you were losing some of these elements—but not all of them, because the phenotype of this patient was not as severe as with others with mutations in this gene."

Using novel computational methods and mouse models, Gabe was able to obtain evidence of rearrangements in these DNA elements—ie, enhancers—nearby. "One thing that concerned me was that I spent 4 years proving that there was an enhancer that was actually being activated by Hedgehog signaling, which was driving the expression of *Sox9*," Gabe noted. [The hedgehog family of signaling molecules play a critical role in transmitting development signals.] "It took me 4 years to prove that there was 1 enhancer, but we speculated that there must be dozens of such regulators." He believed that there had to be a way to determine where such enhancers were without the *a priori* knowledge he had in this case, ie, critical phenotypes and mouse models that gave him this evidence. "There had to be a way to figure out where these elements were. So that was my first foray into genomics," Gabe explained.

"At the time, there was a very new technology that was just published by a group at the Sanger Institute that I thought could help me find these other elements." This molecular cytogenetics team, led by Nigel Carter, DPhil, investigated methods to detect changes in the numbers of chromosomes and genes to learn more concerning the causes of particular inherited diseases in humans. Gabe explained that the new technology, called Chip on Chip, relied on using antibodies to capture proteins bound to the DNA elements of interest, which

were then frozen. The "captured" DNA would then be hybridized against a microarray chip that included genomic regions of interest, in this case, the regions in Chromosome 17q. "When hybridized, you would see in the analysis where the DNA elements were located, therefore identifying all putative elements in a single experiment without bias." However, he noted, the technology ultimately did not pan out for multiple reasons: "It wasn't very scalable or reproducible, it didn't really work very well at the time, but that was really the beginning of genomics and with DNA for me. And it was really essentially our first approach, not unlike microarray technology, to capture a lot of DNA or RNA information all at once, rather than in a very focused experiment like we'd always done in the past."

He realized at the end of his PhD that folks who are geneticists tend to go into pediatrics to study the same kind of rare diseases that he was currently studying or perhaps internal medicine, but he found that he did not want to focus on very rare disease. Rather, he wanted to focus on common disease "and make an immediate impact in this space. My goal was to bring genetics and genomics into the clinical space. It always has been, and it still is." He felt that the best way to accomplish this was in the cancer field through pathology. "There are 2 ways [to enter the field of cancer research]: one is through internal medicine and oncology, and the other is through pathology. Both pathologists and oncologists are experts in cancer, but they are experts in different ways. Oncologists are experts in the treatment of cancer, and pathologists are experts in the diagnosis of cancer, and they're both experts in the biology of cancer."

So that is the path that Gabe chose. When he became a resident, he decided to go to Washington University in St. Louis, known as one of the best training programs in this area. And right around that time, next-generation sequencing (NextGen Sequencing or NGS) was developed. Also known as high-throughput sequencing, NGS enables researchers to sequence DNA and RNA much more quickly and inexpensively than Sanger sequencing, representing a paradigm shift that revolutionized the study of molecular biology and genomics. Gabe realized that this NextGen sequencing was a new method that would be transformative. "Even though the chemistry is almost essentially identical to Sanger sequencing," he explained, "with NGS we're turning a 2-dimensional process into a 3-dimensional one, where we can perform massively parallel sequencing and capture sufficient data, allowing us to draw conclusions independent of explicit *a priori* knowledge." In addition to greatly reducing cost, it has dramatically increased the throughput of genomic sequencing, enabling simultaneous screening of thousands of genetic locations (loci) for disease-causing mutations. "Now we can sequence everything all at once if we want," Gabe noted, "and figure out what you sequenced later."

Gabe subsequently decided to do a postdoc to learn NextGen sequencing in the lab of Robi Mitra, PhD, a new faculty member at Washington University's Center for Genomic Sciences. While in the lab, Gabe learned computational methods and coding as well as how to use Unix (a multi-user computer operating system). As he emphasized, "To make sense of these data, you really had to create the software yourself, honestly: it really did not exist. If you needed to make some sort of analysis of NextGen sequencing results, you needed your own program to achieve this."

