

## Circulating Cancer Cells and Their Clinical Applications

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Minimal residual cancer is defined as “the presence of tumor cells that are not detectable by the current routine diagnostic procedures used for tumor staging in cancer patients after surgical removal of the primary tumor.” Data from European and North American groups have demonstrated the prognostic impact of disseminated tumor cells (DTCs)<sup>9</sup> in the bone marrow of breast cancer patients. Circulating tumor cell (CTC) detection and enumeration in peripheral blood have been examined in prospective multicenter studies of metastatic breast, colorectal, and prostate cancers and have been associated with decreased progression-free and overall survival. An increasing number of clinical research studies are validating these observations and extending them to other cancers and to earlier disease stages. CTCs are highly heterogeneous, and their molecular characterization is important, not only to confirm their malignant origin but also to follow immunophenotypic changes with tumor progression and identify diagnostically and therapeutically relevant targets that will help stratify cancer patients for individualized therapies. The rarity of CTCs—and thus the very limited amount of available sample—presents a formidable analytical and technical challenge. Recent technical advances in CTC detection and characterization include reverse-transcription quantitative PCR (RT-qPCR) methods, image-based approaches, and microfilter and microchip devices. CTCs represent a promising new diagnostic field for advanced-stage patients in that the sensitive CTC-detection platforms allow monitoring of disease and treatment efficacy. The development of single-cell technologies might allow profiling of these cells for the purpose of adapting treatment regimens. CTC-detection and characterization techniques hold promise for playing a role as a “liquid biopsy” that will allow physicians to

follow cancer changes over time and to tailor treatment. Current research on CTCs is focusing on the identification of novel diagnostic and therapeutic biomarkers produced by these cells. CTCs are promising as novel tumor biomarkers because they are well-defined targets for understanding tumor biology and tumor cell dissemination that can open new avenues for the early detection of metastasis and its successful treatment. We discuss CTCs and their diagnostic potential with 4 leading scientists and clinicians in this field. A suggested reading list on this topic is provided in the Data Supplement that accompanies the online version of this Q&A at <http://www.clinchem.org/content/vol57/issue11>.

**What are the current analytical methods for detecting CTCs? How reliable are these methods? Does it matter which method is used clinically?**



**Klaus Pantel:** Current analytical methods for detecting CTCs always include an enrichment step and a detection step. Enrichment of CTCs can be based on size (filtration devices), density (e.g., Ficoll centrifugation), ability to invade a collagen matrix, and positive immunoselection [e.g., epithelial cell adhesion molecule (EpCAM)

antibody-based enrichment of CTCs] or negative immunoselection (i.e., depletion of leukocytes by CD45 antibodies). The subsequent approaches used to detect CTCs are (i) immunocytochemistry with anticytokera-

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<sup>9</sup> Nonstandard abbreviations: DTC, disseminated tumor cell; CTC, circulating tumor cell; RT-qPCR, reverse-transcription quantitative PCR; EpCAM, epithelial cell adhesion molecule; CK, cytokeratin; EPISPOT, epithelial immunospot (assay); PSA, prostate-specific antigen; FDA, US Food and Drug Administration; DAPI, 4',6-diamidino-2-phenylindole dihydrochloride; Cq, quantification cycle; HER2, human epidermal growth factor receptor 2; CDER, Center for Drug Evaluation and Research; MGB1, mammaglobin 1.

tin (anti-CK) antibodies; (ii) RT-PCR targeting various epithelial mRNAs, including CK-19 mRNA; and (iii) epithelial immunospot (EPISPOT) assays detecting tumor-specific proteins released by CTCs [e.g., prostate-specific antigen (PSA)].

