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Double Mutations in PIK3CA Gene Increase Patient Sensitivity to Targeted Therapies, Study Finds

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NEW YORK – About 12 to 15 percent of patients with breast cancer have multiple mutations in the PIK3CA gene, a new study has found, and about 95 percent of them are double mutations.

The recently published study found that patients with double or multiple mutations in this gene appear to respond robustly to PI3K inhibitors, suggesting that the multiplicity of mutations could serve as a biomarker for determining which patients will respond best to treatment, and highlighting the importance of looking beyond single-nucleotide variants when trying to identify best responders to cancer treatment.

Activating mutations in PIK3CA are frequent in human breast cancer and have been implicated in other cancers, such as ovarian and urothelial tumors. Previous studies have identified many distinct cancer-associated PIK3CA mutations, including hotspot single-amino acid substitutions, which are considered oncogenic in multiple cancer histologies. In breast cancer, PIK3CA mutations are present in 40 percent of estrogen receptor-positive, HER2-negative primary and metastatic tumors.

The US Food and Drug Administration has approved three PI3K inhibitors for the treatment of lymphoma or leukemia, and only one drug to treat a solid tumor — the alpha-specific PI3K inhibitor alpelisib (Novartis' Piqray) was approved by the FDA in May in combination with fulvestrant for treatment of hormone receptor-positive, HER2-negative breast cancer.

As such, researchers are actively studying PI3 kinase inhibitors in an effort to expand their use to more cancer patients. While conducting clinical trials involving PI3K inhibitors, Memorial Sloan Kettering Cancer Center Medical Oncologist Neil Vasan and his colleagues noted that a small subset of patients had a deep and prolonged clinical benefit from alpelisib. They conducted single-molecule real-time sequencing on the Pacific Biosciences RS II platform to try to tease out the biology driving the exceptional responses in these patients and found they harbored multiple PIK3CA mutations in *cis*, or on the same allele.

In their study, published recently in *Science*, the researchers explained that double PIK3CA mutations are most often clonal, resulting in a single protein with two mutations in the case of *cis* mutations and resulting in two proteins from separate mutations in the case of *trans* mutations.

They explained that these double mutations hyperactivate PI3K signaling, thereby disrupting normal cellular growth and proliferation mechanisms, and driving tumor growth. In turn, tumor cells with these double mutations become reliant on the PI3K pathway for survival, which also makes them great targets for PI3Kα inhibitors and more sensitive to treatment than single-mutation cells.

By contrast, mutations in *trans*, which result in two different proteins, were less sensitive to alpelisib than *cis* mutants, and were no more sensitive to treatment than tumors with a single major mutation. In their experiments, the researchers found that mutations in *trans* don't increase cell signaling and growth proliferation any more than single mutations do.

"Patients with double-PIK3CA-mutant tumors had a longer median [progression-free survival] than did patients with single-mutant tumors or wild-type tumors," the authors wrote. "In model systems, the double mutations hyperactivate PI3K signaling and enhance tumor growth. Preliminary analysis of clinical trial data suggests that breast cancers with double mutations are more responsive to PI3 kinase inhibitors than those with a single mutation."

According to Vasan, double or multiple mutations have been observed in other genes as well, but are often associated with treatment resistance, not sensitivity. They're also quite infrequent.

In contrast, double mutations in PIK3CA cancers are present in about 10 percent to 15 percent of tumors across all histologies, according to Vasan. This includes breast cancer, colorectal cancer, endometrial cancer, and even some rare histologies.

"We see this very consistent percentage [of double mutations] and we see these in primary untreated tumors," he said. "This tells us that [they aren't] simply due to drug resistance — it's due to tumor heterogeneity, and we're seeing it as probably an early event."

Indeed, when the researchers analyzed a publicly available cancer-patient cohort of about 70,754 patients across different histologies, they identified 4,526 PIK3CA-mutant tumors, 576 (13 percent) of which contained multiple PIK3CA mutations. They were also able to recapitulate these findings using a cohort of 28,139 patients from Memorial Sloan Kettering, which was enriched for metastatic tumors across different cancer types. There, they identified 3,740 PIK3CA-mutant tumors, 451 (12 percent) of which contained multiple PIK3CA mutations. In both cohorts, breast, uterine, and colorectal cancers had the greatest number of multiple-PIK3CA-mutant tumors.

