Capturing Variant Data from the Blood

Using the HiSeq® 2500 System, researchers at the MD Anderson Cancer Center are studying genetic variation in exosomal DNA found in the blood of pancreatic cancer subjects and gaining insight into the potential of liquid biopsies in diagnosing and monitoring the disease.

Introduction

More than 2000 years ago, when a person had cancer, a Roman or Greek doctor examined their "humors" or bodily fluids, including blood. The focus then was on analyzing the excess or deficiency of the fluids. At that time, medicine was highly individualistic and it was said that each patient had his or her own unique humoral composition. Today, medical research, specifically cancer research, seems to be returning to these origins. At least that is what scientists like Hector Alvarez, MD, PhD believe. Dr. Alvarez is a physician scientist leading the Liquid Biopsy platform in the Pancreatic Cancer Research Center at the MD Anderson Cancer Center. He is searching for innovative, less invasive ways to diagnose and monitor pancreatic cancer.

According to the World Cancer Research Fund International, pancreatic cancer is the 12th most common cancer in the world, with more than 300,000 new cases diagnosed worldwide in 2012.[1] Pancreatic cancer is one of the few cancers in which survival has not improved over the last few decades.[2] The continued high rate of mortality is likely due to a lack of tests that can detect the disease in its earliest stages.[2]

Dr. Alvarez is developing laboratory protocols, devices, and software for liquid biopsies that could find early biomarkers in the blood or other bodily fluids. Using next-generation sequencing (NGS) with HiSeq 2500 System, he and his team are performing deep analysis of blood samples from pancreatic cancer patients to identify subtle tumor events associated with the disease and its progression. His focus is on finding biomarkers that could help to diagnose pancreatic cancer.

Messages in the Blood

Historically, most cancers are diagnosed after a tissue biopsy. It starts with a clinician removing a small piece of tissue from a suspicious lesion or mass. A pathologist then looks more closely at cells in that tissue to determine whether they are cancerous. When the organ in question is deep in the abdomen, like the pancreas, such procedures require image-guided needle biopsies that run the risk of missing important tissue abnormalities elsewhere in the tumor.[3]

"Pancreatic tumors are very difficult to reach and it's hard to sample the tissue," Dr. Alvarez said. "That means we often don't have access to important information that could help with treatment and improve survival rates. When the tumor is in a place that does provide access and a biopsy yields evidence of cancer, only about 20% of patients are amenable to surgery. Usually, it's too late and the tumor has grown so much that the patient is not a candidate for surgery."

This has led many clinicians to call for a liquid biopsy test, where a vial of blood could provide all the necessary clinical information for doctors to make reliable diagnoses and identify targeted interventions. A liquid biopsy would rely on genetic information from the tumor circulating in the blood or other bodily fluid. "As tumor cells die, the cells and surrounding tissues release DNA content into circulation," Dr. Alvarez said. "The genetic alterations in this DNA might help clinicians understand what is happening in the tumor, or if it has metastasized and spread from the initial, primary tumor site to other areas of the body."

In June 2016, a large-scale study of more than 15,000 cancer subjects was presented at the 2016 American Society of Clinical Oncology (ASCO) meeting. It found that the Guardant360, a type of liquid biopsy that tests for 70 different mutations, was accurate and might be a reliable alternative to conventional biopsy methods.[4]

Dr. Alvarez was not surprised by the study results, but believes that more research is required to create a truly clinic-ready test that physicians can rely on. "At the MD Anderson Cancer Center, we are committed to developing a platform that has comprehensive meaning for clinicians," Dr. Alvarez stated. "We're looking to develop a test that is clinic-ready, with a short turnaround time. Our work suggests that the genetic material shed through extracellular vesicles might provide that kind of information."[5]

Hector Alvarez, MD, PhD, is a physician scientist leading the Liquid Biopsy platform in the Pancreatic Cancer Research Center at the MD Anderson Cancer Center in Houston, Texas.

NGS, Actionable Intelligence, and Exosomes

Three years ago, scientists were in the early stages of research to prove that liquid biopsies were possible. Today, Dr. Alvarez emphasizes "we're doing more than just basic research. We're trying to find the right protocols for a test that delivers results that are accurate, clinically relevant, and that support physicians in determining what kind of therapy is needed."