All enrichment methods are biased because tumor cells are heterogeneous and some fraction of the CTCs might be lost (e.g., immunoselection with EpCAM antibodies cannot catch EpCAM-negative CTCs). Detection with anti-CK antibodies is currently the most validated and standardized approach, which also allows morphological interpretation of positive events. Different detection methods lead to different results, as shown by the comparative analysis of the same patient samples with different technologies. Thus, the clinical results largely depend on the technology used to detect CTCs. The technology that has produced the largest amount of clinical data on the prognostic relevance of CTCs in breast, prostate, and colon cancers is the US Food and Drug Administration (FDA)-approved CellSearch system (Veridex).



**Howard I. Scher:** At this point, there is no standardized definition of a CTC, and the various techniques used to enrich and characterize these cells do not measure or report the same “CTC” biomarker. The broad range of assays and devices in use and in development include those based on: physical differences, e.g., density gradient

centrifugation, filtration, or the plasticity of CTCs relative to nonmalignant cells; cell surface antibodies conjugated to magnetic beads, microposts, or ferrofluids to “positively select/capture” tumor cells; and depleting the nonmalignant cell population first, leaving CTCs behind—a process called “negative selection.” The various enrichment steps are then followed by different methods to detect and characterize the cells via various molecular or cytometric techniques.

Assay reliability must consider 2 components. The first is the analytical performance of the assay. In most reports, details of the analytical validation steps that have been performed are lacking. Many of the assays have been studied only in a single-laboratory setting, and most of these assays are not CLIA certified. Knowing both the capability of the assay and what it is actually measuring and reporting is critical before proceeding to clinical testing. The second component is the clinical evaluation, i.e., the level of evidence that has

been generated to date with respect to the context in which the test might be used in the clinic.

Does it matter which method is being used clinically? Yes, and the first consideration is the “context of use” for which the test is being developed. Stated differently, what is the medical decision that the test result is needed to inform? Contexts of use include diagnosis, prognostication, prediction, response indicators, or efficacy/response surrogates. As examples, knowledge of a cell count does not inform the choice of one specific therapy over another, whereas detection of a kinase mutation in CTCs could. No single test will provide information on all these contexts.



**Leon Terstappen:** At present, the CellSearch system is the only validated system for CTC enumeration and as such is the only system that can be used in the clinic. Although a variety of analytical methods are being explored for the detection of CTCs, only a few are available for routine laboratory use. The definitions used to define

CTCs vary greatly between the different methods and result in a large range in the reported numbers of detected CTCs, as well as in the proportion of patients in which CTCs are detected. As a consequence, not only does a CTC-detection method need to be accurate and reproducible, but prospective clinical studies also will need to be conducted to determine the implications of the detected CTCs for each analytical method.



**Evi Lianidou:** Currently there is a plethora of analytical methods for detecting CTCs. However, as indicated earlier, the main analytical approach toward the detection of CTCs always includes 2 steps: (a) isolation/enrichment and (b) detection. CTCs are rare events that follow a Poisson distribution, and this fact has to be taken into

account for their detection. The sample volume of peripheral blood used for their isolation is critical, especially in the case of early disease. The most widely used enrichment approaches are based on (a) the different

density of CTCs, a feature exploited with such methods as centrifugation in the presence of Ficoll; (b) filtration; and (c) immunomagnetic isolation (positive or negative) through antibodies specific for epithelial markers such as EpCAM or leukocytes (CD45), respectively. A combination of enrichment methods is also used, e.g., filtration devices in combination with EpCAM-positive isolation, Ficoll enrichment, and then positive immune-magnetic isolation. Detection approaches are based on (a) imaging (immunocytochemistry and immunofluorescence) through the use of specific markers for CTCs such as CKs (mainly CK-8, -18, and -19), leukocytes such as CD45, and cell viability via 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) staining—mainly by the FDA-approved CellSearch system; (b) molecular methods based on gene expression of specific markers such as CK-19; and (c) methods based on the detection of proteins secreted by immobilized CTCs, such as the EPISPOT assay. Despite the fact that most of these methods are highly specific and sensitive, thus far there are no extensive studies designed to compare their efficacy when using the same clinical samples. This is an important issue for their clinical use since, especially in early disease, differences in analytical sensitivity between these methods play a very critical role.

