BEAMing Sheds Light on Drug Resistance

Josh Lauring and Ben Ho Park

Targeted therapies against somatically altered genes are currently used for the treatment of many human cancers. The nascent technology known as BEAMing has the potential to increase the clinical utility of these agents because it allows for the detection of cancer mutations in peripheral blood, providing a rapid assessment of tumor mutation status. Clin Cancer Res; 17(24); 1–3. ©2011 AACR.

In this issue of Clinical Cancer Research, Taniguchi and colleagues (1) describe the use of a novel technology called BEAMing (for Beads, Emulsification, Amplification, and Magnetics) to query for a common second-site epidermal growth factor receptor (EGFR) mutation (T790M) that confers resistance to small-molecule EGFR inhibitors in patients with lung cancer, using blood as the source for mutant DNA detection. As background, somatically mutated or altered genes have led to effective targeted therapies and improved biomarkers for clinical decision-making. As the use of such targeted therapies becomes more widespread, there will be a great need for companion diagnostic technologies that can reliably and quickly identify patients with cancers that harbor the mutation/genetic alteration to be targeted. Particularly challenging is the fact that the ascertainment of mutational status in solid tumors often depends on biopsies of metastatic sites, followed by conventional genotyping assays with limited sensitivity. Such biopsies can be difficult to obtain, even in a research setting, resulting in a great impetus for developing sensitive, noninvasive diagnostic tools to assay for the mutational status of a tumor and molecular predictors of response.

BEAMing is one such technology. It relies on single-molecule PCR at a massively parallel scale, similar to next-generation DNA sequencing technologies (refs. 2 and 3; Fig. 1). The mechanics of BEAMing start with amplification of a predetermined locus of interest by conventional PCR. Next, the PCR product is added to hundreds of thousands of oligonucleotide-coupled beads in oil, and an emulsion is created such that most of the beads will bind only a single DNA molecule. A second round of PCR is then performed. After de-emulsification and magnetic capture are completed, single-base primer extension or hybridization with mutant-specific probes is performed with different fluorescently labeled nucleotides for either wild-type or mutant sequences. Finally, flow cytometric analysis of hundreds of thousands of beads allows for the detection and quantification of wild-type or mutant alleles. Because BEAMing is a digital PCR technique that analyzes one allele at a time, it is highly sensitive for the detection of rare mutant alleles within a large population of wild-type alleles. This is the exact molecular environment found in circulating plasma of cancer patients, i.e., rare circulating tumor DNA (ctDNA) intermixed among a vast pool of noncancerous DNA.

Taniguchi and colleagues (1) applied the BEAMing technology for the first time to the analysis of EGFR mutations in non–small cell lung cancer. Activating mutations in EGFR are found in ~10% of Caucasian patients with non–small cell lung cancer and up to 50% of Asian patients, and they are enriched in females, never-smokers, and patients with adenocarcinomas. Such patients are now routinely offered therapy with the small-molecule EGFR tyrosine kinase inhibitors (EGFR-TKI) gefitinib and erlotinib. Unfortunately, resistance to these agents universally occurs. In 50% of cases, resistance is associated with the emergence of a second-site gatekeeper mutation in EGFR: T790M (4, 5). The authors studied two cohorts of patients with stage III or IV non–small cell lung cancer whose diagnostic biopsy specimens revealed activating EGFR mutations. Cohort 1 had progressive disease after treatment with an EGFR-TKI, and cohort 2 had never received an EGFR-TKI. Overall, BEAMing had a sensitivity of 72.7% for detecting the known activating EGFR mutation in plasma ctDNA collected at a single time point. In the progressive disease cohort, a T790M resistance mutation was identified in 43.5% of patients, a value near the predicted 50%. This is the first use of BEAMing to detect a drug-resistance mutation, although other technologies were used for that purpose in previous studies (6–9). Because BEAMing is both qualitative and quantitative, Taniguchi and colleagues were able to calculate the ratio of T790M mutations to the original activating EGFR mutations for each patient, with ratios ranging from 13.3% to 94%. One would predict that when this ratio
numerous studies involving large populations of patients need to be answered in further depth. This will require the need for adjuvant systemic therapies. However, many questions will also arise. For example, it is currently unclear whether a low level of detectable ctDNA in plasma has clinical meaning or usefulness. In a small number of patients with colon cancer who were studied retrospectively, detectable mutant DNA predicted eventual overt disease progression (13); however, it is clear that more studies are needed. Likewise, in the study by Taniguchi and colleagues, T790M resistance mutations were found in patients with progressive disease, but we do not know at what quantitative level such mutations have clinical significance.

Studies such as that of Taniguchi and colleagues are important steps in the application of new genotyping technologies to clinical cancer care. However, this approach entails some notable limitations and caveats. As the authors note, theirs was not a prospective study, and they did not analyze plasma ctDNA at serial time points. It is not known whether any of the patients who had T790M resistance mutations after EGFR-TKI treatment harbored such mutations at low levels in their primary tumors before treatment, as was previously described (11, 12). To address this issue in future studies, investigators could also use BEAMing on the primary tumor biopsy or surgical specimen because its high sensitivity theoretically would enable detection of this presumably minute drug-resistant population. This would give a clearer picture of the potential preexisting tumor heterogeneity that may be below the current level of detection with conventional techniques, and it may also give insight into the sensitivity of BEAMing ctDNA for detecting microscopic amounts of tumor. If, for example, a T790M population of cancer cells is present at <1% in the primary tumor at the time of diagnosis/surgery, would this minority population be detectable by BEAMing of plasma?

Other potential applications of BEAMing include noninvasive diagnosis, screening, and detection of micrometastatic disease postoperatively to aid decisions regarding the need for adjuvant systemic therapies. However, many questions need to be answered in further depth. This will require numerous studies involving large populations of patients across different tumor types treated with a full range of therapies. In addition, such studies will necessitate the analysis of BEAMing using serial time points to better elucidate the factors that influence the generation and stability of ctDNA and to fully understand the sensitivity and specificity of BEAMing as related to a given tumor type and response to specific classes of therapies. Other questions will also arise. For example, it is currently unclear whether a low level of detectable ctDNA in plasma has clinical meaning or usefulness. In a small number of patients with colon cancer who were studied retrospectively, detectable mutant DNA predicted eventual overt disease progression (13); however, it is clear that more studies are needed. Likewise, in the study by Taniguchi and colleagues, T790M resistance mutations were found in patients with progressive disease, but we do not know at what quantitative level such mutations have clinical significance.

Thus, perhaps the most powerful utility of BEAMing is its ability to quantitate the amount of cancer-specific ctDNA. BEAMing has the potential to enable investigators to assess tumor burden and tumor heterogeneity with great sensitivity and to address important questions with greater precision. Is a patient with no detectable ctDNA after surgery cured? Can we rapidly ascertain whether a patient is benefiting from a chosen therapy? Does early detection of resistance mutations allow for meaningful therapeutic interventions? These questions will undoubtedly be answered as BEAMing and other technologies mature. Ultimately, the ability to detect microscopic amounts of tumor burden will better inform clinicians and patients, and allow for a tailored approach for the treatment of human cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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