**Tumor-only Sequencing May Misguide Therapy But Many Labs Omit Matched Control**

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NEW YORK (GenomeWeb) – Researchers at Johns Hopkins University and Personal Genome Diagnostics have found that sequencing both tumor and normal tissue from the same patient is essential for accurately identifying clinically actionable mutations in their tumors.

Despite these findings, for practical and economic reasons many diagnostic laboratories currently only sequence tumor samples, and some do not believe the tumor-normal approach is necessary.

In a study published in *Science Translational Medicine* today, the Hopkins team, led by Victor Velculescu of the Sidney Kimmel Comprehensive Cancer Center, found that three quarters of patients whose tumors they sequenced had alterations in actionable genes, and three percent carried previously undetected cancer predisposition mutations. However, analyzing only the tumor but not matched normal tissue yielded many false-positive alterations that were not specific to the patient's tumor, including in actionable genes.

Tumor-only analyses, they concluded, "may lead to inappropriate administration of cancer therapies with substantial effects on patient safety and healthcare costs."

"Of course we all want precision medicine," Velculescu said in a teleconference to discuss the study's findings. "But one conclusion from these analyses is that we cannot have precision medicine without precision genomics. We can't expect physicians to provide the right therapy to the right patients if we can't obtain accurate results in our diagnostic tests."

However, companies such as Foundation Medicine and many institutional molecular pathology labs performing diagnostic tumor profiling – for the most part, cancer gene panels – currently do not sequence matched normal controls for a variety of reasons, including cost and lack of reimbursement, the need for enhanced consent for germline sequencing, logistical challenges, and a belief that analyzing tumors alone is sufficient, though some, such as Memorial Sloan-Kettering Cancer Center, have started to move to tumor-normal analyses.

"The goal is to use this study to start a conversation in the pathology and oncology world on how we can improve these kinds of tests to more accurately identify this information, and ultimately, do what's right for our patients," Velculescu said.

Thousands of cancer patients in the US, most with advanced disease, have their tumors analyzed by next-generation sequencing each year, and most of these tests do not sequence normal tissue, which can help filter out germline mutations. "We expect this number to increase to potentially one million patients, representing all late-stage cancer patients in the future," Velculescu said.
Physicians use the tumor test results, along with other factors, to decide on the best treatment for their patients, in particular to match them to the growing number of targeted therapies and clinical trials. "Using accurate versions of such tests will be essential to make the appropriate therapeutic decisions and to better understand the effect of new therapies," he said.

Up until now, there had been no systematic studies of how analyses of matched tumor-normal pairs differ from tumor-only analyses in their ability to accurately identify somatic tumor mutations, so Velculescu's team set out to perform such a comparison.

For their retrospective study, the researchers analyzed a total of 815 tumor-normal pairs from 15 tumor types, including brain, breast, colorectal, lung, melanoma, pancreatic, and blood cancers. Tumor tissue included formalin-fixed samples, frozen tissue, cell lines, and patient-derived xenografts, whereas normal samples came from blood, saliva, unaffected tissue surrounding the tumor, and cell lines.

The researchers performed either exome sequencing or targeted sequencing of 111 clinically relevant genes, using Illumina HiSeq and MiSeq instrumentation, and analyzed either the tumor sequence data alone or data from both the tumor and normal sample. This bioinformatic analysis was followed by clinical annotation to identify mutations in actionable genes that were either associated with FDA-approved cancer therapies, therapies described in published prospective clinical studies, or ongoing clinical trials.

Using the tumor-normal approach, the researchers found that about three quarters of patients had potentially actionable alterations. Of those, two thirds had changes associated with current clinical trials, and one third had mutations linked to either established or investigational therapies. This result by itself suggests that "cancer genome analyses are likely to be useful for physicians and patients," Velculescu noted.

The researchers also looked for germline alterations in about 85 cancer predisposition genes and found that about 3 percent of patients carried such mutations, more than half of which were predicted to be pathogenic or likely pathogenic. "This occurred despite the fact that these patients had no known family history of cancer, and these observations suggested that such tests may provide a simple way to identify such individuals and their affected family members," Velculescu said.

The most surprising result came from their comparison of tumor-only and tumor-normal analyses. For that, the researchers reanalyzed tumor data alone from 58 targeted sequencing cases and from 100 exome sequencing cases, representing a range of tumor types.

To filter out germline mutations, they utilized a variety of bioinformatic approaches, including an unmatched normal control, the dbSNP database, and the 1000 Genomes Project database. In the exome cases, "we found that even after filtering of well-known germline alterations, approximately two-thirds of the remaining mutations were part of the patient's normal inherited genetic variation, and not tumor specific — in other words, they were false positives," Velculescu said.

For the targeted sequencing cases, which focused on actionable genes, about a third of the mutations were false positives. But because each patient harbored several such alterations, about half the
patients were affected by the inaccurate results. "One in two patients receiving tumor-only gene panel sequencing is potentially at risk to receive a treatment that may be inappropriate," Velculescu said, which could result in serious side effects, as well as increased costs "from misguided medicines."

In addition to providing false positives, the tumor-only analysis inadvertently removed some actual somatic mutations, because those mutations occurred in germline variant databases, and the analysis failed to distinguish between somatic and inherited cancer predisposition mutations, which could lead to further testing in family members.

