Looking Beyond Single-Drug Approaches to Precision Oncology Research

At Avera Health, Dr. Leyland-Jones and Brandon Young rely on transcriptomics to uncover multiple drug-susceptible tumorigenic pathways.

Introduction

At the Center for Precision Oncology at Avera Health, a regional health system based in Sioux Falls, South Dakota, Brian Leyland-Jones, MD, PhD and his team conduct clinical research to identify, profile, and validate biomarkers with the potential to diagnose disease and inform cancer treatment decisions. Working closely with Dr. Leyland-Jones is Brandon Young, Director of Molecular and Experimental Medicine Sequencing at the Bowden Precision Oncology Clinical Laboratory in La Jolla, California. Together they are performing DNA and RNA next-generation sequencing (NGS) studies, as well as proteomic tumor analysis to design experimental drug combinations.

The transcriptomic element of their approach is essential. They don't want to be limited to the "traditional" monopharmaceutical approach of matching a single DNA mutation to its corresponding drug. "Much in the same way that triple drug combinations are effective for HIV and tuberculosis, we think that triple or greater drug combinations, could be efficacious in cancer treatment," Mr. Young said. "RNA sequencing (RNA-Seq) enables us to determine whether a particular biological pathway is involved," Dr. Leyland-Jones added. "By moving from basic DNA up to transcriptomic signatures of activated pathways, we can get much closer to what is actually going on at the protein level within the cancer."

iCommunity spoke with Dr. Leyland-Jones and Mr. Young to discuss their research objectives, ongoing RNA-Seq studies, and their vision for the future of NGS in cancer medicine.

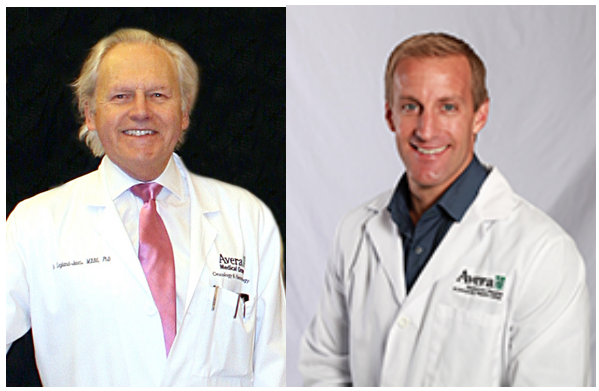
Q: What is the principal focus of your research?
Brian Leyland-Jones (BLJ): Tumors are clonal, and there is clonal dependency between each of the tumor-driving cell populations. We're looking for combinations of three or four drugs that will close down all the clonal drivers. It's a very aggressive approach. Except for Dr. Razelle Kurzrock, who developed one of the largest Phase 1 clinical trials in the United States,[1] most academic centers use sequencing to match subjects to single-agent therapy studies.

Q: Why did you join Avera Health?
BLJ: My team of 11 people approached Avera Health in late 2013 to see if it would be willing to accommodate our sequencing expertise. We were looking for a cutting-edge oncology environment and support for the development of a cancer subject sequencing database. We've now sequenced about 700 individuals.

Q: Why is your sequencing lab in San Diego, rather than South Dakota where Avera Health is headquartered?
BLJ: I've had an affiliation with Brandon Young for close to 10 years. One of the challenges of being in South Dakota is how tough it is to recruit highly specialized genomic and clinical trial people in this area. However, if you're working on the mesa in La Jolla, you're sitting right next to Illumina, hundreds of other biotech companies, as well as the University of California, San Diego, the Scripps Research Institute, the Salk Institute, and the Sanford Burnham Prebys Medical Discovery Institute. Our La Jolla location enables us to engage in collaborations with other biotech companies in the San Diego area. The Worldwide Innovative Networking (WIN) Consortium also uses our La Jolla lab as the global hub of its precision oncology laboratories.

Q: What is the value of sequencing tumor RNA?
Brandon Young (BY): DNA sequencing has traditionally been easier to perform. Conventional programs such as NCI-Match* and Stand Up To Cancer aim to correlate a single DNA mutation with a single drug therapy. Our multidrug approach requires more information. We need information from multiple methods to determine which drugs might be used in combination effectively.