Gabe was influenced by the progress that was being made at Washington University, "including the launch of the Genomics and Pathology Services, where I believe we were the first academic institution to do a hybrid capture, a large gene panel with NextGen sequencing to capture this kind of information from patients. So, I got to be there for the development of that."

Ultimately, he joined the faculty at Washington University, but did not stay there for long. Having multiple opportunities, he decided to enter industry and accepted a position as medical director for Molecular Health, which as noted previously, specializes in the development of analytic software and informatics approaches that are necessary to make sense of these complex data sets captured with NextGen sequencing.

> *"The issue is no longer how to carefully craft an experiment so that the results can answer your question, but how to carefully craft the ANALYSIS of your results so that you can make sense of the data without drowning in it."*[1]

A Catch-22 Scenario

Speaking about Molecular Health's overall mission and goals, Gabe said that "The mission of Molecular Health is to create software applications to allow physicians to make sense of complex clinical data, and right now, we're focused on cancer and genomic data. But, ultimately, the position of the company is to go well beyond both cancer and genetic data." Gabe noted that when he was first brought on board, "We were not only focused on the development of software, but we decided to showcase that software by starting a commercial laboratory and medical service, which I had been running. The company has refocused solely on

software and software development. The service we were offering was very comprehensive and included a medical review by experts. So you were not just getting a test result, you were receiving an interpretation from a real expert into what these complex results mean."

I told Gabe that I had been particularly interested in the fact that Molecular Health was offering such specific medical expertise and just how valuable a service this was, since, as he'd stated at the beginning of our conversation, there is simply no way that we can expect all oncologists to become experts in how to communicate genomic and genetic information and how to interpret it. "Yes," he said, "so it's a Catch-22 scenario, because there are not enough people like me and other molecular pathologists who are really well-grounded in NextGen sequencing as well as in genetics and genomic principles. You can try to make them accessible to as many people as possible—and I think that, in the future, it will be an entirely new subspecialty of medicine. But today it's difficult to have those people available to everyone."

Gabe continued: "But one way that you *can* make them accessible to everyone is with software that enables you to better understand and interpret the results, that can make people who are not quite experts good enough to understand the information that's coming out of the system. So, I would say that that's the direction of the company, and it's something that I'm helping the company do. I'm not sure whether in the future, even in the long-term future, that's sufficient, but it's certainly the biggest dent we can make with this real problem. Yet I do think that there is this future of genomic medicine, that there are going to be people with these skill sets who are more widely available, and I think that they have a critical role to play."

I responded by emphasizing that I appreciated the development of software to enable the delivery of reports that make clinical sense to the ordering physicians, assisting them in their decision making. So lacking an actual clinical consult with a molecular pathologist, they would still have the report explaining the genetic variants identified, their significance, as well as clinical recommendations, and perhaps clinical trials that would be appropriate for these patients.

He agreed, noting that providing such data interpretation is a large part of what they offer and that his focus with the company is "Also, creating a clinically verified or validated knowledge set—that is, a knowledge database of what variants mean in different disease types, so that there can be an automated interpretation for the high-yield, commonly seen or more well-characterized variants, where experts would tend to agree on the significance. That may be helpful for most patients. There's always potentially going to be cases where you need a little bit more insight, but it's a great first step into this field to make it more accessible and understandable to people."

VUS Results: Challenges for Clinical Management

Variants of unknown significance (VUS) are results where DNA alterations are detected, but there is not currently sufficient data to classify whether it is neutral or deleterious. For example, with the *BRCA1* and *BRCA2* genes,[2] initially sequenced and characterized in the 1990s and, by far, the most comprehensively studied human genes, multiple deleterious mutations have been identified that result in a significantly increased risk of developing breast cancer, ovarian cancer, and several other cancers. However, new VUS in *BRCA1* and *BRCA2* continue to be identified.

