

**Title:** Affinity and size capture of circulating tumor cells: A platform for increased sensitivity.

**Author Block:** *Peter J. Maimonis, Keith Merdek, Kurt Dietenhofer, Lucy Yen, Gary Palmer.*  
On-Q-ity, Inc., Waltham, MA.

**Abstract Body:**

**Background:** The potential clinical value of evaluating circulating tumor cells (CTCs) from blood is clear. CTC analysis can aid in cancer diagnosis, prognosis, and treatment prediction. However, since CTCs are rare, current analysis is limited by low efficiencies. Variability of expression of cell surface antigens by CTCs make capture mechanisms based on antibody affinity less than ideal. We report here data from a CTC platform that combines affinity capture with size filtration capture.

**Methods:** A spiked cell model was used, involving whole EDTA blood spiked with SKBR-3 human breast cancer cells. Two microfluidic chips were analyzed, a standard chip (T7) with constant gap size between posts, and a gradient chip (MA3) with a gap-size gradient between posts starting at 40  $\mu$  and decreasing to 12  $\mu$ . Chips were coated with either an anti-EpCAM monoclonal antibody or with no Ab. An anti-cytokeratin (CK) Ab in combination with a nuclear specific fluorescent stain on an automated cell imaging platform was used to identify CTCs.

**Results:** 45% of cells were captured by size alone (MA3 with no Ab), 16% of cells were captured by affinity alone (T7 with MAb), and 65% of cells were captured by a combination of antibody affinity and size gradient (MA3 with MAb). This increased capture efficiency has been confirmed in clinical samples from patients with metastatic breast cancer, with a greater than 40% increase in capture efficiency utilizing both mechanisms. Whole blood from healthy donors run through either chip type yielded virtually no CTCs. FACS analysis showed that 75% of cultured SKBR-3 cells express high levels of EpCAM. This expression level will vary, as will, presumably, the relative contributions of the antibody capture mechanism and the size capture mechanism. A "heat map" analysis can demonstrate visually the relative contributions of each and these will be presented.

**Conclusions:** The MA3 microfluidic chip captures CTCs using a combination of affinity and size filtration. This allows for the capture of CTCs with varying expression levels of EpCAM by exploiting the size differential between these CTCs and other blood elements. This dual-capture mechanism may allow for more efficient capture than affinity alone. Further work evaluating this platform in late-stage breast cancer patients is ongoing.