CTCs and CTC clusters in breast cancer patient-derived xenograft models

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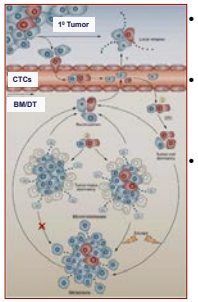
ABSTRACT

Breast cancer (BC) patient-derived xenograft (PDX) models represent a continuous and reproducible source of circulating tumor cells (CTCs). Using various BC PDX models, we describe the utility of CTCs and CTC clusters in detecting tumor-specific mutations and our preliminary results in understanding their predictive value for treatment response and long-term outcomes. CTCs were detected in 300-450 µl of blood of PDX-bearing mice using the RareCyte technology adapted for small blood volume. CTCs were isolated without cell surface marker-based enrichment and identified them as DAPI$^+$, human Cytokeratin (CK)$^+$, and mouse CD45$^-$. Collective expression of cell surface markers (EpCAM, EGFR, and HER2) was assessed using a cocktail of target-specific antibodies in CTCs and primary PDX tumors. Individual CTCs and tumor cells from primary and metastatic tumors were isolated using CytePicker® for single cell analysis of tumor-specific mutations. Single CTCs (1-41 per mouse) and CTC clusters (1-2 per mouse) were detected in the blood of one ER+/PR+/HER2- (BCM-4888) and two triple-negative BC (TNBC, BCM-4272 and BCM-3887) PDX models. The *PIK3CA* T1035A mutation found in primary tumors of BCM-4888 was also detected in isolated CTCs and PDX primary and metastatic tumor cells. As a proof-of-principle experiment, we have evaluated numbers of single CTCs and CTC clusters at baseline and after treatment with 4 weekly cycles of vehicle (N=5-6) or chemotherapy regimens (N=2-3) [docetaxel or carboplatin or their combination] in TNBC PDX models BCM-4272 and BCM-3887. Preliminary analysis from these studies suggests dynamic and differential effects of chemotherapy regimens on single CTCs and CTC clusters, potentially reflecting the genetic characteristics of tumors and their unique response to the selected chemotherapy agents. Ongoing experiments in additional mice and PDX models will determine the predictive role of CTCs and CTC clusters in treatment response and long-term outcomes such as recurrence-free survival. In conclusion, we have demonstrated that RareCyte technology detects CTCs from small volumes of blood without the use of cell surface marker-based enrichment method. Furthermore, CTCs and CTC clusters can be used to assess the presence of tumor-specific mutations. Ongoing studies will fully reveal the potential of CTCs and CTC clusters as surrogate markers of treatment response and outcomes within PDX models.

BACKGROUND & OBJECTIVE



- Circulating tumor cells (CTCs) are considered precursors to metastases
- CTC clusters, defined as a group of 2 or more CTCs, are thought to contribute to higher risk of metastases.
- Here, we describe the utility of CTCs and CTC clusters in detecting tumoral mutations and our preliminary results in a preclinical chemotherapy trial using breast cancer patient-derived xenograft models.

Adapted from Pantel et al., *Nat Rev Clin Oncol*, 2009.

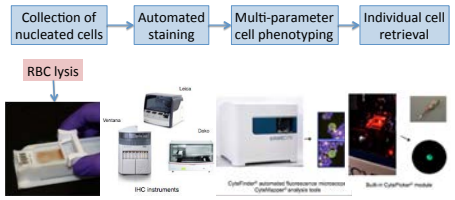
Table 1: CTC detection in selected BC-PDX models.
Adapted from Giuliano et al., *Breast Cancer Res*, 20015.

PDX line	CTC detection rate (%)	# of CTCs per 20K nucleated cells*	Lung metastases rate (%)
BCM-3887	3/4 (75)	3-92	14
BCM-4272	3/3 (100)	<1-25	29
BCM-4888	5/5 (100)	3-28	67

* 20K nucleated cells typically represented ~20µl blood
Abbreviations: 20K- 20,000; BC- breast cancer; CTC- circulating tumor cells; PDX- patient-derived xenograft

METHODS AND TECHNOLOGY

Figure 1. Workflow for processing blood samples from BC-PDX-bearing mice.



RESULTS

Figure 2. Representative single channel and composite images of CTCs isolated from BC-PDX-bearing mice.

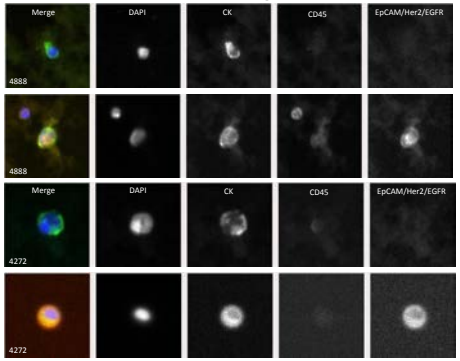


Image shows morphology of recovered CTCs. Column 1 is a composite image; column 2 is DAPI staining of nuclei; column 3 is human CK staining; column 4 is mouse CD45 staining; and column 5 is staining with a cocktail of EpCAM, EGFR and HER2.

