

## Biology and clinical relevance of circulating tumour cells

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Assessment of circulating tumour cells (CTCs) provides a novel approach to interrogating cancer biology and CTCs have potential to serve as clinically useful biomarkers. The presence of CTCs and their role in metastasis has long been hypothesised but it is only in recent years that technology has allowed reliable isolation of CTCs leading to a renewed interest in this area. CTCs are commonly found in the blood of patients with cancer but are rare in the blood of healthy volunteers and those with benign disease (1). As technologies for CTC isolation have evolved, so too has the ability to characterise CTCs at the molecular level, paving the way to better understand metastasis biology; and CTCs could serve as a minimally invasive 'liquid biopsy' for longitudinal monitoring throughout the course of a patient's treatment. This approach could be particularly useful to describe tumour evolution, assess pharmacodynamic biomarkers for drug development and reveal inherent or acquired treatment resistant mechanisms in a way that has been historically challenging due to difficulties in obtaining successive biopsies in lung cancer patients.

A plethora of novel technologies have emerged over recent years for the detection of CTCs (2,3). Most techniques exploit physical or biological properties of tumour cells that distinguish them from the overwhelming majority of blood cells. Physical properties include differences in cell size, density, deformability and electrical charge whilst biological properties include the expression of specific surface proteins, invasion capacity and viability. In general, CTC isolation techniques involve stepwise enrichment of the tumour cell population followed by a more specific procedure for their detection and isolation. The CellSearch® system (Veridex, Raritan, New Jersey), for example, immunomagnetically enriches CTCs, based on EpCam

expression then fluorescently labels cells with DAPI (a nuclear marker), cytokeratin (an epithelial marker) and CD45 (a marker to exclude white blood cells) for identification of CTCs by multicolour fluorescent imaging (1). This semi-automated technique has been extensively validated (1,4) and has been FDA approved for prognostic use and as an aid to monitoring patients with breast, prostate and colorectal cancer (5-7). CellSearch® is considered the 'gold standard' to which new technologies are now compared.

We carried out prospective clinical trials demonstrating the prognostic use of CTC enumeration, by CellSearch®, in patients with non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) (8,9). In NSCLC, twenty-one percent of patients were positive for CTCs ( $\geq 2$  CTCs/7.5 mL blood, range, 0-149) and patients with 5 or more CTCs per 7.5 mL blood exhibited significantly worse overall survival (OS) compared to those patients with less than 5 CTCs (median OS, 4.3 months versus 8.1 months respectively,  $P < 0.001$ ) (8). In SCLC, CTCs were more prevalent (85% patients positive) and abundant (range, 0-44,896 CTCs/7.5 mL blood). Patients with  $\geq 50$  CTCs exhibited significantly worse OS compared to patients with  $< 50$  CTCs (median OS 5.4 months versus 11.5 months, respectively,  $P < 0.001$ ) (9). The prognostic cut-off level was higher in SCLC due to the presence of much larger numbers of CTCs in this disease type. In addition, clusters of cells, termed circulating tumour microemboli (CTM) were identified in a third of SCLC patients with CTCs and the presence of just one microembolus was independently associated with worse survival. We hypothesise that this may be due to cells within CTM having an increased propensity for survival within the bloodstream, supported by the complete lack of apoptotic or proliferation markers, the latter also making them less susceptible to chemotherapy (9). In both NSCLC and SCLC, repeat CTC counts after a single cycle of chemotherapy provided additional prognostic information (8,9).

A change in CTC number during therapy has also been shown to be associated with response. In a phase II study of 41 patients with NSCLC receiving erlotinib and pertuzumab, CTCs isolated by CellSearch® were evaluated alongside radiological assessment (10). A decrease in CTC number with treatment

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correlated with clinical response as assessed by FDG PET and CT and was associated with longer progression free survival. CTCs have also been evaluated as a tool to assist in diagnosis of lung cancer. In a study of 150 patients undergoing diagnostic assessment for a lung mass (125 malignant and 25 benign), CTCs were detected by CellSearch® in 31% of patients with lung cancer and in 12% (3/25) of the patients with non-malignant disease, albeit in much lower numbers (1-2 CTCs/7.5 mL blood) (11). This suggests that CTC enumeration by CellSearch® is neither sensitive nor specific enough to be used as a diagnostic biomarker at present.

