Clinical utility of circulating tumor cells in non-small-cell lung carcinoma: are we there yet?

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Over 140 years ago, the discovery was made that tumor cells morphologically resembling those in the primary tumor of a patient can be found in the patients' circulation (1). The notion that these cells in the circulation are probably shed from the patient's primary tumor was confirmed upon advancement in cytogenetic techniques that demonstrated that these circulatory cells can also carry genetic abnormalities detected in primary tumors (2). However, it is only recently that these circulating tumor cells (CTCs) have started being investigated for their possible use as a marker for diagnosis or prognosis in cancer patients. The progress has been slow because CTCs are extremely rare. In addition, like primary tumors or metastatic spreads, from which they originate, these cells can be extremely heterogeneous. The development of new technologies for CTC isolation over the last decade, spurred great interest in CTCs, but the variability of these methods and the lack of reproducibility across different laboratories made data interpretation difficult (3). So far, the only validated assay, considered to produce reliable and reproducible CTC enumeration across clinical studies, is the CellSearch system (Veridex, Raritan, NJ). This technology received the U.S. Food and Drug Administration (FDA) approval to be used as an aid to monitor patients with metastatic breast, colon, and prostate cancer, due to demonstration of prognostic significance of CTCs in these indications, where changes in CTC numbers upon therapy have also been shown to be a predictive biomarker.

More recently, prognostic significance of CTCs has begun to be elucidated also in non-small-cell lung carcinoma (NSCLC). Using CellSearch for CTC enumeration, Krebs and colleagues have demonstrated that metastatic NSCLC patients with fewer number of CTCs before chemotherapy (less than 5 per 7.5 mL of blood) had better overall survival compared to patients with CTCs above this cut off. Further evidence of prognostic significance of CTC number came from a study of resectable NSCLC, showing that the increased CTC number was associated with shorter disease free survival (4). Another group has addressed the utility of CTC numbers in monitoring the efficacy of standard therapy suggesting that decrease in CTCs may be indicative of early response to erlotinib and ertuzumab therapy in this study of relapse/refractory NSCLC (5). This study is an example of what is considered to be one of the most exciting clinical utilities of CTCs, also termed "liquid biopsy" that uses changes in the number of peripheral blood CTCs for real-time monitoring of response to therapy, thereby substituting the need for painful and often inaccessible biopsies of primary tumor. Clinical utility of such monitoring of therapeutic effectiveness lies in the opportunity to rapidly terminate ineffective treatment in order to prevent side effects and unnecessary costs (6), although such utility remains to be demonstrated for NSCLC. In addition, the good correlation between CTC enumeration and imaging data (obtained by PET and CT scans) in this study (5), opens a possible future clinical utility of CTCs in NSCLC as a prognostic factor that can be complementary to conventional imaging or even superior to it. CTC counts may also be used to monitor cancer recurrence/progression by substituting for a CT scan, a less patient-friendly option. However, further studies are needed to explore these possibilities and validate such clinical utilities for NSCLC.

In addition to CTC enumeration, characterization of these cells can provide invaluable insight into tumor properties and the complex biology of tumor cell dissemination. A recent NSCLC study provided evidence that phenotypic analyses of CTCs are feasible, showing that cell surface expression of tyrosine kinases in CTC is possible to assess. Although the expression of EGFR on CTCs in this study was not correlated with clinical benefit (5), these results suggested that EGFR expression on the surface of CTCs may determine whether CTCs can be used as predictive biomarkers in situations where EGFR expression levels would be

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predictive of therapeutic response. The sensitivity of mutational assays appears to be low: only one of the eight EGFR mutations identified by analyses of primary tumor was detected in CTCs, limiting the utility of such assays of monitoring a response to therapy by decrease in mutation-bearing CTCs (5). Thus, comprehensive molecular profiling of CTCs remains hampered by low yield of CTCs obtained by most of the current CTC isolation platforms requiring sophistication of current cytogenetic and molecular assays for DNA, RNA and protein analyses in CTCs in NSCLC. While chromosomal changes and copy number changes analyzed by fluorescence in situ hybridization (FISH) and array comparative genomic hybridization (aCGH), respectively, have been shown to be feasible in CTCs from prostate cancer patients (6,7), these techniques can not be applied in NSCLC due to the very low number of CTCs isolated in NSCLC (generally less than 10 per mL).

