

CCR 20th Anniversary Commentary: Paving the Way for Circulating Tumor Cells

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Cancer cells can enter the bloodstream, form distant metastases, and ultimately lead to death. A study by Allard and colleagues, which was published in the October 15, 2004, issue of *Clinical Cancer Research*, concluded that the CellSearch system could be used as a reliable tool to investigate circulating

tumor cells and their clinical utility, and it spurred a still-growing interest in the field. *Clin Cancer Res*; 21(13); 2883–5. ©2015 AACR.

See related article by Allard et al., *Clin Cancer Res* 2004;10(20) October 15, 2004;6897–904

In the October 15, 2004, issue of *Clinical Cancer Research*, Allard and colleagues (1) introduced the CellSearch system, designed for the enumeration of circulating tumor cells (CTC) in 7.5 mL of blood. The analytical accuracy, reproducibility, and linearity of the system were demonstrated by spiking blood samples with a predetermined number of cells from tumor cell lines. The prevalence of CTCs was determined in blood from 145 healthy donors, 199 patients with nonmalignant diseases, and 2,183 samples from 964 patients with metastatic carcinomas. Whereas virtually no "tumor cells" were detected in the control population, 36% of the blood samples of cancer patients contained ≥ 2 CTCs in 7.5 mL, with a broad range of 0 to 23,618 CTCs per 7.5 mL.

Background

Tumor cells travel through the blood and lymphatic system to form distant metastases. Our original hypothesis was as follows: If we could identify, count, and extract information from these CTCs, we might be able to detect the cancer early, determine its aggressiveness, and monitor and guide therapy. The challenge was to develop a technology that reliably detects CTCs in a tube of blood among the abundance of blood cells. The demonstration that tumor cells indeed could be detected in the blood of cancer patients using a combination of immunomagnetic enrichment, immunofluorescent labeling, and multiparameter flow cytometry formed the basis of our initiative to develop a CTC platform suitable for clinical translational studies and actual use in the clinic (2). The path from proof of concept to an FDA-cleared CTC platform was a challenge and required investors that believed in us, a team capable of developing the platform, and designing and conducting the clinical studies. The results presented in 2004 showed that the system could reliably detect CTCs and enabled

prospective clinical studies that formed the basis of successful FDA submissions.

The first clinical study conducted in patients starting a new line of therapy for metastatic breast cancer (3) was followed by similar studies in colorectal (4) and prostate cancers (5). Studies in patients before and at several time points after surgery for non-metastatic breast (6) and colorectal cancers (7) were started at the same time but only reported recently, as much longer follow-up was needed. All of these studies showed a strong relationship between CTC load in blood and progression-free and overall survival. These results raised interest in CTCs, and the original results have been confirmed and validated in numerous studies since the original 2004 publication. In studies comparing the use of CTCs to serum tumor markers and/or radiographic imaging, the measurement of CTC load clearly showed superiority in predicting the efficacy of ongoing therapy. This evidence, however, did not translate to the measurement of CTCs in routine clinical assessment of patient status. One of the biggest stumbling blocks has been that although persistently elevated CTCs indicate that the current therapy will not provide benefit, the system does not provide an alternative treatment strategy. This level of information is also not provided by other diagnostic tools, such as serum tumor markers or radiographic imaging, and raises a question about to why these tools are used routinely in the clinic.

The CTC Field

Since the original publication of our study, many other studies have shown the ability to detect molecules that represent therapeutic targets on CTCs, and each of these investigations revealed a great deal of heterogeneity among CTCs of individual patients (8). Comparisons of CTC expression of Her2 with the original tissue biopsies showed concordant as well as discordant results, raising a question about whether the expression of biomarkers on the primary tumor or on tumor cells present in the blood will be the better predictor of response to therapy. Prospective studies will need to be conducted to determine whether biomarkers assessed on primary tissue, protein, or gene expression in CTCs in blood will be the best predictors of therapy response. The future will tell us whether support can be found to conduct such studies.