#### *Is there a need for a quality-control system for CTC enumeration?*

**Klaus Pantel:** There is a clear need for a quality-control system for CTC enumeration. Automated CTC detection systems (e.g., CellSearch) have included built-in positive and negative controls that have allowed the distribution of images among participating laboratory centers. Since microscopical detection systems are observer dependent, the development of international standards for CTC enumeration and characterization is of utmost importance.

**Howard I. Scher:** There is more than a need; it is essential. Without quality control, the reported results and the clinical data that might be derived from them are virtually uninterpretable and are of limited to no value. At this time, as indicated earlier, the only CTC assay that has been FDA-cleared for use, CellSearch, defines a CTC as a cell that is morphologically intact, has a nucleus surrounded by cytoplasm after DAPI staining, expresses CK-8, -18, or -19, and is CD45 negative. Noteworthy is that as a part of the validation process, which showed the reproducibility and consistency of the assay in the reference and local laboratories, over 450 breast cancer patient samples, in addition to control samples, were evaluated.

**Leon Terstappen:** Each CTC system should be validated and accompanied with a quality-control system.

**Evi Lianidou:** Numerous single-institutional studies suggest that CTCs can play an important role in risk stratification and monitoring of therapeutic efficacy. These findings need to be evaluated in trials to verify this concept in the clinical setting. Agreement on the standardized detection of CTCs is absolutely necessary. Critical issues include: (a) the standardization of the preanalytical phase, such as sampling itself (e.g., sample volume, avoidance of epidermal epithelial cell co-sampling in case epithelial markers such as CK-19 will be used later for CTC detection), sample shipping (stability of CTCs under different conditions), and storage conditions (use of preservatives or anticoagulants); (b) standardization of CTC isolation through the use of spiking controls in peripheral blood; (c) standardization of detection systems; and (d) interlaboratory- and intralaboratory-comparison studies for the same samples. The development of international standards for CTC enumeration and characterization is also very important, especially in imaging detection systems that are observer dependent. A recent study has shown the feasibility of external quality assurance of CTC enumeration using the CellSearch system. RT-qPCR-based molecular methods can be used in routine clinical laboratories and can be standardized, since the required quality issues, such as quantification cycle (Cq) values, limit of detection, precision, accuracy, and recovery experiments, have been clearly described. Studies that have compared molecular RT-PCR-based methods and immunocytochemistry have shown a significant correlation, whereas in a recent comparison study of the CellSearch assay and a molecular test (AdnaTest BreastCancer), concordant results regarding HER2 (human epidermal growth factor receptor 2) positivity were obtained in only 50% of the patients. In conclusion, a universal internal and external quality-control system for CTC detection and enumeration is urgently needed before their application in the clinic.

#### *For which cancer has the most work been done with CTCs and why?*

**Klaus Pantel:** Breast cancer has been the focus of the international activities on CTCs because it is accepted that early blood-borne dissemination of tumor cells plays an important role in breast cancer, as underlined by the previous work on the clinical relevance of DTCs in bone marrow. More recently, data on monitoring CTCs in advanced castration-resistant prostate cancer has provided important insights of this approach as a “liquid biopsy,” particularly in the context of new therapies.

**Howard I. Scher:** A PubMed literature search for circulating tumor cells and tumor type produced the following numbers of publications: breast, 275; melanoma, 181; lung, 155; prostate, 117; colorectal, 116; sarcoma, 53; head and neck, 41; lymphoma, 41; leukemia, 21; kidney, 22; and bladder, 16. The number of publications is only one aspect of the “work” done in a particular disease area. More important is the quality, which brings us back to the analytical validity of the assay(s) used in the reports and the level of evidence that has been generated to support a specific context of use. One issue is in the detection step. For example, using CellSearch, one finds unfavorable cell counts in upwards of 50%–70% of patients with progressive, castration-resistant metastatic disease; for colorectal cancer, this rate is 15%. In cases where detection rates are low, new assays are needed to increase the proportion of patients in whom CTC biomarkers can be evaluated.

