Circulating Tumor Cells: Getting More from Less

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Recent insights into circulating tumor cells (CTCs) have been driven by numerous technological innovations aimed at isolating, purifying, and analyzing these rare cells. However, the information density within these cells has yet to be truly accessed and exploited for patient benefit. A device reported by Issadore et al. in this issue of Science Translational Medicine proposes a highly sensitive methodology that may both extend CTC capture to a broader patient population and provide greater understanding of biological targets for personalized medical therapies.

**KNOW THINE ENEMY**

An oft-cited translation from Sun Tzu’s Art of War contends that “if you know the enemy and know yourself, you need not fear the result of a hundred battles.” In the field of oncology, our “knowledge of the enemy” continues to grow and evolve, providing insight into mechanisms of tumorigenesis and previously unidentified biologic targets for cancer drug development (1).

Recent advances have provided clues as to how heterogeneous subpopulations of “the enemy” may contribute to the development of recurrent/metastatic cancers and resistance to therapies. Among these advances are technologies that assay a rare population of tumor cells shed into circulation (2)—called circulating tumor cells, or CTCs—from primary and metastatic tumor sites that may both contribute to the development of metastatic disease and reflect the heterogeneity that likely exists between various tumor deposits (Fig. 1). These rare cells have been approximated to average 1 CTC for every 1 billion peripheral blood cells, emphasizing the challenges in their isolation and analysis. CTCs have been collected with the goal of understanding their role in the metastatic cascade and, ultimately, to predict patient outcomes from both the disease as well as treatments for that disease (3). In this issue of Science Translational Medicine, Issadore and colleagues describe an innovative microfluidic assay with high sensitivity and specificity to detect CTCs expressing multiple cell-surface markers (4).

To achieve this goal, the authors designed a microfluidic device using micro-Hall (µHall) sensors to detect the magnetic moment from immunolabeled cells bound with magnetic nanoparticles (4). By varying the size of the nanoparticles, the µHall detector (µHD) was able to sensitively identify and quantify different cell-surface proteins recognized by each particle. A series of validation experiments showed excellent specificity and superior sensitivity to enumerate more CTCs from the peripheral blood of 20 patients with ovarian cancer compared to the commercially available CellSearch assay (Veridex): A median of 1 CTC per 7.5 ml of blood was recovered with CellSearch, versus 57 per the same volume of blood with the µHD.

Issadore et al. further extended this technology to assay for expression of cell-surface markers that are relevant for detection and may be therapeutic targets in other solid-tumor malignancies (4)—for instance, human epidermal growth factor receptor 2 (HER2/neu) in breast and ovarian cancers and epidermal growth factor receptor (EGFR) in lung cancer. In this series of experiments, the authors implanted a human epidermoid cancer cell line into a mouse model and then initiated systemic therapy with an HSP90 inhibitor (geldanamycin) known to alter EGFR expression. Using the µHD, they were able to detect alterations in EGFR expression from tumor aspirates during treatment. These preclinical studies with the µHD suggest that this approach may have adequate sensitivity to detect alterations in cell-surface targets of new molecular therapies, such as EGFR, during a patient’s course of treatment.

Extensive studies on the mechanisms by which metastases develop suggest that heterogeneity exists among tumor deposits, and subsets of these cells have greater metastatic potential (5). This heterogeneity can extend from genomic, proteomic, and functional differences among these cell populations. Subpopulations of CTCs may have greater potential to extravasate into circulation from a tumor and seed a metastatic site, whereas cells that disseminate by means other than circulation, such as aggressive local invasion or lymph node metastases, may be another subpopulation of cells within the tumor milieu. Given these hypotheses, the isolation and evaluation of CTCs will be crucial to understanding the mechanisms by which secondary metastatic sites form.

The enumeration of bulk CTCs from patients with advanced cancer has yet to answer whether these isolated cells are responsible for the initiation of a metastatic site, are cells in transit from already established metastatic sites, or are merely sloughed cell populations with little relevance for the cancer progression or responses to anticancer therapies (Fig. 1). Devices such as that described by Issadore et al. (4) extend the potential of CTC analysis to subpopulations of CTCs and further interrogation of these cells in the process of patient care. However, translation of these technologies into patient benefit requires extensive knowledge of the strengths and limitations of each assay with a particular prognostic or predictive goal in mind—thus, “knowing yourself” to fit within the conceptual frameworks of Sun Tzu and, more importantly, regulatory bodies, which approve such devices for human use.