There are several major networks and pathways in cancer. Much in the same way that we see triple drug combinations for HIV and



Brian Leyland-Jones, MD, PhD is an oncologist/hematologist at the Avera Cancer Institute Center for Precision Oncology and Brandon Young is Director of Molecular and Experimental Medicine Sequencing at the Bowden Precision Oncology Clinical Laboratory.

*National Cancer Institute-Molecular Analysis for Therapy Choice (NCI-Match) is a precision medicine cancer treatment clinical trial.

tuberculosis, we think that triple drug combinations could be efficacious in cancer treatment. Expression profiling with RNA-Seq enables us to determine whether a particular biological pathway, such as one involved in angiogenesis, is upregulated and represents a possible target in a combination approach.

Finding a DNA mutation is not necessarily enough because it might not be expressed all the time. Therefore, RNA-Seq might tell us whether a DNA analysis–based approach would work or not. Directed treatments exist for fusions, so we would want to know whether the fusion itself has actually been expressed. RNA-Seq is a valuable tool that gives us a complete picture of tumor biology. Similarly, we believe that proteomics and protein phosphorylation will be important.

We still think that DNA sequencing is absolutely fundamental and needs to be performed to identify disease states, especially in cancer. RNA-Seq is an important complement that provides information above and beyond simply looking at a mutation and trying to match it up with a drug.

> "We took the same set of clinical samples and compared RNA-Seq with TruSeq RNA Access on the NextSeq 500 System with NanoString nCounter direct hybridization technology."

BLJ: If you're looking at *HER2* copy number, *HER2* fluorescence *in situ* hybridization (FISH) ratio, or quantitative immunohistochemistry (IHC), all of these correlate and benefit from Herceptin. Yet, the best marker currently is at the transcript level. In oncology cases with phosphatidylinositol-3-kinase (PI3K) pathway alteration, our work has shown that the best correlation with activity is at the phosphoproteomic level, as opposed to the DNA level. So, we feel that going further up into either a transcript or a protein level will be much better. We have a collaboration with Andrea Califano at Columbia who has developed transcriptomic signatures of activated pathways. By moving up from DNA and into transcription signatures, we can get much closer to what is actually going on at the protein level within the cancer.

Q: Why do you use sequencing rather than a hybridization approach?

BY: A decade ago, Brian and I began using Illumina microarray technology for retrospective analysis of 10-year–old clinical trial samples. The idea was to go back and compare responders vs. nonresponders. When NGS was introduced, we began using the HiSeq™ 2500 System. We were also one of the original groups to use TruSeq™ RNA Access Library Prep Kit† in a study.

†Currently known as TruSeq RNA Exome

We've analyzed 8000 to 10,000 samples on microarrays and several hundred using RNA-Seq. One of the significant issues is always cost. If you're running 400–500 samples, the cost of sequencing the whole transcriptome adds up. Illumina understood the value of a complementary approach that integrates DNA and RNA sequence information. TruSeq RNA Access was developed to enable those studies to be carried out more economically.

Q: Did you compare the benefits of RNA-Seq versus a hybridization-based approach?

BY: We wanted to be agnostic in our approach, so we conducted platform comparison studies to uncover gaps in our knowledge about the data type, data quality, and costs of using different platforms. We took the same set of clinical samples and compared RNA-Seq with TruSeq RNA Access on the NextSeq™ 500 System with NanoString nCounter direct hybridization technology. We knew that the NanoString nCounter cost per sample was half of the cost of performing RNA-Seq. We wanted to determine whether we could obtain the same expression profiles if we ran the same samples on both platforms.

We found that the correlations were very high, in the 0.98–0.99 range. So, if we compared just the genes on the 770-gene NanoString nCounter PanCancer Pathways Panel vs. the same genes from the TruSeq RNA Access transcriptome sequencing assay, the NanoString cost would be lower and the expression profiles we determined for those 770 genes would be the same.