He believes that the information that can be gleaned from DNA released by special extracellular vesicles, called exosomes, and genetic information from other cellular compartments might be critical to understanding tumor development and metastasis. "There are more than 50 types of extracellular vesicles, with the most well known being apoptotic bodies. There are also smaller microvesicles and still smaller exosomes," he explains. "We were one of the first teams to show that there is enough double-stranded DNA present in exosomes to characterize them at high resolution for tumor profiling."[5]

He and his colleagues studied shed exosomes in two subjects with pancreatic cancer and one with ampullary cancer.[5] They used the HiSeq 2500 System to perform whole-genome, whole-exome, and transcriptome sequencing of the exoDNA and exoRNA. They found robust representation of tumor DNA within the shed exosomes, suggesting that exosome-based liquid biopsies might have the potential for clinical diagnosis, as well as treatment monitoring. While Dr. Alvarez was one of the first to demonstrate the viability and validity of exosome-based liquid biopsies, he says that there might be more important genetic information left to discover.

> "We were one of the first teams to show that there is enough double-stranded DNA present in exosomes to characterize them at high resolution for tumor profiling."

"All extracellular vesicles could be important," Dr. Alvarez said. "First, we believe that the different vesicles are heterogeneous and might change during disease evolution. There are different biomarkers coming from different compartments and these might signal different biological states. Second, it isn't just tumor cells that produce particles or extracellular vesicles. Changes in vesicle DNA might reflect what's occurring in other parts of the body, where cancer might be starting, but the tumors are so small that they would not be evident in regular imaging."

He believes that in the future, these results could aid in diagnosing patients whose cancer is not amenable to a conventional surgical biopsy. "Any step forward on a diagnostic procedure that is not invasive would be a gain for these patients," Dr. Alvarez added.

His liquid biopsy team at MD Anderson is also interested in looking at markers found in cell-free DNA, especially as it pertains to therapeutic response. The different variants and mutations in cell-free DNA might provide genetic data about whether a particular intervention or therapy is working.

> "We're performing whole-genome, whole-exome, and RNA-Seq with the HiSeq 2500 System to identify the wide range of biomarkers that could be present."

"When cells die, the nuclear DNA becomes fragmented and chopped into small pieces of double-stranded DNA that is released into the circulation," Dr. Alvarez said. "It's possible that these small pieces of DNA contain alterations that signal clinical response. There tends to be higher concentrations of cell-free DNA in advanced stages of cancer. That could give us a more complete picture about what the cancer is doing."

Innovations in NGS Technology

Dr. Alvarez' sequencing experience began when he was studying gall bladder cancer at Johns Hopkins University. "As I was working on my PhD thesis, I was introduced to a new technology called serial analysis of gene expression (SAGE). It was the sequencing technology used for the Cancer Gene Anatomy Project (CGAP). It was basically an RNA sequencing technology."

In fact, SAGE data collection was a precursor to NGS. But, like any early platform, it wasn't without its challenges. "We needed to build handmade genomic libraries," Dr. Alvarez said. "There was no kit for library preparation, so it took a long time to create the gigantic libraries we needed to perform sequencing runs. Despite the difficulties, it was a great introduction to the power of genetics."

> "We are very happy that we chose the HiSeq 2500 System. It provides high-quality data, and for this kind of work, data quality is important."

Data quality was the critical attribute as Dr. Alvarez and his team selected an NGS system for their research. "We looked at several competitive systems and decided we were going to use the system that offered the best data quality," Dr. Alvarez said. "We are very happy that we chose the HiSeq 2500 System. It provides high-quality data, and for this kind of work, data quality is important. We're using it to look at and follow various biomarkers."

Besides ease of use, Dr. Alvarez believes that one of the benefits of using NGS and the HiSeq 2500 System, and more recently a NextSeq 500 System, is that both systems enable an integrated genomics approach to studying extracellular biomarkers.

"We're performing whole-genome, whole-exome, and RNA-Seq with the HiSeq and NextSeq Systems to identify the wide range of biomarkers that could be present," Dr. Alvarez stated. "For a liquid biopsy test to be valuable, it needs to assay several biomarkers. If we can identify biomarkers that are coming out of the extracellular vesicles, circulating tumor cells, and cell-free DNA, we could obtain an overall picture of what is happening with this disease across the entire body."

