Letter to the Editor

Clinical Cancer Research

# Detecting Plasma Tumor DNA in Early-Stage Breast Cancer—Reply

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We appreciate the comments of Kodahl and colleagues. However, there are several notable differences between their study and ours (1), which we believe explain the significant dissimilarities in the detection of circulating tumor DNA (ctDNA). Kodahl and colleagues suggest the majority of our positive samples were due to technical mutations from PCR infidelity and preamplification. This suggestion is not consistent with the specificity of our results. In our preop evaluation, we queried all samples for both *PIK3CA* mutations and did not detect any false positive results. Moreover, in our postop samples, we found mutations in only 5 of 10 patients, though in theory all 10 could have had a mutation. Although preamplification can lead to contamination and artifacts, we and others take great care in running positive and multiple negative controls (normal human DNA and water) with every test run to ensure accurate results. We also have four separate physical areas for our work flow to further reduce cross contamination. We and others have found this that greatly mitigates contamination issues inherent to this ultrasensitive technology. Finally, a recent report by Turner and colleagues examining the plasma of early stage breast cancer patients with digital PCR obtained results similar to ours (2).

The most conspicuous difference between the two studies is Kodahl and colleagues' use of circulating serum tumor DNA (stDNA) rather than plasma tumor DNA (ptDNA). To obtain optimal DNA integrity, plasma should be separated from whole blood within 1 to 2 hours after phlebotomy. There are now numerous published studies that demonstrate plasma is a superior analyte versus serum (reviewed in ref. 3) and why we prefer using the "ptDNA" nomenclature. Although some studies have suggested that the total amount of circulating DNA in serum is greater than plasma, most investigators now believe this to be due to lysis of lymphocytes leading to release of normal genomic DNA into the circulation. In our hands, the release of DNA from lymphocytes can increase genome equivalents of a given locus by an order of magnitude. This greatly impedes the sensitivity of the assay since a 0.01% fractional abundance would now artificially be 0.001%. This is akin to requiring a significant more read depth for next-generation sequencing of rare mutation detection. To this point, a recent study by Oshiro and colleagues also demonstrated greatly reduced sensitivity with the use of stDNA for *PIK3CA* mutation detection in early stage breast cancer patients (4).

In sum, we believe that our results are not due to technical mutations and that they support the use of digital PCR to detect ptDNA in early stage breast cancers. We feel the studies by Oshiro and colleagues and Kodahl and colleagues provide further evidence that great care and caution should be taken in using the highest quality analyte when performing ctDNA analyses.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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