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BEAMing Up Personalized Medicine: Mutation Detection In Blood

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SUMMARY

BEAMing is a feasible, accurate and sensitive method for detection of *PIK3CA* mutations in circulating tumor DNA in blood. Mutation status of *PIK3CA* may change between primary tumor and recurrence. The results suggest a new approach for non-invasive determination of current mutation status in patients with metastatic disease.

In this issue of Clinical Cancer Research, Higgins et al. report the feasibility and accuracy of *PIK3CA* mutation detection using free circulating tumor DNA (ctDNA) from peripheral blood samples in a prospective study of metastatic breast cancer patients (1).

The study uses a recently described mutation detection assay called BEAMing (Beads, Emulsification, Amplification, and Magnetics, Figure 1), reported to be sensitive and capable of detecting mutations present in 1 in 10, 000 DNA molecules (2–4). Higgins and co-authors cite 100% agreement in *PIK3CA* mutation status determined by BEAMing when compared to standard sequencing performed on the same tumor samples. More important clinically, there was 100% concordance between mutations detected in paired tumor samples and plasma ctDNA when the samples are obtained from the patient concurrently. The technical feasibility of BEAMing on blood ctDNA is supported by the 100% success in determining the presence or absence of a *PIK3CA* mutation in all patients enrolled in a prospective study. In contrast, only 85% of the patients in this study had adequate and available primary tumor blocks for mutation testing.

The number of new targeted anti-cancer drugs under development and entering into human trials has grown dramatically, and many of these agents are specifically aimed at gene products that bear activating mutations(5). This approach has led to a number of recent successes in the treatment solid tumors including drugs targeting mutant B-raf in melanoma, mutant EGFR or translocation-activated ALK in non-small cell lung cancer, to name a few. To ensure new agents are tested appropriately in the right population and any potential efficacy is recognized, many early phase trials attempt to enrich for patients with tumors containing the "presumed" target of the drug being tested. Caution should be taken when deciding the appropriate time during drug development to limit trial entry on the basis of a companion tumor biomarker, including results of a tumor genotype test. In the absence of strong pre-clinical data showing a tight correlation between a particular biomarker and drug sensitivity, trial eligibility should not be restricted too early (6). For instance, in early phase

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trials of inhibitors of the PIK3CA/mTOR pathway, responses are observed in patients both with and without detected mutations in the presumed target (7). For drugs with a validated role against a targeted mutation, there is need for efficient and cost-effective mutational analysis that informs treatment selection. Many cancer centers are developing gene mutation screening approaches that can be applied to archived FFPE tumor samples (8,9). These centers will undoubtedly encounter the logistical nightmare of identifying which tissue block is most appropriate for tumor DNA analysis, locating the blocks in the pathology archives, transferring the blocks to the correct laboratory for nucleic acid extraction and reporting out mutational analysis in a way that can be used clinically. When primary tumor blocks cannot be located or tumor content is not adequate, an invasive biopsy procedure may be required to obtain tissue from a patient to determine if they are eligible for a particular trial or treatment.

There are potential benefits that BEAMing ctDNA may provide that overcome some of these issues. Obtaining a blood sample is less invasive and risky than biopsy of metastatic lesions. Patients may be monitored for changes in mutation status or mutation burden over time by serial testing of blood during the course of treatment. In the study by Higgins et al, the authors find a difference in *PIK3CA* mutation status, in both directions, between the archived primary tumor and prospectively collected ctDNA in patients with recurrent metastatic disease in up to 27.5% of the cases. All of the changes in PIK3CA mutation status were seen when the recurrence was more than 3 years after the primary diagnosis. Thus, mutation status in the archived tumor sample may not reflect current mutation status in a patient with recurrent metastatic disease, especially those with a long disease-free interval. The current study reports a non-invasive approach to assess mutation status at the time of tumor recurrence.

With tumor genome sequencing, we are beginning to understand the depth and breadth of intratumor heterogeneity, both within the primary tumor, between primary tumors and distant metastases, and between different metastatic sites (10). Higgins and co-authors report two patients in whom two different PIK3CA mutations were identified in ctDNA. This finding suggests “convergent evolution”, where different tumor subclones acquire different mutations in the same presumably “driver” gene. A recent study sequenced multiple areas of a renal cell carcinoma and several metastatic sites. The investigators identified three different mutations in the gene SETD2 present in different regions of the tumor (11). The co-occurrence of multiple PIK3CA mutations in a breast cancer has also been reported (12). BEAMing mutation detection in circulating free tumor DNA may overcome issues of tumor heterogeneity by detection of rare mutations in minor cancer subclones, or in disparate subclones from different metastatic sites, that might not be identified by sequencing from a single site of disease. However, it is not clear how representative circulating tumor DNA will be of the various subclones of malignancy present throughout the body. Some subclones may release tumor DNA more readily than others. For instance, ctDNA might be more representative of bone metastasis compared to brain metastasis. Answers to these questions will require future clinical trials.

