Editorial

Circulating free DNA in the management of breast cancer

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Abstract: Circulating cell-free DNA (cfDNA) is now under investigation as a "liquid biopsy" in the real time management of cancer. In *The New England Journal of Medicine* Dawson *et al.* reported a proof of concept investigation of tumour specific alterations in cfDNA and demonstrate that this fraction termed "circulating tumour DNA" (ctDNA) shows greater correlation with changes in tumour burden than two other circulating biomarkers (CA 15-3 and circulating tumor cell counts) in individuals with metastatic breast cancer receiving therapy.

Keywords: Breast cancer; biomarkers; circulating tumour DNA; circulating tumour cells (CTCs)

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Breast cancer is the most common cancer in women worldwide. Although metastatic breast cancer is currently incurable, there are a number of endocrine, cytotoxic and biological therapies that benefit some patients though determination of tumor burden remains problematic. Treatment response can be assessed by imaging, the serum biomarker cancer antigen 15-3 (CA 15-3) and the FDA approved CellSearch system, which enumerates circulating tumour cells (CTCs). An increase in CA 15-3 levels or a CTC count of ≥5 cells per 7.5 mL blood is associated with poorer prognosis; however, both methods have a sensitivity of only 60% to 70% (1-3) and imaging often fails to rapidly detect changes in tumour burden. There is a need for improved biomarkers with greater sensitivity and specificity to monitor treatment response, help determine the benefit of new and emerging therapies and provide more accurate means for determining prognosis.

Circulating free DNA (cfDNA) first described over 60 years ago (4), has potential as a "liquid biopsy" to monitor cancer in real time. Elevated levels of cfDNA are observed in cancer, particularly in advanced disease, but have also been suggested for the diagnosis of breast (5) and other cancers (6). However, detection of tumour specific alterations in cfDNA [e.g., mutations, loss of heterozygosity (LOH), hypermethylation] has the potential to provide tumour specific markers and has been more widely investigated [reviewed in (7)]. Some studies have suggested that the proportion of cfDNA, which carries tumour specific alterations termed ctDNA, is variable and represents only a small fraction of total cfDNA (8,9). However, there are currently no consensus protocols either for isolation of cfDNA, or for enrichment of this ctDNA, suggested to be largely associated with low molecular weight fractions (10,11), making comparison between different studies and data difficult.

Our group was the first to report emergence of HER2 amplification in cfDNA in patients who were HER2 negative at diagnosis through analysis of cfDNA (12). We also demonstrated whole genome wide analysis of cfDNA using an SNP 6.0 array and reported common tumour-associated copy number variation in cfDNA of 65 breast cancer patients (13). Rapid developments in next generation sequencing have enabled similar genome-wide analysis of cfDNA (14,15) and other recent studies have shown the emergence of acquired resistance to targeted therapies (16,17) through analysis of cfDNA.

The data presented by Dawson *et al.* (18) extend this developing field through the combined use of digital PCR

and targeted deep sequencing (TDS) to assess serial blood samples in 52 patients with metastatic breast cancer while undergoing treatment. Using a combination of a candidate gene approach to screen for somatic mutations in *PIK3CA* and *TP53* and whole genome paired-end sequencing of primary tumour tissue, they identified point mutations and patient specific somatic structural variants, i.e., ctDNA, in 30 of the 52 patients. Results demonstrated a sensitivity of mutant allele detection of 0.1% for digital PCR and 0.14% for TDS. In some patients ctDNA results were discordant with the primary tumour, suggesting tumour evolution and/or emergence of an original minor clone. One critical question to emerge from these data, which will require follow up in new clinical trials, is what level of alteration/mutation in a key driver is sufficient to initiate a switch in disease management? For those patients in whom mutations could not be observed, probably as a result of lack of analysis of all genes, results were inconclusive; the test appears to work well on finding mutations in both the solid tumor and pairing this with the blood sample. Overall, using a modified bootstrap approach Dawson *et al.* demonstrated improved sensitivity of ctDNA over both CA 15-3 (85% *vs.* 59%) and CTCs (90% *vs.* 67%). In 20 of the patients, for whom blood data was available for three or more time points they showed that fluctuations in ctDNA correlated with treatment response observed by imaging. Similar results were also seen for CTC counts and CA 15-3 but again with less sensitivity. Finally, using a Cox proportional hazards model increasing ctDNA levels and CTC counts were both correlated with poorer overall survival ($P<0.0001$ and $P<0.03$, respectively).

Much work is still to be done before sensitive mutation analysis of ctDNA, whether by TDS or another method, becomes routine in the clinic, but rapidly accumulating data presented by Dawson *et al.* and others (12-14,16-18) show the potential of this approach for sensitive and specific serial sampling to provide a "liquid biopsy" of cancer in real time. As our understanding of the genetic heterogeneity of breast and other cancers develops, this will allow for intelligent design of custom assays to survey ctDNA in cfDNA. Alongside this rapidly advancing technology will likely improve in terms of throughput, sensitivity, cost and ease of use. In the not too distant future, ctDNA/cfDNA analysis has the potential to revolutionise the management of common cancers and as we move into the era of personalised medicine but these tests require standardization so they are reliable and reproducible in the same manner as CTC tests. Despite low numbers of patients in this study, in aggregate it appears that ctDNA is reliable in detecting tumor burden but their role in the clinic will take years to establish, so incorporation into prospective clinical trials would be optimal. Whether such tests can replace or adjunct traditional imaging is unclear, and ctDNA/cfDNA is likely to be as heterogenous as the original tumors themselves.

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