However, cost versus coverage was another matter. When we combined the PanCancer Immune Profiling Panel and the PanCancer Pathways Panel, we obtained transcription data for a total of about 1200 genes. The cost of those combined PanCancer panels was the same as running TruSeq RNA Access. However, TruSeq RNA Access covers those 1200 genes and another 20,000 genes that are involved in those pathways. It also provides the ability to look at fusion calling.

> "RNA-Seq is the right method to choose if you want to give yourself the chance to go beyond what we might currently say is "clinically relevant" and have additional data for collaborations and to look back on later."

The richness of the data we obtained from TruSeq RNA Access, for the same amount of money and the same amount of RNA starting material, signaled to us that there was more opportunity gained by choosing the Illumina platform. RNA-Seq is the right method to choose if you want to give yourself the chance to go beyond what we might currently say is "clinically relevant" and have additional data for collaborations and to look back on later.

Q: Why did you choose the NextSeq 500 system?
BY: When we set up our La Jolla sequencing lab four years ago, our original plan was to use a HiSeq 2500 System. Around that time period, Illumina introduced the NextSeq 500 System. Brian and I reviewed its specifications and capabilities and concluded that the NextSeq 500 System would become the sequencing workhorse in molecular pathology labs. That's why we chose it for our lab.

Q: What benefits does the NextSeq 500 System provide?
BY: In our previous experience with core facilities, we had struggled to use the full capacity of our HiSeq 2500 System economically. We might get 4, 5, or 10 samples in one week, and 3 samples the following week. You need 8 samples to fill the lanes on a HiSeq, but when you need to provide rapid turnaround, you can't sit and wait for samples to accumulate so that you can batch them. We realized that we could have two independent NextSeq systems for the price of one HiSeq system. One NextSeq system could be running DNA, and one could be running RNA, or we could run RNA and DNA together, and we didn't have to wait until we had a large number of samples.

Therefore, much of our decision to choose the NextSeq 500 System was about having the flexibility to match the throughput of our RNA and DNA sequencing instrumentation to the way samples come in, and our ability to provide fast turnaround. In addition, because we are focused on capture technologies and whole-transcriptome analysis, we didn't have a significant requirement for whole-genome sequencing. Some groups can really benefit from the high throughput and cost effectiveness of a HiSeq 2500 System, or even the NovaSeq™ 6000 System. However, labs like ours will find a greater benefit from the NextSeq 500 System which provides rapid turnaround and the ability to run fewer samples at a time economically.

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Q: How do you handle formalin-fixed, paraffin-embedded (FFPE)-preserved samples?
BY: Back when we originally started this, I would jokingly say that I had a love/hate relationship with pathology departments who supplied us with samples. They always used FFPE to preserve samples. I came from a research background and wanted pristine, fresh-frozen samples. Over the past five years, we've evolved our thinking and understand the value of having FFPE samples. FFPE preservation enables us to address tumor heterogeneity. A pathologist can examine an FFPE slide and define the areas of highest tumor content. We can cut out that particular section for analysis, and we can also take adjacent normal tissue from a slide and use that as a comparator.

Q: Has the quality of FFPE samples improved?
BY: One reason FFPE samples are more viable for us is the quality of the FFPE samples that we now handle. Unlike the challenges faced by people looking at decades old samples from the 1960s and 1970s, we typically are extracting RNA and DNA within a month of when the biopsy was taken. Also, the deparrafinization protocols we use now are gentler and leave more RNA intact. It has been a very long time since I have seen a sample that is so degraded that the fragments are less than 100 base pairs and we can't analyze it.

With TruSeq RNA Access, and even whole-transcriptome kits, the first step is usually to fragment the RNA. FFPE preservation fragments the RNA, saving us a step in the assay. We often find that our extracted RNA is well preserved enough that we still need to run the fragmentation step. FFPE protocols and Illumina library prep kits have evolved in such a way that FFPE tissues are no longer as much of a challenge.

