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Screening Blood Samples for Cancer-driving Mutations More Comprehensive Than Analyzing Traditional Tumor Biopsy

- Sophisticated test of DNA in blood yielded a more informative summary of cancer mutations.
- Testing blood provided a more complete picture than probing tumor samples from the same patient.
- Blood-based summary of mutations could help tailor targeted treatments.
- Test has the potential to become standard part of cancer care.

WASHINGTON, D.C. — Researchers using a tool called BEAMing technology, which can detect cancer-driving gene mutations in patients' blood samples, were able to identify oncogenic mutations associated with distinct responses to therapies used to treat patients with gastrointestinal stromal tumors (GIST), according to a researcher who presented the data at the AACR Annual Meeting 2013, held in Washington D.C., April 6-10.

Data from a subanalysis of the phase III GIST–Regorafenib In Progressive Disease (GRID) trial indicated that this blood-based screening technology may provide physicians with a real-time, comprehensive picture of a patient's tumor mutations, according to George D. Demetri, M.D., director of the Ludwig Center at Dana-Farber Cancer Institute and Harvard Medical School in Boston, Mass.

"Our results show that it is possible to sum the total of all of the heterogeneity in a cancer and get a clear picture of the entire tumor burden, using a simple blood sample," Demetri said.

In this era of targeted cancer therapies, the goal is to focus cancer treatments on a specific molecular target. However, as researchers discover more about cancers and their heterogeneity, they are finding many patients have anywhere from one to dozens of different mutations in their tumors.

"It is a real issue that when you do a biopsy on one tumor, and then biopsy a different tumor in that same patient a few inches away or on the other side of the body, you may get a different answer when you do the molecular analysis," Demetri said. "With this blood test, you get a robust summary statement about all the different mutations present across the different tumors in

the body. I believe this testing technology has promise to become a standard part of care in the next five to 10 years."

Data from the main analysis of the phase III GRID study showed that the molecularly targeted drug regorafenib significantly improved progression-free survival compared with placebo for patients with GIST. The researchers hope these results will ultimately lead to the drug's approval by the U.S. Food and Drug Administration (FDA), according to Demetri. The drug is intended to treat patients with advanced GIST whose disease has failed to be controlled by the only two other FDA-approved therapies for GIST, imatinib and sunitinib (Sutent).

Demetri and colleagues conducted an exploratory analysis on patients in the GRID study to assess GIST genotypes. They isolated DNA from archival tumor tissue, which was then analyzed for mutations in two genes, KIT and PDGFRA, which generate the cancer-driving proteins that are the targets of imatinib, sunitinib and regorafenib. The researchers believed that primary mutations would be detectable using traditional analysis, but that those mutations that developed after treatment with imatinib and sunitinib would not be detectable. They then took blood samples drawn at study entry after failure of both imatinib and sunitinib, and analyzed them for mutations via BEAMing technology.

Mutations in the KIT gene were detected in 60 percent of the blood samples compared with 65 percent of the tumor tissue samples. However, when focusing their analysis on secondary KIT mutations, which are the mutations that drive resistance to targeted therapies like imatinib and sunitinib, the researchers found mutations in 48 percent of blood samples compared with only 12 percent of tissue samples. In addition, nearly half of blood samples in which secondary KIT mutations were found harbored multiple secondary mutations.

Importantly, regorafenib was clinically active compared with placebo in patients with secondary KIT mutations.

According to Demetri, the results show a clear association between the presence of different cancer-driving gene mutations in patients' blood samples and clinical outcomes.

"By using this technology, we hope to develop the most rational drug combinations and better tests to match patients with the most effective therapies going forward," Demetri said.

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Abstract Number: LB-295

Presenter: George D. Demetri, M.D.

Title: Detection of oncogenic kinase mutations in circulating plasma DNA and correlation with clinical benefit in the phase III GRID study of regorafenib vs placebo in TKI-refractory metastatic GIST

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Background: GRID is a phase III study for patients with advanced gastrointestinal stromal tumors (GIST) following failure of imatinib (I) and sunitinib (S) who were randomized to receive either the multikinase inhibitor regorafenib (R) or placebo (P). R demonstrated a highly significant improvement in progression-free survival compared with P (HR 0.27, $p<0.0001$). A preplanned retrospective biomarker analysis was conducted to assess GIST genotypes in GRID patients and to explore the possible impact of different driver oncogene mutations on clinical outcomes

Methods: DNA was isolated from archival tumor tissue and analyzed for KIT mutations via Sanger sequencing. The expectation was that primary KIT mutations would be detectable in archival tissue but that secondary KIT mutations may be undetectable in tissues obtained before treatment with I or S. To overcome this potential limitation, plasma samples drawn at GRID study entry, post I and S failure, were used as a source of circulating DNA for evaluation of GIST oncogenic mutations (KIT, PDGFRA, BRAF) via BEAMing technology.

Results: KIT mutations were detected in 83 of 138 (60%) plasma samples and 64 of 99 (65%) tumor tissue samples analyzed. Primary KIT exon 11 and 9 mutations were identified in approximately 42% and 18% of the tissue samples, respectively. The frequency of the canonical exon 9 mutations was similar for plasma and tissue samples, showing consistency between mutation-detection technologies. With limitations of tumor-based assays, a lower incidence of secondary KIT resistance mutations was detected in patient-matched archival tumor tissue compared with plasma samples: resistance mutations were detected in 12% of tissue samples vs 48% of plasma samples. Most (76%) secondary KIT mutations detected in plasma DNA were located in the KIT activation loop encoding structural alterations known to mediate resistance to I and S. Nearly half of the plasma samples in which secondary KIT mutations were identified harbored multiple secondary mutations, consistent with the results of previous studies on fresh tumor biopsies taken following resistance to both I and S. R was clinically active compared with P in all KIT mutational subgroups evaluated (HR 0.27 in patients with KIT exon 9 mutations; HR 0.25 in patients with secondary KIT mutations identified via plasma DNA).

Conclusions: In GIST patients from the GRID trial, driver oncogenic mutations and secondary oncogenic mutations leading to I and S resistance are readily detectable via BEAMing of circulating DNA from plasma. BEAMing may provide a real-time assessment of tumor genotype in GIST and other cancers using blood-derived circulating DNA, that may be more comprehensive than tumor sampling. GIST patients with a wide spectrum of primary and secondary mutations in oncogenic kinases benefit from treatment with R.