During the ASCO pre-meeting, Gabe had said that it was extremely irresponsible not to share potential knowledge about the existence of these variants or what some of these variants can mean—a perspective that truly resonated with me. He had given the example of an epidermal growth factor receptor (*EGFR*) point mutation of unknown significance. In the United States, approximately 15% of patients with non-small cell lung cancer have mutations in *EGFR*. Certain *EGFR* mutations have been identified that may predict a positive response to particular agents, known as tyrosine kinase inhibitors, that target *EGFR*. Gabe noted that if an *EGFR* VUS were identified that was likely damaging and in a known regulatory domain of the protein, you could make a reasonable clinical judgment about the mutation's potential significance. I asked whether he could expand on this critical perspective.

"The standard right now is that we're looking at NextGen sequencing and precision medicine through the prism of non-complex clinical laboratory testing," Gabe noted. "We want our laboratory testing to be precise, right? With most of this testing, there's 1 value that we care about where we want to know with a high degree of reliability and precision that we have the right answer."

"For example," Gabe continued, "if you're having a blood test for hemoglobin, you want to know whatever that number result is. As a physician, you know how to interpret that, and you want to know from a laboratory perspective that that is the accurate reading. We know that there is a normal range of distribution of signal, below which and above which is abnormal and within that range is considered a normal range. That's a typical laboratory test. The problem is that with NextGen sequencing, it's not one test."

"NGS Assays are so complex they should not be considered 'tests.'" [1]

"Rather," Gabe said, "you are testing for every nucleotide position, for everything you're sequencing, which could potentially be mutated in a number of different ways. So, in reality, the number of variables in that test, depending on the size of the panel that you're testing and the kind of testing that you're doing, can seem to approach the infinite. And when you're looking at all that data, it's not a test result like other chemistry tests, it's not a yes or no or normal or abnormal, it's a very complex relationship of multiple variables all at once—and up to thousands at a time if you're doing an exome sequencing capture. So you're really practicing medicine by interpreting the complexity of the data to summarize what that data means."

In other words, as he explained, "It may be similar to a primary care physician's interaction with a new patient. A patient encounter can be broken down to a series of variables, and each variable seen as a 'test': the way they look, their chief complaint category and description, every component of the physical exam, the lab test that was ordered, everything you do with that patient. But the reality is that the doctor does not see it that way. The doctor looks at the patient as a whole, he thinks about what's best for the patient in light of all the evidence presented before him—and that's really how I see the interpretation of these complex data. You also have to consider the patient, their history, and their family history sometimes while reviewing the sequencing data. You need to consider all the genes that are sequenced, what the variants are, what the disease is—and the fact that the same variables in different diseases do, in fact, give you different answers. So looking at this exercise as a lab test is overly simplifying a complex process. Practicing medicine by interpreting results is how you're going to get the most out of this and the most out of precision medicine."

The sophistication of the analysis also raises the specter of confronting the challenge of VUS. Gabe continued, "From a traditional laboratory test perspective, if you see a variant that you've never seen before, you don't know what it means because there's no evidence that it means anything, so it's reasonable to ignore it. That's not the right approach. Instead, the correct approach is 'What is this patient's disease? Let's look at this with suspicion. What is the patient's age? What is the patient's sex?' A *BRCA* variant for breast cancer in a 36-year-old means something very different than it could in an 87-year-old with prostate cancer. And then you can go into more details."

Gabe then posed the following questions:

- What is this gene's function? Is it a tumor suppressor? Is it an oncogene?
- Even if it's never been seen before, is this variant likely damaging protein function? If so, why?
- What domain of the protein does this mutation occupy? Is it in an inactivating domain? Is it in an activating domain?

He then emphasized the following: "You have to consider all of these. And even if the gene itself has not been reported to have multiple mutations, if it is a regulator of another gene that is known to have critical importance, how is that going to affect this patient?"

"If you consider all these factors, then I think that you can make sense of these types of variants. You will do so not with absolute certainty, which is expected with laboratory tests, but with reasonable clinical judgment, which is what's expected when you see a patient."