Despite the strong argument the Hopkins study makes for tumor-normal sequencing, most cancer diagnostic labs currently sequence only tumors. Among them is Foundation Medicine, which offers two 315-gene tumor sequencing tests: FoundationOne for solid tumors and FoundationOne Heme for hematological cancers and sarcomas. Last year, the company provided almost 24,000 test results to physicians, most of them for FoundationOne.

"The paper is really not relevant to what we do at Foundation Medicine," Vince Miller, the company's chief medical officer, told GenomeWeb, because the firm uses a different bioinformatic approach than the Hopkins and PGD researchers for teasing out true somatic actionable mutations and separating them from the false positives.

Foundation Medicine applies "very sophisticated techniques beyond what's done [in the paper] to call out only those things that are unambiguous drivers of the cancer," and includes only those mutations as actionable in its reports, Miller explained.

The overwhelming majority of the false positive mutations reported in the Hopkins study appear as variants of unknown significance on the last page of Foundation Medicine's report. "We utilize the most stringent criteria for calling what appears on the first page [of the report] and could lead to potential therapeutic intervention," Miller said. "Everything else, even if there is a chance it might evolve to be something actionable, goes onto the last page."

In addition, he said, the company has information on cancer mutations from more than 35,000 samples it has analyzed so far, "so we have significantly more insight into some of these variants than others."

In its collaborations with pharmaceutical companies, Foundation Medicine has also sequenced tumor-normal pairs. And while there are valuable insights to be gained from this approach, its drawbacks — including the increased cost of sequencing, the logistics of acquiring a normal sample, and obtaining consent to analyze it — currently outweigh its benefits. "Certainly, with our approach, [it] does not make sense," he said.

Analyzing a germline sample would require a more complex consent form, for example, as well as genetic counseling, which he said would "quickly overwhelm the system."

Other molecular pathology laboratories agree that tumor-normal sequencing is the optimal approach but don't pursue it for economic reasons. "Most labs are forced to do tumor-only testing because of
the way reimbursement works," John Pfeifer, vice chair of clinical affairs in the Department of Pathology at Washington University School of Medicine, told GenomeWeb. "If we could get paid to do tumor-normal testing, I think we would do it. The main obstacle is reimbursement."

Pfeifer helps oversee the Genomics and Pathology Services (GPS) laboratory at WashU, which sequences more than 1,000 tumor samples per year, the majority from patients with advanced cancers. The lab's current cancer panel sequences 150 genes but only reports results for the 40 genes for which it receives reimbursement.

Another challenge for implementing tumor-normal testing, he said, is the more involved consent required for germline testing.

"Labs are aware that tumor-normal is optimal, as this paper clearly establishes, but understanding that tumor-only is limited, they changed the way they do that testing to mitigate some of those limitations," Pfeifer said.

For example, the GPS laboratory has tweaked its bioinformatic pipeline to alert it to tumor mutations that may in fact be germline cancer predisposition mutations, so clinicians can recommend germline testing to those patients and their families.

Velculescu acknowledged that insurance does not fully cover the cost of the additional sequencing required for including a matched normal control. He said the cost of most tumor sequencing tests is on the order of several thousand dollars, and adding matched normal samples would cost "more" but not twice as much.

Some institutions have already moved toward tumor-normal sequencing after deciding that the benefits do outweigh the challenges. Memorial Sloan-Kettering Cancer Center in New York, for example, sequences tumor and matched normal samples for its MSK-IMPACT assay, a clinical 400-gene panel it launched about a year ago and has performed for almost 4,000 patients so far.

"We thought long and hard about it," Michael Berger, associate director of the MSKCC Center for Molecular Oncology, told GenomeWeb. "There are compelling benefits, and there are big challenges" to offering tumor-normal profiling in clinical setting, but "we still wanted to go ahead and do it because of the benefits," he said.

Tumor-normal sequencing has increased the sensitivity and specificity for calling somatic mutations, he said, and has improved the lab's ability to interpret them. "When you do just the tumor, you are using databases and other information to help you filter out what's likely germline, but you can never be sure in an individual case what is germline and what is somatic," he explained.

For example, Berger and his colleagues have sequenced a number of tumors from patients that had previously had tumor-only testing from Foundation Medicine and other labs, and has found discrepancies between their own and the earlier report, which "are simply due to the fact that the other lab had not used a matched normal and wasn't able to filter out inherited variants," he said.
MSK-IMPACT requires patients to sign a consent form that explains the risk of finding an incidental germline mutation, even though MSKCC currently does not return germline results but only uses the normal sample to filter out inherited variants.

Starting next month, MSKCC plans to start returning germline mutations in cancer susceptibility genes along with the somatic tumor mutations to patients interested in receiving the extra information. That will require additional consent, and patients will need to watch an instructional video prior to undergoing testing. Only patients with a positive germline test result — which Berger said is expected in only a small fraction of patients — will see a genetic counselor.

The logistical challenges of implementing tumor-normal sequencing have been “trickier than I might have expected,” Berger said, for example handling tumor and normal samples arriving from different labs and matching them up for sequencing.

Most of the additional cost that is incurred from sequencing two instead of one sample per patient is currently shouldered by MSKCC and philanthropic resources, he said, because except for a small number of tumor types, it is not reimbursed by insurance companies or Medicare.