Editorial

Circulating tumor cells as a liquid biopsy in small cell lung cancer, a future editorial

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Circulating tumor cells (CTCs)

CTCs are shed tumor cells that have entered the bloodstream and are able to survive in the blood environment often by endothelial mesenchymal transition (EMT) (1-4). It is thought that CTCs mirror tumor heterogeneity of both the primary tumor and metastases, making them an excellent candidate that reflects the behavior of cancer. CTCs may replace invasive biopsies of the original tumor as a diagnostic tool, lowering the diagnostic burden placed on patients. Follow-up of number of CTCs will give physicians the opportunity to monitor therapy efficacy and observe relapses in time. A major prerequisite is that enough CTCs are being detected; in small cell lung cancer (SCLC) a variable amount of about 20 to 20,000 CTCs are found in 7.5 mL blood.

CTCs numbers in SCLC

Hou *et al.* showed that CTCs are prognostic for survival in SCLC patients, and observed decreasing CTCs after therapy in responding patients (5). These findings were confirmed in 2012 (6). Hiltermann *et al.* showed that a decrease in CTC counts after the first course of therapy already predicted tumor response (7). Similar findings were found in other studies (8-10). CTC enumeration is therefore a very promising biomarker for chemotherapy efficacy. Molecular characterization of CTCs may help us to understand mechanisms of metastasis and resistance, hopefully leading to better treatments in this disease where chemotherapy and radiation are still the only known effective treatments (11).

CTCs and copy number variation (CNV) or aberration status

SCLC has a very high mutation rate, as shown by Peifer *et al.* when by sequencing 29 SCLC exomes, 2 genomes and 15 transcriptomes of SCLC tumors ,they observed 7.4±1 protein-changing mutations per million base pairs (12).

In 2013 Ni *et al.* described CNV patterns in CTCs of lung cancer patients that are highly reproducible for individual patients (13). Tumor subtyping in adenocarcinoma and SCLC could be made on basis of CTC CNV. These patterns were not affected by drug treatment as described in one SCLC patient. For the 23 genes with significantly increased mutation frequencies in response to chemotherapy, six genes (*ALPK2*, *KIF16B*, *TP53*, *MYH7*, *TTLL2*, *PAK2*) were enriched and possibly involved in resistance. In 2017 Carter *et al.* were the first to demonstrate the predictive value of copy number aberrations (CNAs) in CTCs in 31 patients with SCLC (14). SCLC patients receiving chemotherapy were classified as either chemorefractory [progressive disease (PD) within 90 days after completion of chemotherapy] or chemosensitive (PD after 90 days). First, 88 baseline CTC samples from 13 patients were used to create a CNA-based classifier by combining CNA status with clinical response to chemotherapy. They studied the known 13 gene signatures with frequently altered genes in SCLC (8 amplified and 5 deleted genes). No segregation between chemosensitive and chemoresistant status was observed, which is in line with the

findings of Rudin *et al.* and Peifer *et al.* (12,15). Afterwards, the CNA classifier was expanded, and using this new 16 CNA profile classifier the concordance between predicted and clinical outcome (chemosensitive *vs.* refractory) was 83%. CNV patterns in CTCs were also not influenced by chemotherapy. Initial chemosensitive patients who became chemoresistant had similar CNV patterns in their CTCs. One of the issues is that the CNV test is not sensitive enough and not the right test to detect the many known resistance mechanisms for platinum and etoposide at DNA or protein level.

The 16 CNA profile classifier was subsequently validated in a new set of 18 patients with 112 CTC samples. The classifier correctly assigned 15 out of 18 patients (83.3%) to either the chemosensitive or chemorefractory group. However, the prediction became worse when 1–4 individual CTC calls were in disagreement with the majority of CTCs. This CTC heterogeneity remains an important issue for biomarker studies and therefore a substantial number of single cells are needed to perform robust treatment predictions.

Hurdles in CTC detection

Isolation and detection of CTCs is based on the different physical and biological properties of CTCs compared to normal cells. Different methods to identify and isolate the CTCs (including different definitions of a tumor cell) make comparisons of studies difficult. At this time, the Cell Search system—based on the expression of the epithelial cell adhesion molecule (EpCAM)—is still the only FDA approved system. Other techniques such as the ISET platform (RareCells Inc.) which is based on size and the Clear Cell system (Clearbridge, BioMedics, Singapore) that separates cells by sorted weights are becoming more commonplace, competing with the cell search method (16,17). It is not yet clear how CTCs obtained by different techniques mirror the characteristics of the original tumor and/or its metastases best. Different CTC characteristics should be compared to identify the most important cell traits that determine patient outcome. In the Cell Search system however, identified CTCs are fixed and can't be used for further cell culturing and molecular characterization of CTCs enclosed in a cartridge after enumeration is a challenge. Another way to isolate CTCs is microsieve filtration where single cells are deposited into microwells (18), from which living tumor cells can be isolated for immunocytochemistry or culturing, which is developed by VyCap (VyCap, Deventer). Another filtration technique from the same company was described by de Wit *et al.* who used a silicon microsieve on cell waste obtained after Cell Search to detect EpCAM-CTCs on which they carried out FISH (19). The ISET system and the Clear Cell system both offer, after the different methods for filtration or isolation, further characterization of CTCs by molecular analysis, FISH, immunofluorescence or culturing (6,17,20-25). These additional CTC applications will offer more detailed information.

Alternatives to CTCs as liquid biopsy

Currently, circulating tumor DNA (ctDNA), and tumor RNA derived from platelets are other biomarkers to evaluate tumor response and that may be useful for follow-up. Cell free DNA (cfDNA) are nucleic acids detected in body fluids. In cancer patients, at least a part is ctDNA, thought to originate from dying tumor cells. These approaches may be complimentary to CTCs, where high ctDNA and CTC numbers of untreated patients could indicate a high burden of disease.

Fernandez-Cuesta *et al.* studied ctDNA in SCLC patients, identifying *TP53* mutations in ctDNA in the plasma of 53 SCLC patients (49%) and 123 controls (11.4%) (26). This study illustrates that a substantial number of otherwise healthy people showed *TP53* mutations without having cancer. This is an important hurdle in the implementation to ctDNA as a screening test. It showed however that it is possible to identify specific mutations in ctDNA, which could be useful in daily practice.

RNA from the tumor is also found in the blood. In 2011 Nilsson *et al.* showed that tumor RNA was transferred into blood platelets, so called tumor-educated platelets (TEPs) (27). Using TEPs, Best *et al.* distinguished 228 patients with localized and metastasized tumors from six different origins from 55 healthy individuals with 96% accuracy. They correctly identified the source of the primary tumor with 71% accuracy and they distinguished MET or *HER2*-positive, and mutant *KRAS*, *EGFR*, or *PIK3*CA tumors (28).

Although both methods have hardly been studied in SCLC, mRNA and ctDNA can be detected in plasma and can be used to detect specific mutations or translocations. CTCs may deliver more information than mere plasma ctDNA or mRNA in platelets. Different cellular surface markers can be stained such as PD-L1, a target for

checkpoint inhibitors in lung cancer or *delta-like 3* (*DLL3*), a ligand in the NOTCH signalling pathway that shows increased expression on SCLC tumor cells in biopsies and perhaps also on CTCs. The meaning of CTC expression of these markers has not yet been clarified.

In conclusion, in patients with SCLC, liquid biopsies like CTCs may play a major role to determine tumor biology in a non-invasive way. Standardization and validation of CTCs and cfDNA assays are important issues realized by the current EU/IMI consortium CANCER-ID (www.cancer-id.eu).

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Footnote

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