A liquid biopsy-based test to detect BRAF V600 mutations in advanced cancers is comparable to standard invasive tests

May 23 2016

Testing for BRAF V600 mutations in cell-free DNA from plasma using the PCR-based IdyllaTM BRAF Mutation Test was feasible and concordant with standard tests of archival tumor biopsy samples and had a short turnaround time of 90 minutes, according to a study published in *Molecular Cancer Therapeutics*, a journal of the American Association for Cancer Research.

"Cell-free DNA is released to the circulation from cancer cells undergoing apoptosis or necroptosis in the primary or metastatic cancer lesions and can be detected in the blood," said Filip Janku, MD, PhD, assistant professor of Investigational Cancer Therapeutics (Phase I Clinical Trials Program) at The University of Texas MD Anderson Cancer Center in Houston.

"Unlike performing tissue biopsies, obtaining blood samples to isolate cell-free DNA is a minimally invasive approach with less risk to the patients at a lower cost," Janku added. "The samples can be collected at multiple time points and provide valuable information about the genetic changes that occur during the course of the disease, as this is not a static process.

"We developed IdyllaTM BRAF Mutation Test, a fully integrated quantitative allele-specific real-time PCR-based test that uses a single disposable cartridge. We demonstrated that testing for BRAF V600

mutations in plasma cell-free DNA using this test is feasible, has comparable sensitivity and specificity to other PCR or next-generation sequencing methods, and has an unprecedented short turnaround time," Janku said.

Janku and his colleagues used plasma samples collected from 160 patients with a range of advanced cancers with known BRAF V600 mutation status determined using paraffin-embedded tumor tissue samples. The most common tumor types were colorectal cancer and melanoma.

The researchers analyzed the plasma samples for BRAF V600 mutations using the Idylla system and found that the test had 88 percent concordance with the results from the standard tests that used paraffin-embedded tissues, in samples collected at baseline. The concordance was 90 percent when results from samples collected at any time point during the course of the treatment were compared.

The new test had 73 percent sensitivity and 98 percent specificity, with a positive-predictive value of 96 percent (meaning that it correctly predicted a positive result 96 times out of 100) and a negative-predictive value of 85 percent (meaning that it correctly predicted a negative result 85 times out of 100).

The researchers also found that the amount of BRAF V600 mutant cell-free DNA, as detected by the Idylla system, was predictive of overall survival of the patients: In patients with a BRAF-mutant cell-free DNA percentage of 2 or less, overall survival was 10.7 months, compared with 4.4 months in those who had more than 2 percent of BRAF V600 mutations in their samples.

The amount of cell-free DNA present in the plasma by itself was not predictive of survival outcomes.

In patients whose baseline samples were negative for BRAF V600 mutations, the time to treatment failure (TTF) was 13.1 months after treatment with BRAF and/or MEK inhibitors, as opposed to those whose baseline samples were positive for the mutation, in whom the TTF was three months. Patients whose baseline samples were negative for BRAF V600 mutations were 69 percent less likely to see their therapy fail, compared with those whose baseline samples were positive for the mutation.

"Our results suggest that high amounts of BRAF-mutant cell-free DNA before therapy is a negative prognostic biomarker for survival and outcomes of targeted therapy. It was somewhat counterintuitive since one would assume that more BRAF-mutant copies in the circulation would rather predict better outcomes with BRAF-targeted therapies," Janku said. "We also showed that a decrease in the amount of BRAF-mutant cell-free DNA in sequentially collected plasma samples correlates with better treatment outcomes."

As limitations to the study, Janku said that the team investigated only BRAF V600 mutations, which are clinically relevant to a limited number of patients with certain tumor types. Second, the survival and treatment outcomes analyses were retrospective and need to be validated in future prospective studies, he added. Also, the clinical utility of cell-free DNA mutation testing remains to be proven in prospective clinical trials in which therapeutic interventions are tailored on the basis of patients' respective cell-free DNA mutation statuses, Janku cautioned.

More information: F. Janku et al. BRAF Mutation Testing in Cell-Free DNA from the Plasma of Patients with Advanced Cancers Using a Rapid, Automated Molecular Diagnostics System, *Molecular Cancer Therapeutics* (2016). DOI: 10.1158/1535-7163.MCT-15-0712