**TECHNOLOGIES FOR ISOLATING CTCs**

Over the past 15 years, various technologies have been developed to permit the isolation and analysis of CTCs (6–8) and can generally be divided into positive and negative selection methodologies. Positive selection techniques to isolate cells are based on expression of cell-surface markers, such as the epithelial cell adhesion molecule (EpCAM), and include the CellSearch assay, the Herringbone-CTC chip (9), and flow cytometry-based approaches (Fig. 1) (3). The CellSearch assay is U.S. Food and Drug Administration (FDA)–approved and defines CTCs as those events that are EpCAM-positive, negative for a lymphocyte marker (CD45), positive for a nuclear stain (DAPI), and positive for cytokeratins 8 and 18. However, positive selection technologies are criticized for their reliance on cell-surface expression of EpCAM to capture (and define)
Aspects of heterogeneity

CTCs because some tumors down-regulate expression of this marker during epithelial-mesenchymal transitions (EMTs), whereas other tumor types, such as renal cell carcinoma, do not commonly express EpCAM.

To address this limitation, negative selection technologies are being developed that isolate CTCs on the basis of physicochemical properties—such as size, density, or surface charges—that distinguish them from other blood cells (10–12). Issadore et al. took a different approach by assaying for multiple cell-surface markers with their μHD (4). Using multiple markers provided specificity similar to flow-based approaches while minimizing cell manipulation.

Various technologies are now identifying both concordant and discordant results with regards to enumeration of CTCs that remind us of the fundamental questions regarding the biology of these cells and how they should be defined in the context of cancer. For example, Armstrong et al. proposed that a subset of EpCAM-positive CTCs expressing CD133 has greater invasive potential and may be a more relevant population to isolate and study (13). Is it possible that this population of cells may hold greater prognostic import than cells that do not express these markers? According to some recent reports, some CTCs may also lack surface expression of EpCAM and would likely be missed by the CellSearch and other positive-selection assays (14). In this case, these technologies would be underreporting CTC burden in patients with metastatic cancers.

Can we gain greater information density on the underlying genomic and proteomic heterogeneity among these rare cells? Danila et al. found that CTCs from patients with prostate cancer can be analyzed for expression of cancer-specific genes, including androgen receptor (AR) and the gene fusion TMPRSS2-ERG (15). Magbanua and colleagues identified different amplification events in AR in CTCs from patients with prostate cancer and from archival patient samples (16). These inter- and intrapatient variations suggest a greater underlying complexity in CTCs, including heterogeneous subpopulations and progressive genomic aberrations typical in later-stage/metastatic disease.

As often occurs with advances in medicine, greater underlying complexity can inhibit or impede the translation of these advances into therapeutic benefit for our patients. This complexity can also impede the broad adoption of new technologies if they are unable to either account for or predict individual patient responses and outcomes. The degree to which these current technologies can answer these critical questions is unclear, and validation studies are ongoing. Issadore et al. reported detection of CTCs from patients with various histologic subtypes of ovarian cancer, with either stage III or IV disease (4), that had not previously been identified. Furthermore, the authors also identified higher CTC burdens from patients with cancers with platinum chemotherapy-resistant disease versus platinum chemotherapy-sensitive disease. These results raise critical questions as to whether the presence of these previously unidentified cells is relevant for recurrent cancers or patient outcomes. Ultimately, whether these cells will further our understanding of the progression of ovarian cancer is unknown, but their identification is the first step in developing a new cell-based biomarker.

CTCs AS BIOMARKERS?

Ultimately, the great hope in the continued investigation of CTCs is the development of an easily accessible biomarker (in blood, for example) that could be repeatedly interrogated to inform physicians on an individual’s tumor biology, predict which therapeutic interventions may be of greatest benefit, monitor treatment responses to therapy, and/or assay for mechanisms of therapeutic resistance to guide subsequent therapies (Fig. 2). The potential simplicity by which CTCs could be repeatedly assessed for these purposes would

Fig. 1. Heterogeneity amongst tumor sites. Heterogeneity among primary tumors and metastatic sites is well established. Emerging evidence suggests that this heterogeneity extends to subpopulations of circulating tumor cells. Whether these subpopulations originate from the evolving mutations within the primary tumor or from metastatic sites is unclear and likely is both patient- and tumor-stage–dependent, with advanced disease showing greater heterogeneity.
be a clear advantage over commonly used biomarkers, such as imaging modalities or painful tumor biopsies.

It is within this conceptual framework that we must consider how CTCs can aid in patient care now and what we should expect in the coming years. First, the distinction between predictive and prognostic biomarkers must be understood to develop a clinical niche for such an assay. Although a prognostic biomarker attempts to provide a risk assessment for clinical end points, such as disease progression, recurrence, or survival, a predictive biomarker attempts to assess the likelihood of a disease response from a therapeutic intervention. These markedly different concepts must drive experimental design for advancement of any new technology proposing to assay CTCs. The CellSearch assay is the defined gold standard in CTC enumeration based on rigorously conducted prospective trials (17). Yet, no current CTC-based assay has been shown to have a predictive capacity to drive therapeutic decision-making, although numerous research efforts are under way.

