Topic: CTCs / CECs (circulating tumor cells / circulating epithelial cells)
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Circulating tumor cells detection in peripheral blood of advanced non-small cell lung cancer patients

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Introduction
Circulating tumor cells (CTC) in the bloodstream are relevant biomarkers for several solid tumors, including non-small cell lung cancer (NSCLC). The aim of this study was to establish a practical methodology for detection and enumeration of CTC from advanced NSCLC patients using flow cytometry.
Methods
Culture NSCLC cell lines spiked into normal blood at decreasing concentrations were enriched using RosetteSep^{TM} CTC Enrichment Cocktail Containing Anti-CD36 followed by a traditional gradient centrifugation with Ficoll-Plaque PLUS. Extracellular staining was carried out using an allophycocyanin (APC)-conjugated EpCAM mAb and a R-phycoerytrin-Cyanine 7 (PE/Cy7)-conjugated CD45 mAb. Then, cells were exposed to the Fix&Perm kit to perform intracellular staining using both a R-phycoerytrin (PE)-conjugated pan cytokeratin (CK) and the Hoechst 33342 staining dye solution.
Results
The fine-tuned CTC enrichment method and the flow cytometry analysis showed a linear correlation between the output cell count and the input cell number from zero to thousands of cells. The next step to evaluate the efficacy of our CTC detection strategy was to analyze the presence of CTC in the peripheral blood from a treatment-naïve advanced lung adenocarcinoma patient with high tumor burden. After the informed consent was signed by the patient, we processed 30 mL of peripheral blood sample resulting in the detection of 399 CTC ($CD45^-$, $Hoechst^+$, $EpCAM^+/CK^+$, $EpCAM^+/CK^-$ and $EpCAM^-/CK^+$ cells). After three months of induction therapy with carboplatin, pemetrexed and bevacizumab, the number of CTC in 30 mL of peripheral blood decreased down to 10. This result correlated with the clinical evolution of the patient, who showed stable disease. The third blood sample was obtained after several maintenance cycles. At that time, the number of CTC in blood had doubled from 10 to 19 and the patient presented radiologically and clinically confirmed disease progression.
Conclusions

This CTC enumeration and analysis methodology by flow cytometry has shown a good correlation with the state of the NSCLC disease and may be of great benefit to oncologists. The efforts in development, assessment and analysis of CTC derived from NSCLC patients as a noninvasive biomarker should lead to more effective and better tailored anti-tumor therapies for individual patients, thus resulting in their improved life expectancy.