The FDA clearance document for CTC enumeration with CellSearch states: “The presence of CTC in the peripheral blood, as detected by the CellSearch™ Circulating Tumor Cell Kit, is associated with decreased progression free survival and decreased overall survival in patients treated for metastatic breast, colorectal or prostate cancer. The test is to be used as an aid in the monitoring of patients. . . . Serial testing for CTC should be used in conjunction with other clinical methods for monitoring” ([http://www.accessdata.fda.gov/cdrh\\_docs/pdf7/K073338.pdf](http://www.accessdata.fda.gov/cdrh_docs/pdf7/K073338.pdf)).

Clearance, however, does not mean that the results can be used as an efficacy response “surrogate” for survival in regulatory filings. That particular question can be addressed only by embedding the biomarker question in survival-based phase 3 trials. Such an initiative is ongoing in baseline and posttreatment follow-up CTC enumeration in the phase 3 registration trails of abiraterone acetate (Ortho Biotech, a Division of Cougar Biotechnology), in the trial of MDV3100 (Medivation), and in the trial of ipilimumab (Bristol-Myers Squibb) and TAK-700 (Millenium). A formal briefing document outlining this initiative has been filed with the Center for Drug Evaluation and Research (CDER) branch of the FDA, and analyses of the association of CTC enumeration with survival is ongoing.

The prognostic significance of CTC number after treatment is also being studied in breast cancer as a response indicator to change treatment. Of particular interest in lung and colorectal cancers is the ability to detect kinase mutations that predict treatment resistance, both at the start of therapy and on therapy. For these contexts, the test provides for taking an easily acquired blood sample for profiling tumor at the time a treatment is considered, potentially replacing the need

for an invasive and costly biopsy that is difficult to perform repeatedly.

CellSearch as currently configured cannot be used to detect CTCs in patients with melanomas and renal cell carcinomas, because these tumors do not express EpCAM. To detect CTCs in these contexts, some groups are studying CTC detection using qPCR-based methodologies for melanoma-specific antigens. On the basis of promising phase 2 data, phase 3 trials are ongoing.

Ultimately, the level of research activity will be determined by the unmet need that the test will be used to address, the technical performance of the assays available, and the results from the sequence of trials required to generate sufficient evidence to enable routine use in a clinical-practice setting.

**Leon Terstappen:** Prospective multicenter studies have been conducted for metastatic breast, colorectal, and prostate cancers. The sponsor chose to conduct studies in the 3 most frequent carcinomas.

**Evi Lianidou:** CTCs are mostly studied in breast cancer. The reason is that the clinical relevance of CTCs in the bone marrow of breast cancer patients has been clearly shown. Since early blood-borne dissemination of tumor cells plays an important role in breast cancer, a lot of work has been done in this type of cancer with both bone marrow and peripheral blood samples. By using RT-qPCR, our group has shown that in early breast cancer the detection of peripheral blood CK-19 mRNA-positive cells is an independent prognostic factor for a reduced disease-free interval and overall survival for node-negative breast cancer patients. Moreover, the detection of peripheral blood CK-19 mRNA-positive and mammaglobin 1 (MGB1) mRNA-positive cells before adjuvant chemotherapy predicts poor disease-free survival, whereas the detection of CK-19 mRNA-positive CTCs in the blood after adjuvant chemotherapy is an independent risk factor indicating the presence of chemotherapy-resistant residual disease. The importance of CTC enumeration in advanced breast cancer has been shown by using the CellSearch system. More recently, data on monitoring CTCs in other types of cancer, such as advanced castration-resistant prostate cancer, have provided important insights as a “liquid biopsy,” particularly in the context of new therapies.

