MultiGen's Modified Sanger Sequencing Takes Aim at Detecting Cancers, Infectious Diseases

Apr 28, 2017 | Leo O'Connor

Premium

NEW YORK (360Dx) – An allele-specific multiplex sequencing (ASMS) assay that can identify low concentrations of relevant drug targets could provide clinical support in identifying and prescribing appropriate chemotherapeutic drugs, and it has potential for routine clinical use in a far broader scope of applications, according to its developer MultiGen Diagnostics.

In a study conducted with the department of pathology at Mount Sinai School of Medicine, and published online in the *Journal of Solid Tumors*, MultiGen reported that its ASMS test identified and confirmed the BRAF p.V600E mutation with a 1,000-fold increase in sensitivity compared to competing PCR-based and next-generation sequencng methods.

Testing for BRAF p.V600E is an expected practice prior to starting drug therapy for melanomas, papillary thyroid cancer, and other cancers, and the presence of the mutation predicts for better treatment success with BRAF kinase inhibitors such as Zelboraf (vemurafenib), MultiGen said.

Its Founder and Chief Scientific Officer Thurai Moorthy said in a statement that the ASMS assay, which is a modification of traditional Sanger sequencing, provides a sensitive companion diagnostic for the detection of cancer mutations, and that the BRAF mutation project is a "generic demonstration" of capabilities that could immediately translate into "many more patients qualifying for potentially curative treatment."

Roger Hodkinson, MultiGen's director of medical affairs, who is also an author of the online study, said that the assay enabled detecting target mutations at picogram concentrations, and it detected positive results in samples that had been previously reported as negative by Thermo Fisher Scientific's SNaPshot Multiplex System and Ion Torrent NGS platform.

The research team used SNaPshot and Ion Torrent to analyze 83 DNA extracts from formalin-fixed paraffin-embedded samples. All of the samples that tested positive for BRAF p.V600E also tested positive by ASMS. Of 46 samples that tested negative for BRAF p.V600E — 20 from

SNaPshot and 26 from Ion Torrent — ASMS detected BRAF p.V600E-positive results in 10 of the SNaPshot-negative samples and in 18 of the Ion Torrent-negative samples, the study said.

Hodkinson noted that the content of the published paper provides a glimpse of the clinical potential of MultiGen's modified Sanger sequencing platform, and that several potential applications exist.

While the company has published previously about clinical use of the technology to test for infectious diseases, this was the first paper to describe its application in oncology. "We're looking to scale that up and launch it as a commercial product through a subsidiary," he said, "and to accomplish that, we are currently looking for appropriate management and seeking investment."

He said that a key part of the firm's value proposition is its ability to multiplex "traditional Sanger sequencing, the gold standard for DNA identification," enabling its use in "routine clinical applications."

MultiGen's modified Sanger sequencing platform simultaneously sequences multiple DNA targets. The target DNA can be from the same genome, different genomes, or a combination of both, the firm said. The sequencing reaction is the same as that of conventional sequencing methods, except that its primers are of different molecular weights, and that enables separation of fluorescence-labeled, truncated DNA molecules generated from different amplicons. When groups of fluorescence-labeled truncated molecules are passed through capillary electrophoresis, they separate without an overlap, generating a nucleotide-specific sequence for each pathogen.

Sanger sequencing may be the method for DNA identification that all others are compared against, but it has its limitations, including sensitivity, said Dahui Qin, medical director of the molecular diagnostics lab at the Moffitt Cancer Center in Tampa, Florida. "It's not the perfect test," he said.

As part of his role as medical director, Qin evaluates multiple technologies with a view to purchasing and implementing those that are most suitable for his lab's clinical requirements.

By modifying its design, "MultiGen has increased the sensitivity of the Sanger technique," he said, "and that drew my attention and made me think that this could be something we'd use."

Qin noted that he has not purchased the MultiGen platform, because its existing pyrosequencing and NGS platforms are sensitive enough to identify BRAF mutations. However, he said that he is watching the progress of the MultiGen modified Sanger platform.

"MultiGen's paper is about applying the technology to identify BRAF mutations," he said, adding that if the firm can demonstrate its capability to detect other gene mutations, such as the T790M mutation, which is a gatekeeper mutation of the epidermal growth factor receptor (EGFR), he would consider using it.

In 2014, specialty laboratory Physician's Choice Laboratory Services acquired the assets of MultiGen for an undisclosed amount.

The deal included access to and control of the commercialization of MultiGen's platform technology, which PCLS said at the time "greatly reduces the cost of delivering targeted molecular diagnostics and genetic mutation analysis."

Hodkinson noted that the firm is expanding applications within cancer testing. In the near future, his firm expects to publish a paper about its work in multiplexing cancer markers, "looking not just at BRAF, but also at a mix of markers required to analyze a particular tumor."

He noted that a particularly useful component of the MultiGen approach will be in helping clinicians diagnose syndromes. "Real clinical medicine is all about creating a list of possible explanations for the presenting syndrome — we call it the differential diagnosis — and then prudently investigating as many of the options as possible to identify the cause of the disease or condition," Hodkinson said. The firm's multiplexed panels have been designed to address that issue head-on, he noted.

MultiGen currently provides six modified chemistry platforms that form the basis for assays it is developing for infectious diseases, oncology, pharmacogenomics, and drug development.

With the comparison study behind it, the firm plans to demonstrate results with additional clinical trials. "With suitable partners, we'll do trials related to a particular cancer with a larger number of patients," Hodkinson said.

The firm expects that its analyte-specific reagents will soon be made available for purchase and use "by sophisticated oncology institutions and laboratories to test samples in house, using their own Sanger sequencers," he said.

Potential customers include CLIA-certified clinical laboratories that build and use laboratory-developed tests, he said, adding that the cost of doing testing with MultiGen's technology is competitive with that of existing PCR and other molecular testing, and far less expensive than NGS testing.

He noted that the firm's greatest challenge is establishing a strategic focus. "We have a technology that is powerful and applicable to many questions in infectious diseases, oncology, and other areas, and as a small company we need to be very careful what we chose to work on," he said.

For example, the firm expects that its technology will be of use to pharmaceutical companies seeking to select the most appropriate people for clinical trials. At the same time, he said that it is poised to enter a collaboration with the Centers for Disease Control and Prevention in Atlanta to conduct genotyping of Hepatitis C and identify markers that may be important for selecting an "appropriate curative therapy for that condition."

"It's a technology that can go in a variety of directions, and right now we are being very careful as to which ones we chose to demonstrate," he said.