I noted that during the ASCO pre-meeting, there was a great deal of discussion about managing this mutational data and the fact that many labs are not currently sharing this information, either because they do not know what to do with it or because that data is constantly changing. However, there was mention of a research study among patients who, when asked if they were interested in such VUS data, a majority said they were. (There were some differences in percentages among different patient types, such as for those who were cancer patients and those who were not: in general, cancer patients were more interested in this information.) I shared with Gabe how striking it was to me that so many in the audience seemed surprised that most patients were interested in receiving such information on VUS.

Gabe said that he wasn't surprised. "I knew that that was going to be the outcome, where most physicians think that patients would not want to know information that they think is irrelevant to them. But I believe that, ultimately, it all stems from a physician's lack of understanding of this new space. 'If I don't understand this, why would the patient want to know stuff that I don't even understand?' Of course, being a patient and having been on the other end of this, you probably saw this differently: you probably thought 'I want to know everything that I could possibly know.' And the reality is

that what I'm saying *is* potentially problematic. There's so much complexity, and there's a scalability problem. You have so many patients you have to see every day, so many cancers that are sequenced every day, so the amount of work necessary to address all these data and concerns that I'm talking about can be very, very high.

"So, perhaps," he continued, "the question is not 'what's best for the patient?' as much as it is 'what is a reasonable standard of practice?' And, I think, that's where I kind of disagree with where we are. It may be a reasonable expectation of a practitioner to stop at the VUS. Is that what's best for the patient? Probably not. And it would be different, too, if we were talking about disease types where, assuming everyone were the same, we know we have reasonable current standard treatments—such as for diabetes. If you're not giving someone standard of care, you better have a really, really good reason. But we're now talking about cancer and, typically, high-stage, stage IV cancers, where treatments in general are not very effective. It's this kind of Bayesian analysis issue," Gabe said. "You can't pretend that you don't have these data. You do have these data: you can choose to ignore it, which I think is folly, or you could do your best with the data to try to predict the best possible therapy for your patient."

We then spoke more specifically about Gabe's current work to develop algorithms to characterize some of these VUS for clinical assessment. "Yes, creating these kinds of tools can also honestly make my life easier as a molecular pathologist. So the idea would be that [these tools function to] point you in the right direction based on *a priori* thinking from a team of physicians and medical scientists who are looking at all of these variables, assessing what they mean, and what they could mean in different disease types, so that when you see this mutation and you have this phenotype, the system informs you 'here's a potential scenario,' 'here's a potential treatment,' 'here are some clinical trials.' So the software would make the pathologist's life easier, and it could make the oncologists' lives easier by providing information that they can understand and comprehend and by providing critical interpretation. But it's also giving them access to all the other information that I mentioned for variants that aren't well-described: Where in the protein is it? What is the role of this protein in this pathway, and is this pathway targetable? What is the directionality of the mutation? Is it activating or inactivating this pathway?"

Gabe went on to explain, "The reality is, again, that in medicine, doctors know a lot of 'stuff,' but they're not necessarily experts in the science and in the biology behind all the stuff they know. We talk about biomarkers and their importance, but, in reality, what is a biomarker? A biomarker is a very superficial understanding of what's actually happening. We talk about *EGFR* mutations—for example, *L858R* is the most common mutation in lung adenocarcinoma. And we talk about treating that biomarker with erlotinib, gefitinib, or afatinib. But that's not really what we do, right?"

But rather than "treating that biomarker," Gabe continued, "The reality of it is that the gene is transcribed into an RNA molecule, which is translated into a protein, and that protein then carries out some cellular function, whether it's signaling function or some other function—and with the drug, we're actually targeting the protein. It's these series of epistatic relationships that we have to think about in precision medicine to make it successful." [Epistasis refers to the phenomenon where the effect of one gene is dependent on the presence of one or more modifier genes.] "So even though we see, for example, a deletion in a gene called *CDKN2A*, and [if] we [had not] known what that means or does, from a physician perspective we may not know what that gene is—and that gene is a tumor suppressor of the cell cycle pathway that acts to suppress *CDK4/6* signaling—you could potentially treat a patient bearing that mutation with a CDK4/6 inhibitor, even though the mutation is not in *CDK4* or *CDK6*."

