

Background

- To date, the isolation of circulating tumor cells (CTCs) from patients with renal cell carcinoma (RCC) has been met with limited success.¹
- This is due to the fact that most available CTC isolation technologies rely on the positive selection of cells using the surface protein EpCAM, an epithelial marker which is expressed in a minority of RCCs.²
- ApoStream is a novel technology which utilizes dielectrophoresis and microfluidics for the antibody-free isolation of CTCs.³
- In this study, we developed a novel method for detecting RCC CTCs using the ApoStream platform and fluorescence *in situ* hybridization (FISH) for loss of the *VHL* gene. This assay was then tested in a cohort of patients with metastatic clear cell RCC (ccRCC).

Methods

- The optimal operating frequency for enrichment of RCC CTCs was determined using fluorescently labeled 786-0 cells spiked in blood cells from healthy donors.
- In parallel, conditions were optimized for performing FISH for the *VHL* gene on isolated cells.
- Following assay development, CTCs (defined as any non-diploid cell) were enumerated in a cohort of patients with untreated or progressive metastatic ccRCC as well as healthy donors.

Figures / Data

Figure 1. Theory of ApoStream operation

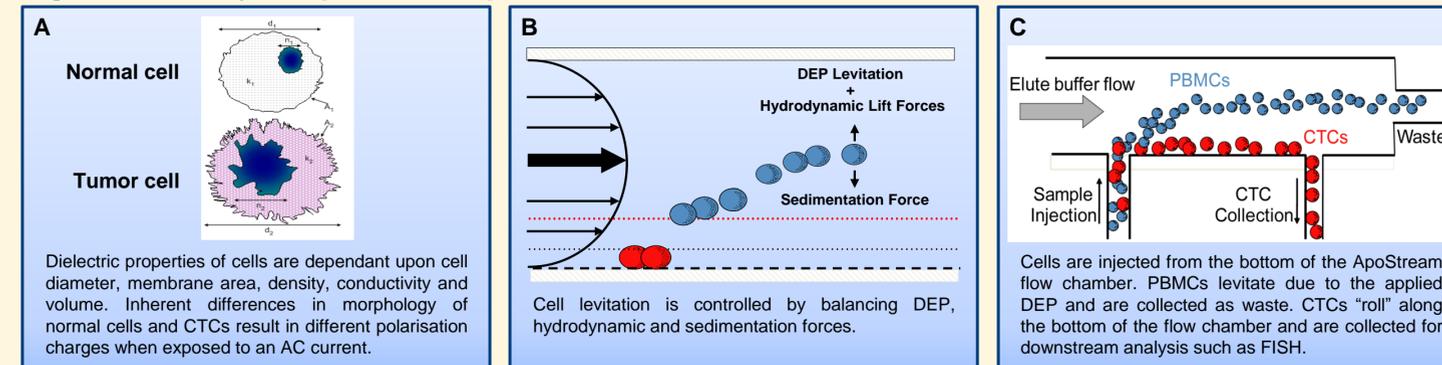


Figure 2. Crossover frequency optimization for recovery of 786-0 cells

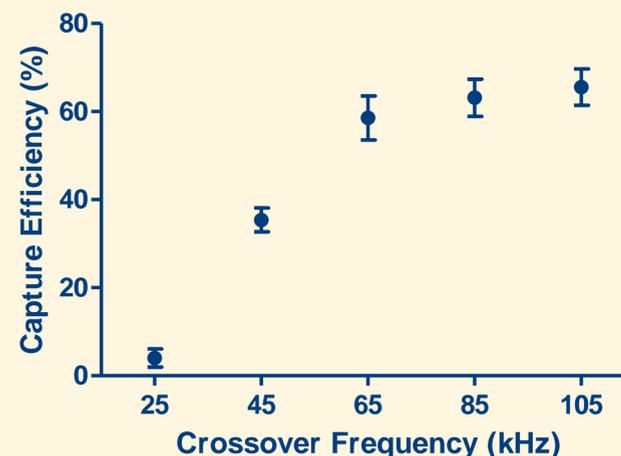


Figure 3. Assay validation in patients with metastatic ccRCC

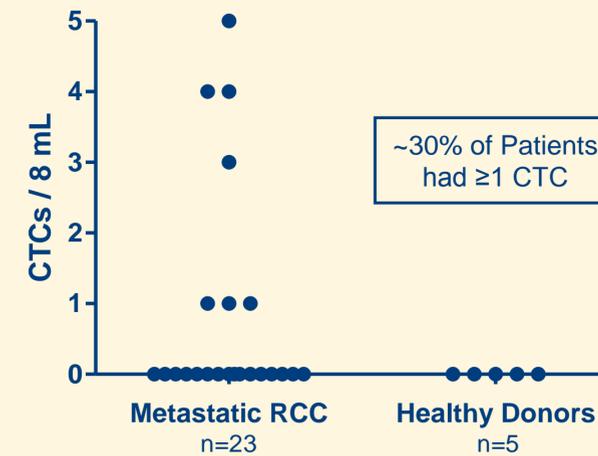
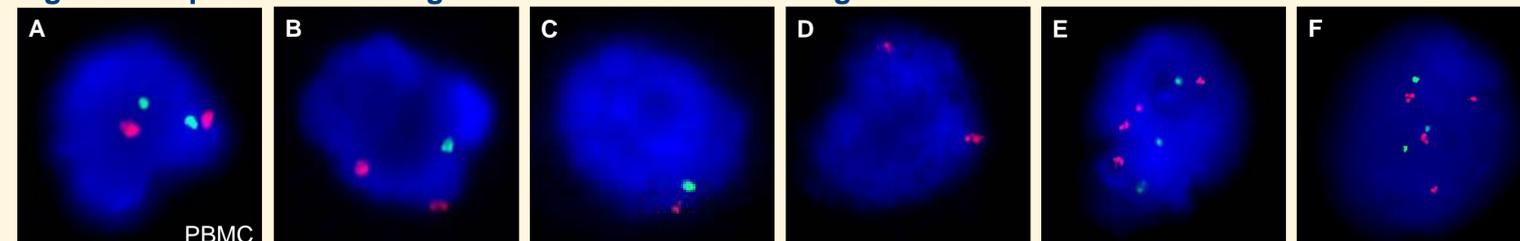


Figure 4. Representative images of isolated cells following FISH



Pink probe = centromere of chromosome 3. Green probe = *VHL* gene located at 3p25. (A) Diploid wild-type cell. CTCs with (B&C) heterozygous and (D) homozygous loss of *VHL*. (E&F) Polyploid CTCs with loss of *VHL*.

Conclusions

- Antibody-independent isolation with dielectrophoresis and subsequent FISH for the *VHL* gene is a promising novel method for CTC detection in patients with metastatic ccRCC.
- Future work aims to validate this assay in larger patient cohort.

References

- Bluemke K, *et al.* Detection of circulating tumor cells in peripheral blood of patients with renal cell carcinoma correlates with prognosis. *Cancer Epidemiol Biomarkers Prev.* 2009; 18(8): 2190-4.
- Went P, *et al.* Expression of epithelial cell adhesion molecule (EpCam) in renal epithelial tumors. *Am J Surg Pathol.* 2005; 29(1): 83-8.
- Gupta V, *et al.* ApoStream, a new dielectrophoretic device for antibody independent isolation and recovery of viable cancer cells from blood. *Biomicrofluidics.* 2012; 6(2): 24133.

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