**What are the main clinical issues and unmet needs to be addressed with CTCs?**

**Klaus Pantel:** (i) Estimation of the risk for metastatic relapse or metastatic progression (prognostic information); (ii) stratification and real-time monitoring of

therapies; (iii) identification of therapeutic targets and resistance mechanisms (biological therapies).

**Howard I. Scher:** Focusing on prostate cancer in particular, key issues in drug development and patient management are the difficulties of assessing treatment effects. This is because skeletal metastases, which are difficult to analyze quantitatively, represent the most frequent site of metastatic spread and one of the major causes of disease-related morbidity, and although serial PSA measurements do guide management, there are circumstances where a patient may be responding favorably when the PSA is going up or not be responding when it is decreasing. Early-response indicators that can be rapidly assessed in phase 2 trials are essential, both for the individual patients, as well as to inform the decision to proceed to more-advanced phase 3 testing.

An additional problem is that it is difficult to obtain metastatic tumor for molecular profiling from bone, the most common site of spread, and even when it is feasible, few of the assays used have been analytically validated. To utilize a targeted therapy requires a demonstration that the “target” is present when treatment is considered: biopsies are invasive, costly, and hard to repeat.

Virtually all prostate tumors are initially responsive to androgen ablation but eventually progress to a castration-resistant state (referred to as “castration-resistant prostate cancer” or CRPC), which is almost invariably lethal. PSA concentrations are frequently used as an indicator of progressive disease as well as a response indicator for both hormonal and cytotoxic agents. However, posttherapy PSA changes are not a surrogate end point for overall survival. These findings suggest the need to identify and define outcome measures of efficacy that more accurately reflect the true clinical benefit.

**Leon Terstappen:** A tool to determine which therapy or combination of therapies promises to be the most effective for the individual patient. A tool to effectively determine the effectiveness of the treatment once administered.

**Evi Lianidou:** Main clinical issues: (a) clinical studies to show that CTC detection can lead to a change in the management of cancer patients that results in an improved clinical outcome (the Southwest Oncology Group 0500 randomized phase 3 trial is especially designed to test the strategy of changing therapy vs maintaining therapy for metastatic breast cancer patients who have increased CTC levels at the first follow-up assessment and is expected to be completed soon); and (b) personalized medicine: the use of CTCs for stratification of patients and real-time monitoring of therapies.

Unmet needs to be addressed: (a) cross-validation of findings between labs; (b) molecular characterization of CTCs will enable the identification of novel therapeutics that will target micrometastatic spread and elucidate their connection to cancer stem cells.

#### *Is detection of heterogeneity among CTCs important?*

**Klaus Pantel:** CTCs and DTCs show a marked heterogeneity in terms of genetic aberrations and gene-expression patterns. To determine this heterogeneity is important, e.g., to estimate the “aggressiveness” of the residual tumor load and to obtain information on the selection of particular CTC clones during therapy (e.g., HER2 status of CTCs undergoing anti-HER2 therapy with trastuzumab).

**Howard I. Scher:** Yes. Sensitivity and specificity are an issue with specific CTC-enrichment techniques, owing to heterogeneity of the tumor in cell size, density, and marker expression. Consequently, some tumor cell loss is likely to occur, irrespective of the enrichment technique used. Primary tumors are known to be heterogeneous, and CTCs possess a molecular cytogenetic profile reflective of this phenomenon. The selection of multiple markers in detecting CTCs is critical. In addition, a mutation that is predictive for response to a specific drug may be detected in a population of CTCs, but if the mutation is present in only a small proportion of CTCs, the clinical benefit may be limited. The same consideration applies to protein-based biomarkers.

**Leon Terstappen:** Yes, heterogeneity with respect to the presence of treatment targets in particular may become important to tailoring the optimal cocktail of therapies.