The BEAMing assay is not full gene or even full exon sequencing and will not identify all mutations in a particular gene. The assay is designed to detect specific commonly observed mutations in “hot spot” locations. For *PIK3CA* , such hot spot mutations account for around 80% of all reported mutations. The current study only tested for four of the most common hot spot mutations in PIK3CA. Therefore, a negative result for a BEAMing test cannot be interpreted as an absence of mutation in the gene. This is a feature that is shared with most of the other currently available mutation detection methods being used for clinical mutation screening. The methodologies we will use in the future for cancer mutation assessment will depend on such factors as cost, breadth of mutation detection, specificity and sensitivity.

This study of the BEAMing technology suggests it may be a useful method for sensitive but limited mutation determination from a blood sample. Additional clinical trials are required to confirm the positive and negative value of a BEAMing ctDNA mutation result for prediction of treatment response.

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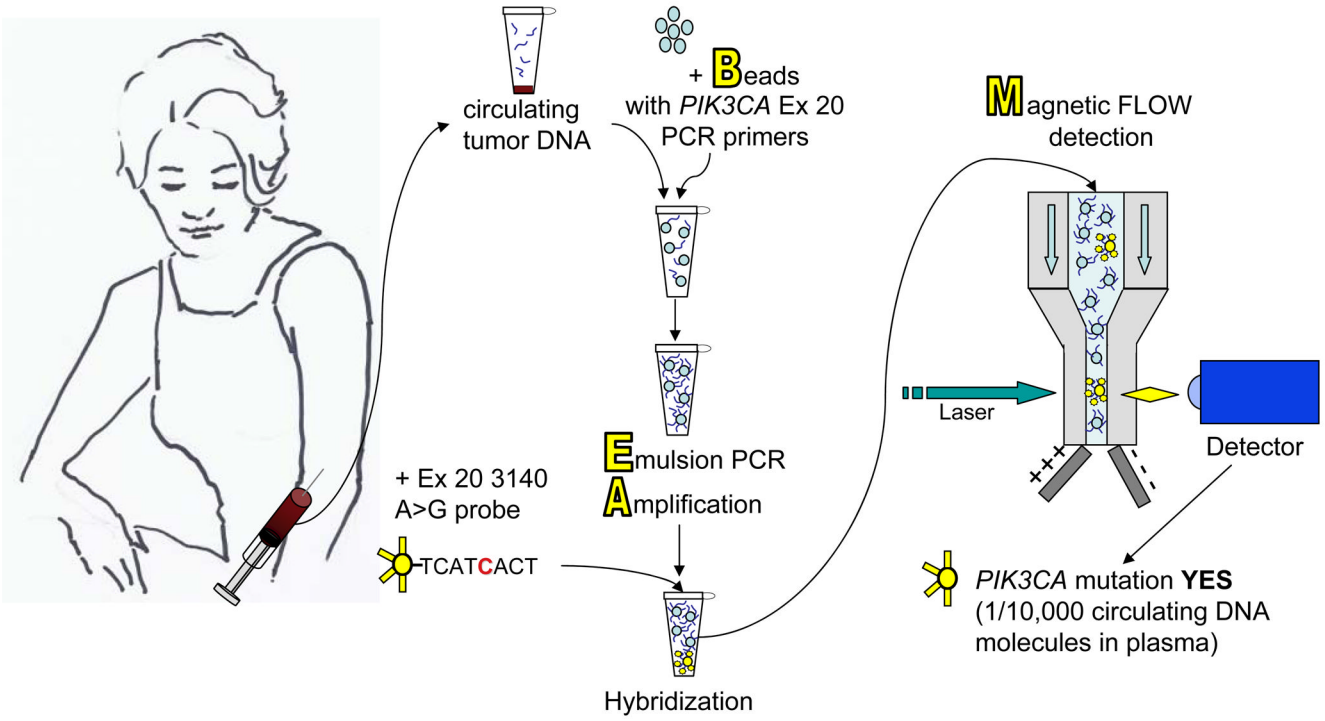

Figure 1.

Mutation detection from blood by BEAMing. Blood samples from patients with metastatic cancer contain free circulating tumor DNA (ctDNA) released into the blood stream from tumor growing at primary or metastatic sites. Specific point mutations in targetable genes of interest such as PIK3CA can be detected in ctDNA using BEAMing technology. Circulating tumor DNA molecules are loaded onto magnetic *B*eads coated with specific polymerase chain reaction primers for the gene of interest. Polymerase chain reaction is performed on the beads in an oil and water *E*mulsion (Emulsion PCR)to *A*mplify the DNA. Fluorescent-tagged probes specific for either the wild type sequence or for particular common point mutations are added and hybridize to the amplified DNA. *M*agnetic flow cytometry of the beads is performed to detect the fluorescent tag and quantify the number of beads containing mutated DNA. The results suggest BEAMing ctDNA in blood plasma is a sensitive and accurate method for relatively non-invasive assessment of current mutation status in patients with metastatic disease.