This led to exploration of different approaches for isolation of CTCs in NSCLC to obtain better yields. Katz and colleagues (8) used FISH probes to isolate circulating cells that contain genetic abnormalities similar to those detected in NSCLC tumor. The number of isolated abnormal circulatory cells was several orders of magnitude larger than that routinely reported for CTCs in NSCLC (which are based on detection of only epithelial cells), thus these authors proposed that the use of this approach may have a role in the management of patients with NSCLC (8), pending the demonstration that these abnormal cells indeed represent CTCs. Genetic characterization of CTCs may lead to identification of future therapeutic targets and provide insight into genetic changes required for metastatic progression, therefore implementation of next-generation sequencing to the analyses of CTCs in NSCLC would represent an important next step. Similarly, the development of new techniques allowing for single cell analyses of CTCs on RNA and protein level will provide further comprehensive insight into CTC biology, improving our understanding of tumor dissemination and providing additional therapeutic targets.

One of the proposed explanations for the low yield of CTCs isolated by current detection methods in NSCLC is that a significant proportion of CTCs may have undergone epithelium-to-mesenchyme transition (EMT) and express EMT phenotype, which includes downregulation of epithelial cell surface markers that current detection methods rely upon. In fact, Katz and colleagues proposed that their method for isolation of circulating cells bearing genetic abnormalities would have included the isolation of CTCs with EMT phenotype and may explain a much larger yield of circulating cells obtained by their approach (9). However, the validity of clinical use of EMT circulating cells remains to be demonstrated, awaiting the development of a reliable method for isolation of circulating EMT cells that would yield acceptable specificity and sensitivity and consistency across different studies. Once this is achieved, their association with clinical parameters will need to be shown to confirm clinical utility of CTCs with EMT phenotype in NSCLC. Currently, significant proportion of patients with metastatic disease are negative for CTCs, thus it remains plausible that future isolation methods that would include detection of CTCs with EMT phenotype, may lead to a larger proportion of metastatic patients with detectable CTCs, as this CTC phenotype is missed by current detection platforms.

Finally, another characteristic of CTCs in NSCLC and other solid tumors, is that their highest numbers in circulation are observed in metastatic disease, limiting their clinical utility when cancer is diagnosed at an earlier stage. Thus far only a single NSCLC study, which used the detection of all circulating abnormal cells, has obtained sufficient yield of circulatory cells to be able to include all stages of NSCLC in the analysis (9). Interestingly, the specificity in this study was not complete, as abnormal cells were also detected in circulation of healthy controls, albeit at a lower level compared to any stage of NSCLC (9).

In summary, a handful of studies using the only FDA-approved method for CTC isolation (CellSearch) have been published so far in NSCLC. While their results are promising, further studies are needed to substantiate clinical utility of CTCs in NSCLC. Never the less, CTCs hold great promise in NSCLC and one of the most exciting potential clinical utilities is real-time monitoring of effectiveness of standard therapy by evaluating chemosensitivity or resistance of CTCs to the therapy of choice, allowing for early termination of ineffective treatment. At present over 40 studies registered on clinicaltrials.gov website (10) are evaluating the use of CTCs in NSCLC clinical studies, therefore clinical utility of CTCs may soon be demonstrated in NSCLC, similar to demonstration of CTCs' prognostic and predictive value shown in breast, colon, and prostate cancer.

In conclusion, we are not there yet, but we are on the way toward fulfilling clinical utility promise of CTCs in NSCLC.

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