According to Dr. Alvarez, the simultaneous investigation of DNA and RNA information is even more important now that cancer researchers understand that tumors are heterogeneous in nature.

> "All the high-resolution information coming from these different compartments could be signaling a better way to determine what is really going on in a particular case of pancreatic cancer."

"Even if you can effectively sample the tissue with a core biopsy or by a fine needle biopsy, what's in your sample might not represent the whole tumor," Dr. Alvarez said. "We believe that NGS performed in the liquid biopsy context could give us a complete picture of what's happening in the entire tumor. That's incredibly powerful. All the high-resolution information coming from these different compartments could be signaling a better way to determine what is really going on in a particular case of pancreatic cancer."

Moving Forward

Dr. Alvarez' goal remains the development of a comprehensive, clinic-ready liquid biopsy platform. He believes it will play a key role in personalizing treatment plans for individual patients, especially for cancers that are difficult to diagnose or difficult to treat such as pancreatic cancer.

"We believe that having a personalized panel of genetic alterations is important," Dr. Alvarez said. "A particular genetic profile could be defined at the beginning of treatment, and then clinicians could follow changes to that profile over time to help them understand the course of the disease."

He and his team are currently following more than 100 pancreatic cancer subjects, comparing exoDNA and cell-free DNA together to measure the effects of different therapies. They hope to publish those results soon. He is also excited that the different identifying markers provided by NGS might also provide some information about mechanisms of tumor resistance and clues to design new immunotherapies in the future.

"In the future, liquid biopsies could have a broad role in precision medicine, including value in prevention, early detection, and disease management," Dr. Alvarez stated. "For example, immunotherapy could benefit greatly from liquid biopsies. Through simultaneous isolation of nucleic acids such as DNA and RNA, we could identify and track neoantigens, which are personalized molecular targets of this very promising therapeutic strategy. We are developing tumor derived exosome enrichment processes to increase the tumor signal in our liquid biopsies. Without liquid biopsies and NGS, continuous monitoring of genetic alterations is not possible for solid tumors."

Despite his optimism about where liquid biopsies could take us, Dr. Alvarez cautions that there is still much work left to do. He and his team will continue to follow subjects over time and measure how the different genetic alterations in cell-free DNA, exosome DNA, and other biomarkers change. He looks forward to seeing what secrets these biomarkers will tell about the nature of pancreatic cancer, and how he and his team could use them to translate those secrets into tools to combat the disease.

"Pancreatic cancer is tough, because it is not a classical solid tumor, like lung, colon, or breast cancer, that allows for much actionability," Dr. Alvarez added. "Our research needs to evolve so that we can understand where we can intervene and make a difference. We'll have to collect enough data to demonstrate liquid biopsies are clinically robust and relevant to care. It will take some time, but we will get there."

Learn more about the Illumina system mentioned in this article:

HiSeq 2500 System, www.illumina.com/systems/hiseq_2500_1500.html

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

References

1. Pancreatic cancer statistics. World Cancer Research Fund International, www.wcrf.org/int/cancer-facts-figures/data-specific-cancers/pancreatic-cancer-statistics. Assessed July 13, 2016.
2. Pancreatic Cancer Facts. Hirshberg Foundation for Pancreatic Cancer Research. www.pancreatic.org/site/c.htJYJ8MPIwE/b.5050503/k.40C9/Pancreatic_Cancer_Facts.htm. Assessed July 13, 2016
3. Hébert-Magee S. Is there a role for endoscopic ultrasound-guided fine-needle biopsy in pancreatic cancer? *Endoscopy*. 2015; 47(04): 291–292.
4. McGinley L. Liquid Biopsy study offers hope for a blood test to find cancer. The Washington Post. June 4, 2016.
5. San Lucas FA, Allenson K, Bernard V, et al. Minimally invasive genomic and transcriptomic profiling of visceral cancers by next-generation sequencing of circulating exosomes. *Ann Oncol*. 2016; 27(4): 635–641.

Illumina, Inc. • 1.800.809.4566 toll-free (US) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com

For Research Use Only. Not for use in diagnostic procedures.

illumina®