Letter to the Editor

Clinical Cancer Research

Detecting Plasma Tumor DNA in Early-Stage Breast Cancer—Letter

Annette R. Kodahl[1,2], Niels Pallisgaard[3], Anne Marie B. Jylling[4], Søren Cold[1], Ann S. Knoop[5], and Henrik J. Ditzel[1,6]

Beaver and colleagues (1) reported that the detection of mutant *PIK3CA* circulating tumor DNA (ctDNA) in early-stage breast cancer patients before and after surgery could improve selection of systemic postsurgical therapies. They reported that 14 of 15 patients with *PIK3CA* mutations in the primary breast tumor had the same mutations detected in presurgical plasma. Further, 5 of 10 patients with presurgical *PIK3CA* mutation–positive ctDNA also showed detectable *PIK3CA* ctDNA postsurgery. We analyzed primary tumors of 162 patients with early-stage breast cancer by ddPCR for the four most common *PIK3CA* mutations (E545K, E542K, H1047R, and H1047L), similar to those analyzed by Beaver and colleagues, and detected mutations in 56 of 162 (34.6%). Subsequently, pre- ($n = 8$) and postsurgery ($n = 56$) serum from the patients with a *PIK3CA*-mutant tumor was analyzed by droplet digital PCR (ddPCR) following a preamplification step (12 cycles). Surprisingly, *PIK3CA*-mutant DNA was detected in no presurgery and only one postsurgery serum sample from a patient with no available presurgery serum. Because these findings contrasted with those of Beaver and colleagues, we analyzed the methodological setup of both ddPCR assays. Beaver and colleagues used nonamplified genomic DNA (gDNA) to determine the lower detection limit and concluded that their assay could detect 1 mutant in 10,000 wild-type molecules, i.e., a fractional abundance (FA) $\geq$0.01%. The FA of mutant *PIK3CA* DNA in the positive plasma samples of Beaver and colleagues ranged from 0.01% to 0.07%, with only two samples exhibiting higher FA of 0.12% and 2.99%, respectively. However, these results were based on preamplified plasma DNA, and we hypothesized that the "positive" plasma samples resulted from technical mutations during the preamplification step due to limited proofreading of the polymerases. This was clearly confirmed in our analysis of nonamplified gDNA and preamplified gDNA (22 cycles) from 5 healthy donors (4 replicates each) using the same preamplification primers, polymerases, ddPCR primers, and setup as Beaver and colleagues. The average FA of mutant DNA for the E545K primers was 0.0024% in the nonamplified samples, but 0.0178% in preamplified samples from the same donors. Similar results were obtained using primers for H1047R, with an average FA in the nonamplified samples of 0.0162% and 0.0674% in the preamplified samples. Our results strongly suggest that technical mutations yielded the "positive" samples in the study by Beaver and colleagues, with exception of two presurgery plasma samples with FAs of 0.12% and 2.99%, and question their conclusion. The use of ctDNA as a potential biomarker in early-stage breast cancer is still uncertain.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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[1]Department of Oncology, Odense University Hospital, Odense, Denmark. [2]Institute of Clinical Research, University of Southern Denmark, Odense, Denmark. [3]Department of Biochemistry, Vejle Hospital, Vejle, Denmark. [4]Department of Clinical Pathology, Odense University Hospital, Odense, Denmark. [5]Department of Oncology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark. [6]Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark.

Corresponding Authors: Annette R. Kodahl, Odense University Hospital, Sdr. Boulevard 29, DK-5000 Odense C, Denmark. Phone: 45-60138716; Fax: 45-66135477; E-mail: annette.kodahl@rsyd.dk; or Henrik J. Ditzel, Institute of Molecular Medicine, University of Southern Denmark, J. B. Winslowsvej 25, DK-5000 Odense C, Denmark. Phone: 45-60113781; E-mail: hditzel@health.sdu.dk

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Reference

1. Beaver JA, Jelovac D, Balukrishna S, Cochran RL, Croessmann S, Zabransky DJ, et al. Detection of cancer DNA in plasma of patients with early-stage breast cancer. Clin Cancer Res 2014;20:2643–50.