"But to really make sense of this—and this is the other problem when we talk about these very small panels—we need a comprehensive view of what is happening in the tumor. In lung cancer, we can just target *EGFR*, or we can just do *EGFR* and *KRAS*, or *EGFR*, *KRAS*, and *ALK*. But the problem is that *EGFR* and *KRAS* by themselves will only tell you a very small piece of the puzzle. There's a series of downstream mediators in cell signaling, and most of the genes have mutations in these gene pathways, but you really need to understand the health of that pathway. Is that pathway being fully activated or not?"

He continued, "As I just discussed with *CDK4* signaling, that's the cell cycle pathway, and it turns out that one of the last regulatory steps of that process is controlled by a gene called *RB1* that acts to inhibit the cell cycle. And if you lose *RB1*, which you can—and which actually does not happen infrequently in tumors—everything I said about *CDK4/6* won't work no matter what you do. Because you cannot inhibit, you won't be able to turn off that signal, the last switch in the process. It can help to think of these as a series of switches that are on or off, and something goes wrong, or those switches are altered: you have to understand the entire circuit before you know how to attack it. And that's why I think, in the future, we need to go beyond small hotspot pan-

els* into more comprehensive ones that give you a wide view into the health of these signaling pathways that are commonly affected in cancer." [*Cancer hotspot panels assess genomic "hot spot" regions that are frequently mutated in human cancer genes.]

Differentiating Tumor-Specific From Inherited Mutations: Is Matched Normal DNA Necessary?

I then asked Gabe about another topic that had generated a great deal of discussion during the ASCO pre-meeting. Several of the panelists emphasized the importance of not only sequencing the tumor, but also sequencing matched normal (MN) DNA—ie, a sample from the same patient's healthy tissue—to aid in determining whether identified VUS are germline (inherited mutations passed on from parents to children) or somatic (mutations specific to the tumor). When I asked Gabe whether Molecular Health sequenced MN DNA, he responded, "I think that's a great point, and there have been publications describing the dangers of somatic-only testing, including that there is a high false positive and false negative rate,[3] but I think that a lot of this is honestly overblown. If somatic-only testing is done well, these issues are mitigated."

He explained that "Depending on how you do the assay, there are ways to know whether the mutations are germline or somatic, even if you're only looking at somatic tissue. Now, you cannot do it with absolute certainty, but you may be able to do it with *reasonable* certainty. And again, if we're running a test where things have to be absolutely true, then this is in no way acceptable—but [in situations] where we need to use our clinical judgment to make decisions, then it's acceptable. The reality is that knowing what the tumor cellularity is and knowing the allelic frequencies of the variants in the sample can tell you whether the variants you observe are germline or somatic. There are some caveats to that: you also have to understand the copy number alterations, which could throw those numbers off. You have to have good-quality DNA and good, even distribution of the DNA capture regions. But if you look at the tumor cellularity, at the copy number alterations in that sample, as well as allelic frequencies, you can with a very high degree of certainty, although not absolute certainty, identify whether it's a germline or somatic variant."

"Again," Gabe continued, "there are special cases where that's not true. For example, if you have a pure tumor population, meaning only tumor cells, then you may not be able to make that distinction. But, in reality, that never really happens anyway [in solid tumors], because tumor samples are obtained from tissue and are contaminated with non-tumor material. But even if it's not absolutely pure, but almost, if you have greater than 90% tumor cellularity, it may make it very difficult to do this."

"And, secondly," he noted, "I would say that testing only somatic samples will give you an indication of whether variants are germline or not. But if you find a germline variant that is important from an inherited cancer syndrome perspective, I would always recommend confirmatory germline testing. You cannot make decisions based on testing that was not intended to make those calls. Even though I'm fairly certain, for example, of a germline mutation in *TP53* in a 35-year-old in certain types of cancer, I would ask that there be confirmatory germline testing prior to a discussion with the patient and family about their risk of inherited cancers."