**Evi Lianidou:** CTCs are highly heterogeneous, as has already been shown through confocal laser scanning microscopy and molecular methods. This is highly important, particularly when therapeutic targets are expressed in CTCs but not in the primary tumor. Since HER2-positive CTCs have been detected in a substantial number of patients with HER2-negative primary tumors, evaluation of HER2 status by assessment of HER2 expression on CTCs is a strategy with potential clinical application. However, the importance of CTC heterogeneity thus far has not been fully exploited clinically.

#### *When is it best to assess CTCs? Before or after primary treatment?*

**Klaus Pantel:** The estimated half-life of CTCs is short. Thus, it can be assumed that the assessment of CTCs after primary therapy (e.g., after completion of adju-

vant chemotherapy) provides information on occult micrometastatic deposits, whereas the detection at primary diagnosis (and surgical removal of the tumor) might be largely determined by the disseminatory capacity of the primary tumor. Although data comparing the clinical relevance of enumerating CTCs at these 2 time points are still missing, the enumeration of CTCs that survived (neo)adjuvant therapy might be more relevant for the patient's prognosis than the detection of CTCs at primary diagnosis.

**Howard I. Scher:** Both, but, once again, the question boils down to the context. For example, if a particular assay detects disease in only 5% or fewer of patients presenting for primary therapy, it would be more appropriate to look for new assays that increase detection rates, because the "failure to detect" CTCs does not guarantee a favorable outcome. That said, there are data from multiple tumor types indicating that the detection of cells at diagnosis is associated with an inferior prognosis, but there are no data yet to support decisions on the definitive treatment of a primary tumor on the basis of the presence or absence of cells. For the context of using CTCs for molecular profiling to guide treatment selection, it is essential to have a pretreatment sample, whereas for the context of response both are important.

**Leon Terstappen:** Before administration of therapy, the presence and number of CTCs should be used to assess prognosis and determine the presence or absence of treatment targets. After the first cycle of therapy, CTCs should be used to determine whether or not the administered therapy is effective.

**Evi Lianidou:** The presence of CTCs in bone marrow has been clearly shown to be of prognostic significance in patients with breast cancer before primary treatment. Our group has shown that in early breast cancer, the detection of CK-19 mRNA-positive CTCs is an independent prognostic factor both before and after adjuvant chemotherapy.

#### ***What is the role of CTCs in "personalized medicine"?***

**Klaus Pantel:** Real-time monitoring and molecular characterization of CTCs, particularly for therapeutic targets (e.g., HER2) or mutations conferring resistance to targeted therapies [e.g., *KRAS* (v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) mutations], may contribute to a better selection of cancer patients who may benefit from expensive therapies with considerable side effects, and these measurements may also allow an early switch to more-effective therapies in individual patients.

**Howard I. Scher:** For many tumor types, the biologic determinants that contribute to progression change over time. In such cases, molecular profiles of the primary tumor obtained to establish a diagnosis or that is removed as definitive therapy may not be informative. In these cases, it is essential to obtain tumor material at the time of first and potentially all subsequent relapses. Similarly, it is known that molecular profiles can also change on a particular therapy. Here, obtaining tumor material for profiling is essential. For most tumors, however, performance of a repeat biopsy after initial diagnosis and treatment is not a part of routine practice and, as noted above, is also invasive, costly, and difficult to repeat. CTCs obtained from a simple, minimally invasive phlebotomy sample obtained in the context of routine patient management can fulfill this unmet need. Further, sequential studies showing the presence or absence of CTCs will likely be shown to provide rapid readouts of treatment efficacy, enabling therapies that are benefiting a patient to be continued and those that are not to be discontinued.

**Leon Terstappen:** In patients in which CTCs can be detected and characterized, CTCs can replace a traditional biopsy.

**Evi Lianidou:** Molecular characterization of CTCs can provide valuable information on the expression of specific receptors such as HER2, activating pathways such as angiogenesis, and specific mutations, such as in the epidermal growth factor receptor, that confer sensitivity to therapies. CTC molecular analysis offers the possibility of monitoring changes during the course of treatment and can serve as a real-time biopsy to guide tailored therapies in the near future.