Q: Have you looked at other ways of distinguishing tumor heterogeneity?
BY: We've dabbled in laser capture microdissection. With laser capture, a good technician can perform microdissection on a few samples a day. The way that the cells are adhered to the capture membrane makes extraction more difficult. Performing the standard microdissection is not quite as selective. However, a good pathologist can circle the tumor section on a haemotoxylin and eosin (H & E) staining slide in 5–10 minutes. It would take me another minute to perform the microdissection by hand with a scalpel or razor blade. In the case of surgical resections, instead of cutting out the parts we want, we cut away the parts we don't want, to obtain tumor content from multiple parts of the specimen.

It then becomes a matter of how deeply you're sequencing. That's something that we work on closely with the informatics team. We want to make sure that we have as many reads as we need based on assay parameters and how many of the reads map to TruSeq RNA Access transcriptome sequences. All these things factor into our confidence in the assay itself and being able to tackle that heterogeneity. From a technical perspective, TruSeq RNA Access does an excellent job of maximizing our ability to focus on the known transcriptome so that we get the greatest benefit from a cost and data perspective.

Q: Have you considered performing single-cell sequencing to determine tumor heterogeneity?
BY: I believe there's unique value in single-cell sequencing, but we're not yet at a point where we can perform it in a cost-effective way. One of the issues is that we're performing RNA-Seq on 100,000–500,000 cells, roughly equivalent to what you would take from a pathology slide. There's no effective way to prepare

that many individual cells from an FFPE sample. I've seen the single-cell data, and it looks great with respect to the way it addresses heterogeneity. However, the sheer number of cells that we would have to isolate and sequence to feel confident from a tumor analysis perspective would be a challenge. Brian and I keep ourselves informed on the development, release, and improvement of new technologies. We'll allow the field to mature, while we focus on our translational research.

BLJ: At this moment, we're performing DNA targeted sequencing of tissue and blood. We use that combination to compensate for some degree of heterogeneity. There are several groups that are performing combined circulating tumor cell (CTC) and cell-free DNA (cfDNA)analyses. We need to answer the questions of where CTCs fit in, and where transcriptomics and proteomics fit in. I think those are more important questions at the moment than analyzing at a single-cell sequencing level.

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Q: What is the future role of sequencing in cancer medicine?
BY: We think DNA sequencing will be standard practice in ten, if not five years. We think it will be the same for RNA.

BLJ: In five years, sequencing will be alongside conventional radiology and pathology in cancer analysis. This is a technology that is rapidly going to be incorporated into the clinic. At a recent patient advocacy conference, Dr. Kurzrock said that in five years it will be considered malpractice if a physician does not perform sequencing of a cancer patient's tissue samples.

I believe that these assays are going to end up in the molecular pathology laboratories of every hospital. Just as we now have biochemistry and hematology assay systems in clinical labs, I think there will be sequencing platforms in the molecular pathology laboratories of every hospital. The introduction of TruSeq RNA Access is preparing for this transition.

As we progress over the years, people are going to move from DNA sequence analysis to transcriptomics and proteomics. Some kind of targeted sequencing panel at the DNA level will always be used. However, assessing fusion, microRNA, and expression levels will become more important.

We feel that Illumina platforms represent where oncology is going to be in another five years.

Learn more about the Illumina systems and products discussed in this study:

NextSeq 500 System, www.illumina.com/systems/sequencing-platforms/nextseq.html

TruSeq RNA Access Library Prep Kit (Currently known as TruSeq RNA Exome), www.illumina.com/products/by-type/sequencing-kits/library-prep-kits/truseq-rna-access.html

HiSeq 2500 System, www.illumina.com/systems/sequencing-platforms/hiseq-2500.html

NovaSeq 6000 System, www.illumina.com/systems/sequencing-platforms/novaseq.html

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1. UC San Diego Health Sciences, Moores Cancer Center Research and Training, Razelle Kurzrock, MD, Senior Deputy Director, Clinical Science. www.healthsciences.ucsd.edu/research/moores/about/leadership/directors-office/Pages/clinical-science.aspx. Accessed September 19, 2017.

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