"As a field we've been so fixated on single-nucleotide variants that we've lost the bigger picture phenomena. I think that this is one of those bigger picture phenomena that, frankly, could have been discovered probably 10 years ago, but people just weren't asking that question," Vasan said. "This really points to the power of looking beyond single-point mutations and thinking about higher-order interaction and higher-order mutational changes."

Further, the researchers believe that the multiple mutations they've identified constitute a completely new mechanism to turn on an oncogene. Because of that, they're planning to use their findings as a model system to examine and reinvestigate the PI3 kinase.

"This gene was discovered in the 1980s, and it's the most frequently mutated oncogene in all of human cancer," Vasan said. "And, so, we think that because this is a way to turn off that really well-known oncogene, that this is a really wonderful opportunity to re-examine everything we know about the kinase."

The findings may have some clinical implications for how mutations are analyzed in certain cancers. As Vasan noted, the data suggest that the vast majority of multiple PIK3CA mutations in breast cancer are in *cis* on the same allele. Ideally in a clinical scenario, a clinician would confirm that PIK3CA mutations present in a patient are in *cis*, but this is logistically challenging, he said, adding, "What is key here is to use a sequencing platform that sequences across the entire gene."

One examination the researchers are undertaking is a continuation of how the multiple mutations could be used as a biomarker for treatment, especially as PI3 kinase inhibitors are being examined for utility in treating a large range of cancers.

"We hope that this might be a good rationale to start looking at these drugs in double PIK3CA mutants or multiple PIK3CA-mutant patients in other cancers," Vasan said. "[We] would be identifying this as a marker for patients who might respond better to PI3 kinase inhibitors."

It is especially important to precisely home in on the best-responder population since PI3K inhibitors can cause serious toxicities. In approving alpelisib in PIK3C-mutated advanced breast cancer, for example, the FDA told doctors to monitor patients for severe hypersensitivity reactions and warned that patients on the drug have reported severe hyperglycemia. "Figuring out who are the patients who are going to benefit the most from this is really an important question in the field," Vasan said.

The new data could also aid in drug development efforts, he added, by identifying the subgroup of patients who are markedly sensitive to PI3K inhibitors in breast cancer, thereby improving the therapeutic window. Further, these findings could be extended to other clinical trials in this space.

He also noted that the presence of multiple mutations in PIK3CA or other genes could also serve as biomarkers for treatment with other drugs. In their paper, Vasan and his colleagues showed that patients with double mutations in PIK3CA responded well to the drug everolimus (Novartis' Zortress). Everolimus is an mTOR inhibitor rather than a PI3 kinase inhibitor, but PIK3CA is in the PIK3/AKT/mTOR pathway.

"I think that there may be other drugs that these double mutants may be really sensitive to," Vasan said, though more research is needed.

There may even be an application for these findings in other diseases. The researchers looked for double PIK3CA mutations in pediatric cancers but didn't find any. However, Vasan said, there is a well-known syndrome in children called PIK3CA-related overgrowth spectrum disorder, which is due to germline mutations in the PI3 kinase pathway, including germline mutations in PIK3CA. If double or multiple mutations exist in patients with that disease as well, then researchers could test out whether they could be treated with PI3 kinase inhibitors.

There may also be double or multiple mutations in other genes that are more relevant for pediatric cancer, he added.

According to Vasan, this latest study highlights the importance of sequencing the whole gene when trying to unravel complex cancer biology and the reasons underlying treatment response.

The double-mutation phenomena with PIK3CA mutations largely went unnoticed for years, he said, because PIK3CA is a large gene and researchers were looking for hot spot mutations and analyzing certain exons, rather than looking at the whole gene. "This has really opened the possibilities for looking at double mutations in other genes as well," Vasan said. "From a detection point of view, this technology has actually been there for a while — it's just that we were asking different questions."

Vasan and his team are undertaking various basic science and clinical studies to explore this phenomenon in further detail.

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