Quantification and downstream analysis of circulating tumor cells isolated using CapioCyte™ liquid biopsy

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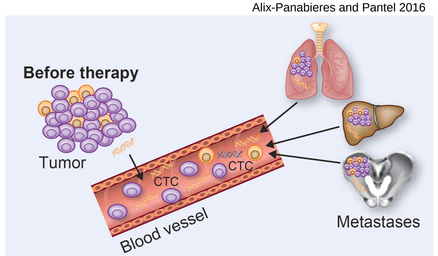
Abstract

Circulating tumor cells (CTCs) have great potential as biomarkers for the diagnosis and prognosis of many cancers. The CapioCyte™ chip isolates live CTCs through a unique combination of biomimetic cell rolling and nanoparticle-mediated multivalent immunorecognition. Recently-published work reported highly sensitive and specific CTC capture from 24 patients in a pilot study [Myung et al., Clinical Cancer Research 2018, 24(11):2439-2547]. Here, we report results from additional cohorts of patients and demonstrate downstream analysis of captured CTCs. Peripheral blood samples were collected from patients undergoing radiotherapy or immunotherapy. Samples were processed the next day by CapioCyte™ chips designed for the immunoisolation of CTCs expressing EpCAM, HER2, and EGFR. Captured CTCs were identified by immunocytochemistry as positive for cytokeratin and negative for CD45. Staining identified CTCs in all pretreatment samples (mean 95 CTC/ml whole blood, SE 54, range 4-680 for 12 recent samples) and immunotherapy (mean 70 CTCs/ml, SE 16, range 39-104). We additionally conducted single cell RNA Sequencing on select samples as a proof-of-concept demonstration for the powerful downstream analysis technique. In conclusion, the CapioCyte™ chip effectively captures CTCs for quantification and downstream analysis requiring viable cells such as RNASeq. The liquid biopsy has great potential to contribute to the diagnosis and personalized treatment of cancer.

Background

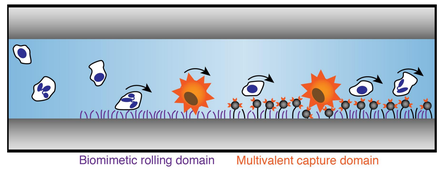
Metastatic cancer occurs when tumors shed cells that take root in other organs and grow new tumors. The number of circulating tumor cells (CTCs) correlates with metastasis and mortality [Pantel and Alix Panabieres 2010; Fidler 1970]

Alix-Panabieres and Pantel 2016

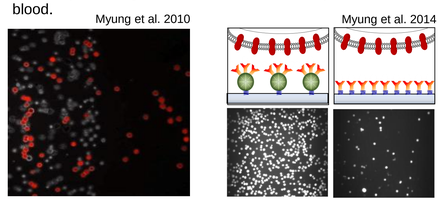


The use of CTCs as clinical markers for cancer is complicated by their relative rarity and the difficulty in separating them from white blood cells (WBCs) and other nucleated cells in the blood stream.

Biomimetic cell capture

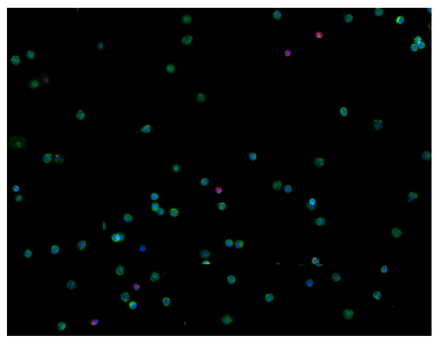


Cells are flowed over an surface coated with E-selectin, which induces biomimetic rolling. This action slows and purifies white blood cells and CTCs. Next, a nanostructured surface containing dendrimer nanoparticles presents a high density of antibodies against tumor cells. These nanoparticles promote multivalent interactions, which greatly enhance sensitivity and specificity resulting in unmatched CTC counts per mL of whole blood.



tumor cells and WBCs in rolling domain | purified tumor cells in capture domain | enhanced capture efficiency with dendrimer-mediated multivalent immunorecognition

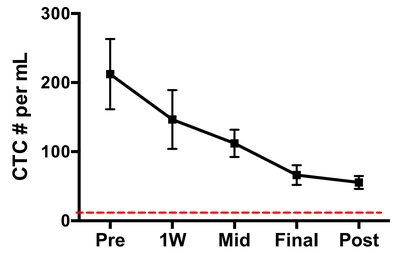
CTC identification and enumeration



Partial image of the capture surface. CTCs are identified as positive for cytokeratin (red) and negative for CD45 (green), with additional restrictions on size, shape, and nuclear positioning.