The most promising applications of CTCs lie beyond their enumeration. CTC characterisation is beginning to unravel intriguing information regarding the biology of metastasis. The concept of epithelial-to-mesenchymal transition is widely reported to be an important process in the metastatic cascade, endowing tumour cells with increased capability to invade and metastasize [reviewed by Chaffer and Weinberg (12)]. Until now this has been difficult to demonstrate from cells within the circulation of patients due to the challenge of isolating CTCs. We, and others have demonstrated, using a filtration-based technology, ISET (isolation by size of epithelial tumour cells; Rarecells, Paris), that a heterogeneous population of cells are present in the blood of lung cancer patients in respect of epithelial and mesenchymal characteristics, providing evidence for the occurrence of this process (13-15). In addition, CTM with maintained cell-to-cell contacts show heterogeneity in EMT markers with some cells showing mesenchymal characteristics and others with epithelial or a combination of both. The relevance of this in guiding future treatments is yet to be determined but highlights the powerful tool CTCs can be in interrogating metastasis biology, shedding light on areas that have previously been challenging to explore.

A further area of high potential for CTCs in lung cancer is in genomic profiling either for known aberrations, such as EGFR mutation or EML4-ALK rearrangement, or as an exploratory tool to identify new oncogenic drivers or gene expression profiles that may inform on novel strategies for personalized therapies. Maheswaran *et al.* (16) utilised a microfluidic device consisting of microposts coated in anti-EPCAM antibodies to detect CTCs in patients with NSCLC receiving gefitinib or erlotinib. EGFR mutational analysis was performed on DNA extracted from CTCs and on matched tumour biopsy specimens. The primary activating mutation, identified from tumour biopsy, was detectable in CTCs in 19/20 cases. Furthermore, serial monitoring of CTCs revealed the emergence of the resistance mutation T790M, which in some cases could also be detected at very low levels pre-treatment. A recent study by Ilie *et al.* (17), demonstrated that the EML4-ALK rearrangement can be detected by FISH in CTCs isolated from patients using ISET. Of 87 NSCLC patients evaluated, five were found to have ALK-gene rearrangement by FISH within primary

tumour biopsies and in all cases, the rearrangement was detected in corresponding CTCs by FISH with corroborating evidence of ALK protein expression by immunohistochemistry. In the other 82 cases there was no evidence of ALK-gene rearrangement or expression of ALK protein either within primary tumour biopsies or within CTCs. A further study assessed the expression of a panel of genes associated with resistance to different chemotherapeutic agents in the CTCs of a group of unselected patients with epithelial carcinomas (18). In patients with metastatic disease expression of this resistance gene panel correlated in 95% of cases with patients who had progressive disease on treatment. These studies clearly demonstrate exciting ways in which CTCs can be applied to the clinic to guide personalised therapy.

Over recent years there has been growing drive in oncology towards personalised medicine to improve patient outcomes, requiring not only the development of new therapies but also ways to determine the optimal biological dose (using pharmacodynamic biomarkers) and to identify which patients are most likely to benefit from a specific therapy (using predictive biomarkers). CTCs have the potential to play a significant role through their use as a source of predictive and pharmacodynamic biomarkers. However, whilst technology has developed at an impressive rate and the promises of CTCs seem tantalising close, the challenge is to translate these new technologies in a robust, reliable form in to the clinic to enable the delivery of meaningful improvements in patient care. The road to biomarker assay validation and the qualification of biomarker tests to prove clinical utility remains challenging and the CTC community needs come together to bring forward promising new technologies and biomarker assays to allow informed clinical decision making for patient benefit.

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