Table 2: Detection of CTCs and CTC clusters in PDX models.

PDX Model	Mouse	Tumor size (mm^3)	Blood (µl)	CTCs	CTC clusters
BCM-4888 (ER+/PR+/HER2-)	1	345	330	6	1
	2	293	400	2	0
	3	361	400	6	1
	4	298	400	0	0
	5	338	330	2	1
	6	375	400	1	0
BCM-4272 (ER-/PR-/HER2-)	1	282	500	1	0
	2	292	415	3	0
	3	330	490	41	2
	4	337	470	12	1
	5	371	500	11	1
BCM-3887 (ER-/PR-/HER2-)	1	NA	520	0	0
	2	NA	520	0	0
	3	1,544	450	2	0
	4	1,104	450	3	0
	5	776	400	0	0

Figure 3. Analysis of *PIK3CA* T1035A mutation in CTCs, primary tumors, and lung metastases from BC-PDX model BCM-4888. Whole genome amplification was conducted on single cells using Rubicon PICOPlex® kit, followed by Nested PCR for PIK3CA mutation, running on an agarose gel, and then sequencing.

Xenograft Line	Transplant generation	Mutation Detected
BCM-4888	TG1	PIK3CA_N345K_T1035A

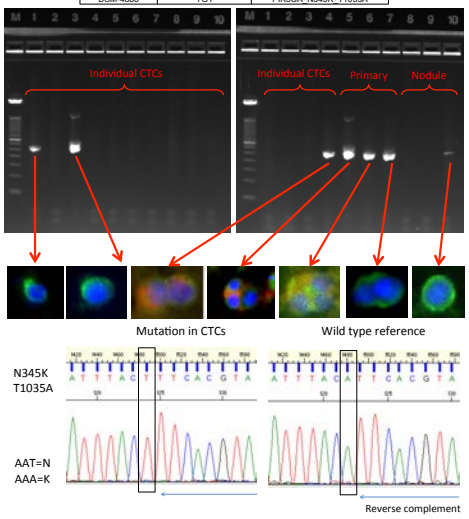


Figure 4. IHC analysis of mammary PDX tumors of BCM-4888 and BCM-4272 models. FFPE blocks were sectioned and evaluated for ER/HER2 dual staining or PR, EpCAM, Ki67, or EGFR staining.

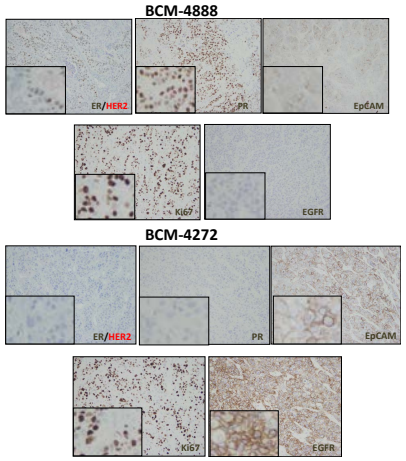
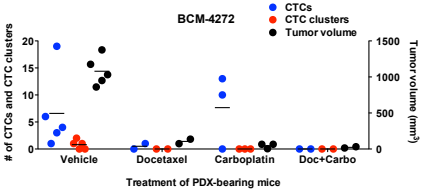
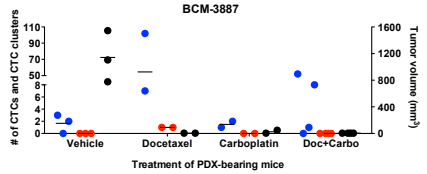


Table 3: Comparison of cell surface marker (EpCAM, EGFR, HER2) expression between CTCs, primary tumor, and lung metastases in BCM-4888 and BCM-4272 models (N=3).

	Number (%) of cells positive for cell surface markers			
PDX model	CTCs	CTC clusters	PDX mammary tumors	PDX lung metastasis
BCM-4888	8/11 (73%)	2/2 (100%)	87/87 (100%)	80/85 (94%)
BCM-4272	5/48 (10%)	0/6 (0%)	NA	NA

Figure 5. Single CTCs, CTC clusters, and tumor volume in 2 PDX models treated with various chemotherapy regimens. Mice were randomized to receive either docetaxel 20-30 mg/kg; carboplatin 50 mg/kg; or their combination. The treatment was given via intraperitoneal route every week for 4 weeks. Tumor volume, CTC, and CTC clusters were evaluated at the end of 4-week treatment (N= 2-5 in each treatment group).





DISCUSSION

1. Biomarker characterization and genomic analysis of individual cells from primary and metastatic tumors as well as circulating tumor cells has been automated using Rarecyte technologies.
2. We have found dynamic and differential effects of chemotherapy regimens on single CTCs and CTC clusters, potentially reflecting the genetic characteristics of tumors and their unique response to the selected chemotherapy agents.

CONCLUSIONS & FUTURE DIRECTIONS

- CTCs and CTC clusters can be used to assess the presence of tumor-specific mutations.
- Ongoing studies will fully reveal the potential of CTCs and CTC clusters as surrogate markers of treatment response and outcomes within PDX models.