#### ***Is the currently available evidence sufficient to use CTCs in the clinic? If so, for which application?***

**Klaus Pantel:** In patients with advanced cancer (in particular breast and prostate cancer), there is sufficient evidence that enumeration of CTCs provides prognostic information and appears to be more sensitive than the current imaging technologies or serum markers (e.g., PSA) used to measure progression. In early-stage patients, the prognostic role of CTCs is still under evaluation. CTC measurements are part of ongoing clinical trials testing new drugs in breast and prostate cancer, and the outcomes of these trials will determine whether CTC detection (and characterization) will become a valuable tool as a "companion diagnostic" (i.e., surrogate marker for therapy response or failure).

**Howard I. Scher:** Yes, the evidence to date does support the use of CTC enumeration for the clinical use for

which it is cleared. The data are insufficient to say that the test can be used in the place of imaging, or as a predictive biomarker to guide treatment, although this question is being addressed prospectively in multiple trials.

**Leon Terstappen:** Yes, for the determination of prognosis and the monitoring of therapy of patients treated for metastatic breast cancer, colorectal cancer, and prostate cancer.

**Evi Lianidou:** There is sufficient evidence that enumeration of CTCs provides prognostic information in patients with certain types of advanced cancer (breast, colon, prostate). CTC detection appears to be more sensitive than current imaging technologies or the classic tumor serum biomarkers used to detect early relapse. In patients with early-stage breast cancer, our group has shown that detection of CK-19 mRNA-positive CTCs is an independent prognostic factor, both before and after adjuvant chemotherapy. However, this is a single-center experience that has not yet been cross-validated in other laboratories, so the prognostic role of CTCs in early disease is still under evaluation. CTC measurement can play a role for the evaluation of the efficacy of novel drugs in breast and prostate cancer. This question is now being tested in clinical trials, and their outcomes will determine whether CTCs can be used as surrogate markers for therapy response.

#### **What is your prediction for the clinical applicability of CTCs 10 years from now?**

**Klaus Pantel:** Real-time monitoring of CTCs to assess therapeutic efficacy will complement current determinations of tumor progression by imaging technologies or measurements of blood serum markers. In addition, the molecular analysis of CTCs for therapeutic targets and/or mutations in pathways conferring resistance to molecular therapies (“liquid biopsy”) may become valuable tools to tailor modern therapies to the individual needs of a cancer patient.

**Howard I. Scher:** In addition to the contexts discussed in the previous question, multiple groups are developing the ability to consistently capture live cells and establish short-term cell cultures. The latter are used to test the antitumor effects of specific drugs to better inform the choice of a therapy most likely to benefit that patient, as well as to better understand mechanisms of resistance and the changing biology of the disease. Single-cell analyses are also under development, and

soon the technological advancements in this area will enable the analysis of sufficient numbers of cells in a real-time setting. In addition to the anticipated technological advancements, it is essential that new trial designs be developed, designed, and executed to streamline the process. This streamlining is essential to shorten drug-development timelines and to minimize the use of survival-based studies that enroll “all comers.” Such studies not only are inefficient and costly but also have the added detriment of slowing drug development and exposing patients to ineffective toxic treatment.

**Leon Terstappen:** CTCs will be used in routine tests to monitor cancer treatment and will be used to determine what therapies might be effective in the individual patient.

**Evi Lianidou:** A combination of advanced imaging systems and molecular characterization of CTCs will be very useful to further refine prognosis, define treatment strategies, and eliminate or reduce the risk of metastasis. The use of modern powerful technologies such as next-generation sequencing will enable the elucidation of molecular pathways in CTCs and lead to the design of novel molecular therapies that target CTCs specifically. Moreover, CTCs might become the preferred method to monitor the efficacy of adjuvant cancer therapies.

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