A Primer on Genomic Interpretation

I asked Gabe whether he could expand upon the significance of allelic frequency and copy numbers when assessing somatic tissue. "So, first, you start with tumor cellularity," he explained, "because not all of the cells that you are looking at are tumor cells. You need to understand the percentage of all the nuclei that are put through the sequencer that represent tumor versus non-tumor. It's an important first step. You don't have to be absolutely certain. It's like a hand grenade or horseshoes: you just have to be close, because it helps you interpret the results later on. And then, when you run the assay," Gabe continued, "you look at 2 things concurrently. One is the allelic frequency, which means that for the variants that are identified, what percentage that cover this particular locus are represented by this variant? If it's 100%, it means that you have a homozygous variant, which is very unlikely to be cancer. If it's 50%, it means heterozygous, also unlikely to be cancer unless, again, if it's a pure population of tumor cells. And copy number variation means where you've added or lost genetic content in the tumor, if you lose a chromosome or you gain a chromosome, chromosomal arm, or chromosomal region that will alter your allelic frequencies in some way."

He gave the following example to demonstrate: "So, you have a sample that's 50% tumor, and by that, I mean that of all the nuclei that were extracted for testing, 50% were from tumor and 50% were from non-tumor [where the latter] could be inflammatory cells, it could be stroma, it could be other contaminants. So you run the test, and you see that there's a *KRAS* mutation with an allelic frequency of 25%, meaning that 25% of the nucleotides at *KRAS* position 12 were mutated. What that implies is completely consistent with the tumor cellularity example I gave you, that you had a mutation that occurred

in all of the tumor cells. And don't forget you have 2 allelic copies at every genetic position (sex chromosomes excluded), 1 copy from mom and 1 copy from dad. So one of those copies is mutated in all of the tumor cells. But because only 50% of the cells are tumor cells, 25% of the alleles are represented in this sample with a somatic *KRAS* mutation at position 12. So it's simple math, and that's why it's important to know the allelic frequency."

Gabe continued with another example: "Now, if you had 50% tumor cellularity and a mutated variant that you've never seen before of *EGFR*, and it's seen at 50%, that means 1 of 2 things in general. Either it's germline and it's a heterozygous germline variant, or it's in 100% of the tumor cells, where both alleles have the same mutation, which is very, very, very unlikely. So in that case, it's much more likely that you have a germline variant, even though you didn't sequence the germline, and you've only sequenced the tumor sample."

"Where copy number alternations could come into it," he explained "you gain and lose genetic content, so it throws those numbers off. So, let's say again we have the same scenario, where you've got a variant in *EGFR* that's germline and never been seen before, and you have 50% tumor cellularity, but you have loss of chromosome 7 (where *EGFR* lies) in the tumor. Depending on which allele is lost (the novel variant or the common allele), you'll have greater or less than 50% allelic frequency, which may make you think you've got a somatic mutation, so that can trip you up. So if you don't look at copy number content—and, of course, cancers tend to have a lot of copy number alterations—you're going to make mistakes in whether it's germline or somatic."

He continued, "Now, you'll remember at the panel, somebody asked [one of the panelists], 'Do you give allelic frequencies?,' which is something that I do. And he said, 'No, I don't give allelic frequencies,' stating that the clinician misinterprets that information, and they were making judgments on whether variants were germline or somatic errantly since they didn't really understand the principles that I'm mentioning to you. So this is an issue."

Who Is Going to Pay?

I then asked Gabe for his thoughts concerning another topic raised at the panel, where there was discussion about CPT codes and the fact that, due to their current lack of specificity for molecular testing, it's very difficult to drill down and learn which specific tests are being conducted. He noted that "CMS [Medicare] decided there was going to be a CPT code, that they were only going to accept 5 to 50 gene panels, and they were only going to reimburse for these gene panels, because they do not see the value in any more than that. Now that's just today. I think that obviously, in the future, they're going to change their minds and not hamstring the kind of work I'm talking about, where you need to know more, not less. They're basically saying, for example, 'Well, as far as lung cancer goes, we only see *EGFR*, *ALK*, and *KRAS* as valuable, or we'll let you do more, but we'll pay you less,' because they haven't seen the value in more comprehensive testing yet."