CTCs may also be useful for drug development. For these purposes, a biomarker can be qualified as either a pharmacodynamic marker that can be assayed for a specific molecular target or drug effect versus a surrogate marker, in which alterations of the marker correlate with a clinical end point that would suggest patient benefit from a particular therapy. The emphasis from the National Cancer Institute/Cancer Therapy Evaluation Program for the concordant development of companion biomarkers with novel therapeutic agents further supports the development of CTC assays as alternative pharmacodynamic biomarkers in early-phase drug development (18). The preclinical studies performed by Issadore et al. suggest that the μHD may have the sensitivity to track changes in EGFR expression on CTCs after treatment with an HSP90 inhibitor that promotes degradation of this cell-surface marker; thus, CTCs could act as a potential biomarker to monitor drug effects.

Nevertheless, the ability of a CTC assay to act as a surrogate biomarker for a clinical end point is a much higher bar to achieve than as a prognostic marker. A report from Scher and colleagues showed that alterations in CTC number, as assessed by the CellSearch assay, correlated with overall survival in a randomized phase III trial testing of abiraterone acetate versus placebo in patients with metastatic prostate cancer (19). This study confirmed the ability to use CTC enumeration as a surrogate marker for overall survival. If this result is confirmed in other phase III trials with this patient population, it may be possible to use CTCs as alternative indicators of therapeutic efficacy without having to wait for overall survival as an end point for drug approval, thus speeding the evaluation of new therapeutic agents.

It is clear that there are several potential uses for CTCs as biomarkers. It is important to recognize that each application will require its own series of validation studies, as mandated by the FDA for all biomarker development as well as for the widespread adoption of a new technology in clinical practice (20). Briefly, the first benchmarks that a CTC biomarker must meet are experimental validation of the sensitivity, specificity, range of detection, and reproducibility of the assay along with initial clinical data and comparisons with existing CTC assays (21). Issadore and colleagues have met the initial validation benchmarks for this technology, including the sensitivity, specificity, and range of detection of CTCs based on four cell-surface markers for patients with ovarian cancer. Further validation experiments will depend on the authors’ development strategy for this technology as a prognostic, predictive, and/or pharmacodynamic biomarker, as well as whether this assay will be developed to assay CTCs from other tumor types.

With both successful validation experiments and a development strategy in hand, qualification studies are then pursued to use the assay under development in Clinical Laboratory Improvement Amendments (CLIA)—certified laboratories. Other than defined specimen acquisition, handling, processing, and storage requirements, the device described by Issadore et al. (4) would need to be replicated in multiple, independent laboratories, including appropriate quality controls and potential sources of assay failure. Such independent validation would also lead to defined standards for data analysis, interpretation, and reporting. The next major hurdle involves large-scale fabrication, training, and dissemination of this technology to a core set of CLIA-certified laboratories to process samples from clinical trials. With these steps in place, a new CTC assay will move forward into large-scale clinical trials.

**OTHER CONSIDERATIONS ON THE ROAD TO THE CLINIC**

With these standards and validation studies in place, an assay can be moved into product development with the appropriate experimental and therapeutic niche in mind. However, cost issues must be taken into account at each step in assay development. To reach qualification studies, the initial investments to develop the assay include device fabrication, automation (if neces-
sary), and training of staff to both process and interpret the assay’s results. Assistance from the National Cancer Institute (NCI) and FDA is available in the form of the NCI Clinical Assay Development Program (http://cadp.cancer.gov/), which is actively soliciting applications for their resources and expertise in these steps. Depending on the goals of the assay under development (prognostic versus predictive, pharmacodynamic versus surrogate), retrospective or prospective analysis of patient samples must be performed. An excellent example of this process includes the High Definition–CTC assay under development by Kuhn and colleagues (22). The costs associated with these are substantial and limiting without broad support from grant agencies and/or private investors. However, the potential cost savings to the health care system can be as important if patients can be stratified to receive or avoid expensive therapies on the basis of these biomarkers.

In summary, experimental validation of the strengths and limitations of a CTC assay has the potential to inform not only our understanding of a patient’s disease but also their response to therapies. Reinterrogation of these cells at earlier time points in a treatment course could then inform us of the potential (or lack of) benefit of a given therapy and enable earlier decision-making to stop an ineffective therapy and move on to alternative choices. This is becoming a key issue as newer agents continue to be approved in multiple tumor types and as cancer resistance to therapeutics increases. We can now begin to imagine a clinical encounter in which continual disease reassessment can truly personalize therapy for patients with advanced cancer, including drug choice, duration of therapy, and quality of life (Fig. 2). Only after such evidence is in place will widespread adoption of a new technology be undertaken to transform the care and lives of patients with cancer. Advancing the μHD presented in this issue of Science Translational Medicine into validation studies and clinical use will require dedicated effort, but the reward is improved care and outcomes for patients.

REFERENCES AND NOTES


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