The 2004 article by Allard and colleagues not only initiated many translational studies conducted with the CellSearch

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technology, but also encouraged a large group of academic research groups and small and larger companies to develop technologies that could improve upon the results obtained with the CellSearch system (8). It is gratifying to see the reports of new approaches for the detection of CTCs. Most of these studies used the results from the 2004 publication as the gold standard. Unfortunately, all are preliminary studies with no multicenter prospective follow-up to determine whether the findings indeed lead to an improvement in sensitivity and specificity of a CTC test that can be used in the clinic. The largest problem in the development of an assay to detect CTCs is that one does not know whether tumor cells are present in a blood sample and, if so, how many. Spiking of cells derived from tumor cell lines is useful to test the analytic performance of the system, but is not a surrogate of tumor cells in cancer patients. Improvements in sensitivity and specificity using the same immunophenotype of cancer cells identified by the CellSearch system (expressing EpCAM as well as Cytokeratin 8, 18, or 19) can only be marginal as the majority of cancer cells with this immunophenotype are likely already captured. Incremental improvements therefore will be needed to detect alternative CTC phenotypes or increase the blood volume analyzed in order to increase the numbers of tumor cells captured. A study in which we analyzed the frequency distribution of CTCs present in 836 metastatic breast, colorectal, and prostate cancer patients predicted that 99% of patients had at least one CTC before therapy when the findings in 7.5 mL were extrapolated to a blood volume of 5 liters (9). Evidence to support these findings is being pursued in the European Union seventh Framework Health Programme CTCTrap (10).

We originally chose the EPCAM antigen as our target for CTC enrichment because it was widely expressed in carcinomas, expressed on the cell surface, and not detected on blood cells. Tumor cells not expressing EpCAM would thus be missed, raising questions about how many CTCs are missed and what distinguishes tumor cells expressing EpCAM from those not expressing EpCAM. In a pilot study in which we analyzed EpCAM⁺ as well as EpCAM⁻ CTCs in non-small cell lung cancer, we found, to our surprise, that CTCs appeared in similar frequencies, but only the EpCAM⁺ CTCs correlated with clinical outcome (11). To our surprise, we also found EpCAM⁺ CTCs in cancers where we did not expect to find them, such as sarcomas (unpublished observations) and small cell lung cancer (8). It has been suggested that EpCAM is downregulated during epithelial-mesenchymal transition; however, a large portion of CTCs express EpCAM, and their presence is associated with poor outcome. Studies aimed at the

identification of alternative CTC phenotypes make use of differences in physical characteristics such as size, deformability and dielectric properties, or depletion of leukocytes (8). The heterogeneity already observed in CTCs identified by the CellSearch system will further increase with the addition of other CTC phenotypes, and more research will be needed to identify the biologic differences between these tumor cells and determine whether specific subsets are responsible for the metastatic phenotype. A considerable proportion of the cells identified as CTCs are undergoing apoptosis and will not be responsible for the formation of metastasis. Also, a much large number of tumor cell fragments, extracellular vesicles, and exosomes are present in the blood of cancer patients, and these still may contain valuable information that can lead to a choice of therapy.

The 2004 article by Allard and colleagues (1) provided the awareness that "a liquid real-time biopsy" for cancer patients was within reach, and a large group of investigators from different fields of expertise are now engaged to expedite our knowledge of the biology of metastasis. We now have a variety of technologies to characterize these tumor cells at the individual cell level and in terms of protein and gene expression, and recent reports have shown that it is possible to successfully expand CTCs in animal models. Hopefully, we will learn from these studies and will translate these research observations into tools that clinicians can use to treat cancer patients more effectively. A concerted effort to validate different methods to isolate, count, and interrogate CTCs, and to bring the liquid biopsy concept into the clinic, has been initiated recently in Europe by the CANCER-ID consortium (12).

Disclosure of Potential Conflicts of Interest

L.W.M.M. Terstappen is listed as an inventor on U.S. patents (No: 5,985,153; No: 5,993,665; No: 6,013,188; No: 6,136,182; No: 6,361,749; No: 6,365,362; No: 6,551,843 B1; No: 6,623,982 B1; No: 6,620,627 B1; No: 6,623,983 B1; No: 6,645,731 B2; No: 6,660,159 B1; No: 6,790,366 B2; No: 6,890,426 B2; No: 7,056,657 B2; No: 7,332,288 B2; 7,863,012 B2; No: 8,329,422 B2) related to the CellSearch system, the rights of which are assigned to Johnson & Johnson, and is the chairman of the department of Medical Cell BioPhysics at the University of Twente, which receives research funding related to the CellSearch system from Johnson & Johnson. No potential conflicts of interest were disclosed by the other author.

Authors' Contributions

Conception and design: W.J. Allard, L.W.M.M. Terstappen
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