"I think that there are 2 issues here," he continued. "One is that they haven't prioritized the groundwork for this field, and it's a very restrictive one at this point. But 2, there's a lot of interpretation that has to happen for us to really understand this process. And there is no professional component for billing for this work. So the reality is that you're creating this system where people are going to drive toward making sure that this is only a test result, that it is some sort of automated system without physician interpretation, because who wants to do that work for free? And the only solution, in my mind, is to work with the American Medical Association and other entities to drive for the creation of a professional component to bill for this kind of NGS interpretation."

My next question for Gabe concerned whether he saw a role specifically for patients and patient advocates to help drive this field forward to help overcome these current challenges. "Absolutely," he said. "Ultimately, there's resistance not just from regulatory agencies to make sure that this develops properly, but also from insurers who don't want to pay for things that they don't have to pay for, even though ultimately, this may end up saving them money." He noted that "There was talk at ASCO from insurers, where they said, 'Hey, when the data are there, we'll start paying for it. We want to see the data; we want to see the mutations.' But what they're not saying is that they also don't want to pay for the testing to get that data. So we're in this sort of Catch-22 again, where everyone wants it to happen, everyone thinks it's in the future, and no one wants to pay for it. But at the end of the day, if the customers are the patients, if they start demanding the kind of testing done and see the value of promoting this, when you have patients who do well because of these approaches to medicine, we should be flaunting these successes. We should make this available to the lay public, so they can see that this really is a paradigm shift in thinking."

Coming Full Circle: Evidence-Based Versus Precision Medicine

We then returned to the provocative question that Gabe

had posed to the pre-meeting panel, which ultimately led to our discussion here. He again noted that, "We've created this system of 'How do we reasonably prove critical data so that we can alter medical decision making?' and, to this end, we've adopted evidence-based medicine. When I was in medical school, it was drilled upon us that, unlike in the past where medical decisions may have been arbitrary, now evidence-based medicine was actually applying empiric evidence and is the epitome of applying scientific principles to medicine. One thing that was not captured or discussed was its limitations, where [evidence-based medicine] worked well and where it didn't. The more you think about it, the more we see it. It doesn't work well in rare disease. Evidence-based medicine is based on statistical inference—it relies on the statistical significance of a variable (usually a treatment) in patients. These studies must be sufficiently powered to draw any meaningful conclusions. But sufficiently powered means that you need many patients who represent the same condition, meaning they are the same physiologically. And, unfortunately, with cancer, even though it's common, every tumor is potentially driven by different alterations and combinations of different alterations. NGS testing has really exposed this, and the more we sequence, the more we see that no 2 tumors are actually the same. This is why evidence-based medicine is so flawed in this space: you have to pretend these tumors are the same to have statistical power, which means ignoring biological facts you have uncovered by sequencing."

Referencing the Bayesian theorem, Gabe explained, "You may need a thousand patients, for example, to have a study for identifying the right use of some targeted therapy in lung cancer. Once you do the sequencing, good luck finding a thousand specimens that have the same molecular signature [despite the large number of lung cancer patients]. There are *many* different pathways that are altered in lung cancer. But if you think, 'Well, I don't care what that mechanism is for disease' and you're going to lump them all together, that's the folly—and likely why so many studies yield poor results with targeted therapies. In order to have enough power to have evidence-based medicine work, you have to have that assumption, and the whole point of evidence-based medicine is to remove physician bias. But now you've created bias. You've created a system where you have to ignore facts to have statistical power, and that's a problem. Evidence-based medicine, in fact, is not scientific evidence, its evidentiary fact. What matters is the relationship between the outcome and the number of times that this was tested, not a scientific rationale. But in order to do that in cancer, you have to ignore the data that we can get from NextGen sequencing, suggesting that the pathomechanisms of all of these—for example, 1000 lung cancers—may be 15 different diseases or a thousand different diseases."

