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RARECYTE

Feasibility of Assessing Circulating Tumor Cells in Patient-Derived Xenograft Tumor Models

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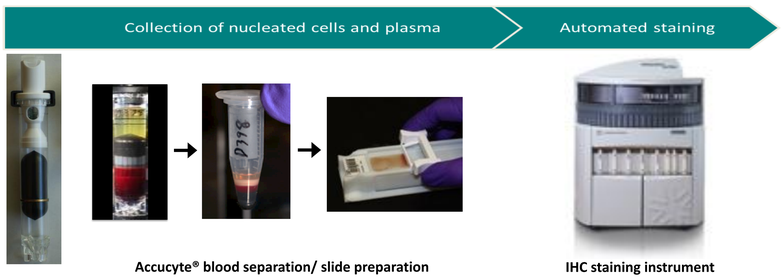
Abstract 646

Background

Patient-derived xenograft (PDX) tumor models more accurately reflect human tumor biology than traditional cancer cell lines or their derived xenografts. Preclinical studies in cohorts of PDX lines emulate clinical trials, with each line representing a unique "patient". Stemcentrx has developed more than 600 PDX lines from various cancer types to use in development of novel therapies directed against the cancer stem cells, which can initiate tumors and drive recurrent disease. In order to better understand the biology of metastasis – the primary cause of cancer mortality – a newly developed RareCyte method for detection of human circulating tumor cells (CTCs) in the blood of tumor bearing mice was independently validated.

Study Design

For platform validation studies, cells from 3 distinct PDX tumor models were counted and spiked into human blood at approximately 10, 50, 200 or 5000 per 7.5 mL. Blood was processed using AccuCyte cell separation and collection (RareCyte) and the nucleated cells spread onto 8 microscope slides. Slides were stained with anti-human cytokeratin, EpCAM and CD45 antibodies and DAPI (nuclear stain) using an automated instrument. A single slide from each spike-in tube was imaged by the CyteFinder digital fluorescence scanning microscope (RareCyte). Cells with morphology and phenotype (cytokeratin+/EpCAM+, CD45–) consistent with human cancer cells were tallied after review of images and compared to expected counts.

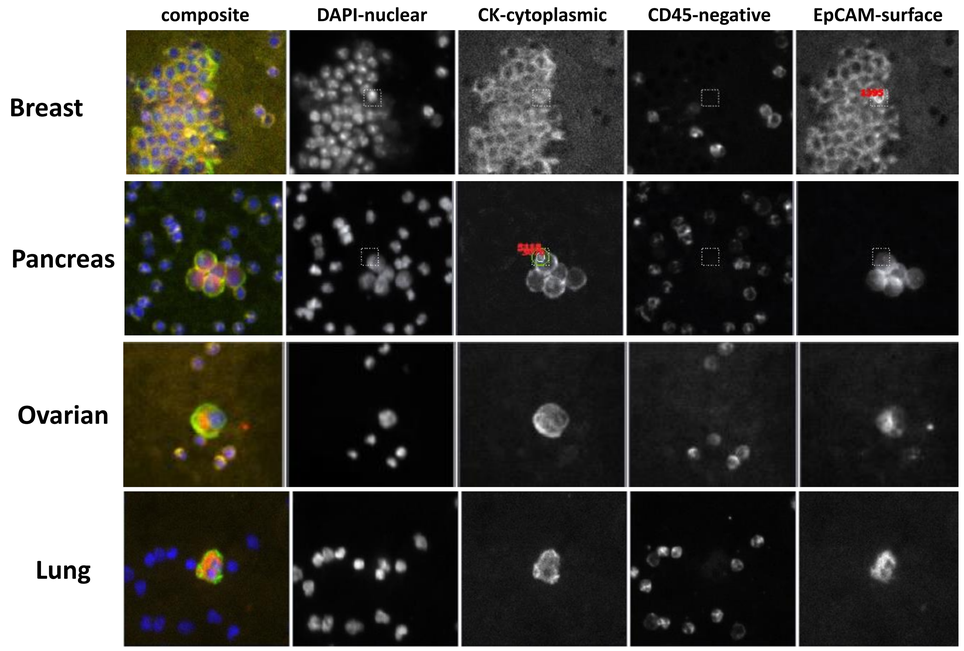


For mouse PDX feasibility studies, 0.5- 1 mL of blood from PDX tumor-bearing mice (~150-2000 mm3) was collected by cardiac puncture. After red cell lysis and washing, nucleated blood cells were spread onto one slide. Cells were stained, imaged and identified as above substituting anti-mouse CD45.

Spike in Experiment Resulted in >90% Cell Recovery in 3 independent PDX models

PDX	LU187			LU149			OV217	Whole blood
spike in	200	50	10	200	50	10	5000	-
expected recovery/slide	25	6	1	25	6	1	625	0
recovery/slide	36	5	2	20	5	1	614	0
# cell cluster	3 (>5 cells)	1 (10 cells)	-	-	-	-	-	-

Circulating Tumor Cells (CTCs) were Detected in Breast, Pancreatic, Ovarian and Lung Cancer PDX models



Conclusion

- There was a high correlation between the expected and experimental counts in the validation study (R= 0.99) that was independent of spike in number
- Regression analysis gave a line of fit with slope 0.95, suggesting 95% recovery efficiency
- CTCs were identified by human epithelial cell marker staining in several different PDX lines.
- Number of CTCs is dependent on PDX line and ranged from 3 to 1000 CTCs
- CTCs ranged from single cells to clusters of cells (>100 cells) and was dependent on PDX lines
- There is no correlation between the number of CTCs and the duration of tumor in mice
- CTCs were detected in PDX models known to spontaneously metastasize
- There is a positive correlation between number of CTCs and size of lung mets.

CTCs were detected in metastatic Breast PDX models. There was a positive correlation between number of CTCs and size of lung mets present.