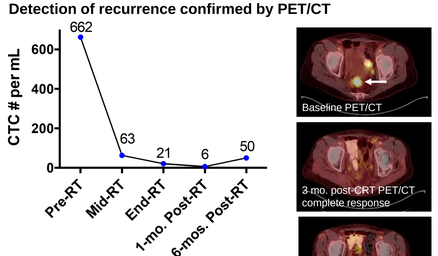
Enumeration of clinical samples

CTC numbers correlate with treatment stage, on average



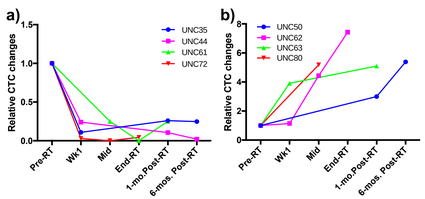
An overall decrease in CTC numbers indicates successful radio-therapy treatments for stage 4a head and neck cancer patients (n=21).

Detection of recurrence confirmed by PET/CT



Rectal squamous cell carcinoma, clinical status: Locoregional recurrence after treatment with 54 Gy radiation + MMC/5-FU chemotherapy.

Relative CTC counts of four patients with oligometastatic cancer responding to treatment (a) and four patients not showing a response (b).

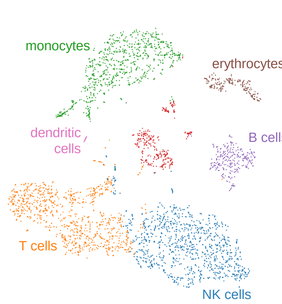


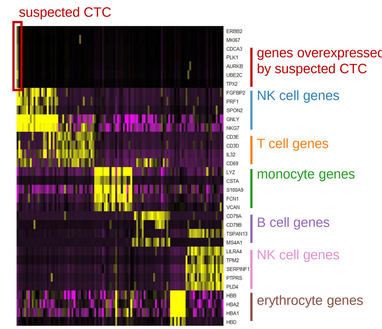
We currently have four IRB-approved studies at UW (2017-0129, 2016-1441, 2016-1555, and 2017-1273) and one at Capio Biosciences (NEIRB third party approval), receiving blood samples in collaboration with clinicians at UW, UNC, Duke University, and the University of Iowa.

Downstream analysis with RNASeq

Cells were recovered from six CapioCyte™ chips by high shear (1 ml/s). Released cells had an estimated 95.5% viability based on trypan blue staining of cell lines. Comparison of clinical samples processed in parallel (one immunostained and counted, one for RNASeq) showed that a median of 86% of CTCs were released from the surface (high 100, low 78). The suspended cells were transported to the UW Gene Expression Center for single cell RNA Sequencing using the 10X Genomics Chromium System. A median of 5472 cells (high 8461, low 1157) out of a targeted 10,000 were successfully paired with a barcoded bead. We observed a median of 257 reads per cell (high 1434, low 128) with read quality of 89.9% (high 92.9, low 87.8). Four of the six samples were selected for full sequencing (Illumina NovaSeq) based on estimated CTC abundance in the sorted samples (high 10.2 CTCs, low 0.5).

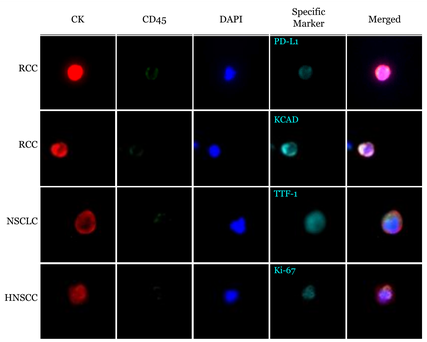
Barcoded cells were clustered using k-means clustering and cloupe software from 10X genomics, pictured above in an annotated tSNE plot. This sample shows a typical distribution of mononuclear white blood cells with some erythrocyte contamination (5.7% of barcodes). A single suspected CTC was identified by expression of HER2 and a gene tied to proliferation (MKI67).





The cell was then processed along with twenty randomly-selected cells from each previously-identified cluster, and clustered according to highly variable genes. Genes identified within the suspected CTC in this sample were associated with proliferation and WNT signaling.

Identification of tumor markers with clinical relevance



Protein expression is quantified and correlated with cytokeratin staining in clinical blood samples using four-channel immunofluorescent microscopy.

Conclusions

The CapioCyte™ chip effectively captures CTCs for quantification and downstream analysis requiring viable cells such as RNASeq. The liquid biopsy technology has great potential to contribute to diagnosis and personalized treatment of cancer.

References

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Acknowledgements

Sequencing was performed at the University of Wisconsin Biotechnology Center. Tyler Duellman and Sandra BonDurant responsible for scRNASeq preparation and Derek Pavelec contributed to sequencing and data interpretation.

This work was supported through DRP program of UW HN SPORE (P50DE026787) from NCI and NIDCR and by the NCI through SBIR grant 1R43CA232924-01..