He continued, "We think of precision medicine and evidence-based medicine as being 2 sides of the same coin, that it's science and evidence telling us how to do things right. But the reality is far from it. Rather, these are 2 opposing perspectives and methods for deciding what proper care is. On one hand, you have evidence-based medicine, which is not based on scientific evidence per se, but based on empirical statistical inference (which is hopefully based on scientific reasoning). On the other hand, you have precision-based medicine, which is based on logic and our understanding of the mechanisms that underlie disease. Now they don't have to disagree; they don't have to be at odds. But the way we practice evidence-based medicine today is not, in my opinion, compatible at all with precision medicine."

"And that may be the paradigm shift that leads to the revolution we need to move forward," he stressed. "We need to stop thinking of evidence-based medicine as this dogma of medicine, of medical science, and instead, start examining its flaws. Let's start thinking about where the system fails and why it doesn't seem to be working in this environment. But we're not having that discussion. And I don't think we're really going to be able to implement precision medicine until we're introspective and until we're willing to look at how we do clinical science and what our limits are—and how we convince people that we've done a good study and proved things to some reasonable degree."

I then asked Gabe the following: "Perhaps the term 'evidence-based medicine' has become overused and has become dogma, and we're not really stepping back and looking at what we really need to do with clinical trials. Are you reassured by some of the new innovative trial designs that *are* being used more and more, such as the MATCH trial, the TAPUR trial through ASCO, and I-SPY2, which also used Bayesian techniques? Do you feel that that's moving in the right direction?"

"Yes," Gabe responded. "I definitely think that there is a way to get evidence-based medicine and precision medicine back on track. We have to work to ensure that in the future, evidence-based medicine and precision medicine are realigned. But [currently], this is the exception, not the rule--and the problem is that it's not just about clinical trial design. It's also about insurance companies not ever having sufficient ev-

idence, because they have this antiquated model of how you can prove something, and they are relying too heavily on significance based on large study sets. That's kind of the approach today, but, hopefully, that changes, since that mentality *has* to change. The reality is that 3 similar patients may have more statistical power based on the magnitude of effect, than having 1000 patients with the same disease type but different biological paths or mechanism of disease. Focusing trials to answer a binary result of a better/worse outcome for 2 drugs will not be as helpful as focusing on treating pathway alterations that are likely driving tumors."

> *"Precision medicine focuses on treating the mechanisms of disease specific to the individual patient before us; this is different from the focus on statistical inference inherent to evidence-based medicine."*[1]

As we ended our interview, Gabe shared the following thought-provoking analogy:

"I was the keynote speaker for a Precision Medicine conference in Cincinnati," he remembered, "and I talked a lot about this particular topic. I tried to explain the problem with an analogy that I think might be helpful: if we took the same approach that we take to clinical medicine, evidence-based medicine—which we hold to be gospel (but may be difficult to really grasp)—and applied it to another common topic that we all understand very well. You have a car, and your car doesn't work. So you want to fix your car. If you use the principles of evidence-based medicine, then you have to create a question and a hypothesis—'How can I best fix my car?'—and create a study design to test your hypothesis. So I think that either I should put gas in my car or change the oil. Those are my 2 options. So now give me 1000 other cars that don't work, and for 500 of them, I'm going to put gas into them, and for the other 500, I'm going to change the oil. I can show that, statistically speaking, putting gas into cars is a better treatment for broken cars than changing the oil. I've just fulfilled the principles of evidence-based medicine. But it's all absurd, right? If your car is broken, you take it to a mechanic, you look under the hood, you figure out what the problem is, and then you treat the problem. So let's say now that I take my car, I look under the hood, and I see that the battery is dead. Do I still put gas in my car?"

Bravo, Gabe! Please accept my wholehearted thanks and gratitude for your time and our fascinating, informative, and thought-provoking conversation. It was truly a joy to speak with someone who is so passionate and eloquent concerning his life's work. Most importantly, thank you for your critical efforts every day on behalf of all of those affected by cancer. ◆

—Debra Madden, Cancer Research Advocate

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