EpCAM-positive Circulating Tumor Cells (CTCs) retain long-survive and migratory capacity following xeno-transplantation

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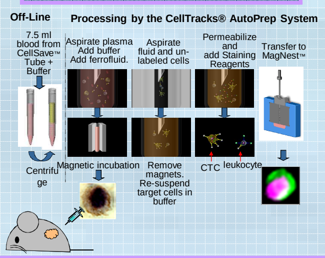
Background

The current technologies' advances allowed to demonstrate an inverse correlation between Circulating Tumor Cells (CTCs) burden and overall survival in solid tumors; changes in CTCs count has been associated to significant change in prognosis as early as the first treatment cycle. Despite these clinical evidences, the tumorigenic potential of epithelial cells rescued from peripheral blood of cancers patients remains to be provided. Several technical and conceptual hitches constraint a definitive successful demonstration of the CTC role in metastatic process, including the lack of an adequate niche to harbor their growth and a consensus about the "gold standard" method to isolate these rare cells.

To address these questions, we investigated whether CTCs isolated ex vivo from metastatic prostate and breast cancer patients are able to growth in NOD/SCID mice.

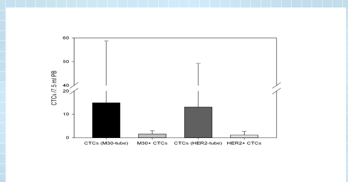
Methods



At present, between the numerous manual or semi automated methods reported to enrich and/or isolate CTCs, only the CellSearch automated platform obtained the FDA approval to be used in clinical settings.

Therefore, we chose this system for CTC counting and parallel enriching. For the enumeration, an event was classified as a CTC when its morphological features were consistent with those of a cell and it exhibited the phenotype EpCAM+, CK+, DAPI+, and CD45-

Between-assay Variability



The plot shows total CTC number (black histograms) measured in a 22 series of MBC patients that undergoes two parallel tests for M30 expression (M30-tube) and HER2 expression (HER2-tube) on CTCs. The total CTC level did not significantly differs in the two test tubes (Wilcoxon signed rank test, p = 0.706), indicating high reproducibility.

Patients' Data

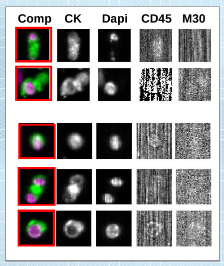
Table 1: Clinical and pathological characteristics of CTC donor cancer patients and CTC detection in xenotransplants

			patients' data									xenograft data		
#	age	sex	histology	T	N	M	Gleason score	site of metastasis	*CTC/huPB (%M30+)*[a]	*OS*[b]	*disease status*	*CTC/muPB*[c]	*DTC/muBM*[d]	*IHC*[e]
1	60	M	prostate adenocarcinoma	-	-	-	ND	bone	66 (2%)	297	AWD	8	1	*pos*
2	78	M	poorly differentiated prostate adenocarcinoma, with neural invasion, G3/G4	-	-	-	7 (3+4)	bone	264 (2%)	105	DOD	2	2	*pos*
3	39	F	Metastatic Breast Cancer	-	-	-	ND		402 (1%)	235	AWD	7	*neg*	*pos*
4		M	prostate adenocarcinoma, with neural invasion	2a	0	x	9 (4+5)	bone, LN	51 (6%)	270	DOD	8	3	*pos*
5	72	M	prostate adenocarcinoma, with neural and vascular invasion, G2	2	0	x	7 (3+4)	bone	2866 (0,2%)	404	AWD	8	1	*pos*
6	68	M	prostate adenocarcinoma, G3	-	-	-	8	bone	253 (3%)	384	AWD	10	*neg*	*neg*
7	see # 2	see # 2	see # 2	-	-	-			224 (0%)			42	2	*neg*
8	56	F	Metastatic Breast Cancer	2	2a	0	ND	bone, liver, LN, lung	207 (2%)	166	DOD	6	2	*pos*

a: Two blood draws were collected from each patient at baseline: the first was devoted to determine the CTC count and the percentage of apoptotic (M30+) CTCs, the second was used for the xenograft assay. The number indicates CTC no./7.5 ml as determined by CellSearch in human peripheral blood (huPB).
b: Overall Survival (OS) : days between the CTC assessment and the documentation of the death or the most recent follow-up evaluation
c: At the time of euthanasia (median 10.3 months, range 6.5-12 months), murine peripheral blood (muPB) was collected to detect the CTCs of the donor. The number indicates CTC no./0.75 ml as determined by CellSearch in muPB. None of the age-matched control mice (n=6) was CTC-positive.
d: At the time of euthanasia murine bone marrow (muBM) was also collected to detect the Disseminated Tumor Cells (DTCs) of donor origin. The number indicates DTC no./0.75 ml as determined in muBM by CellSearch. In the control group (n=6) the DTC mean was 0.4 ± 0.9 (median 0.0, range 0-2 DTCs).
e: Results of IHC for human Cytokeratin (1:50 dilution, clone MNF116, DAKO) in mice spleens.

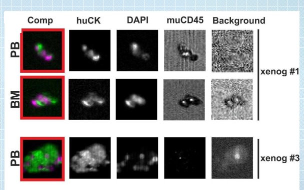
Eight CTC xenografts were then derived from 7 patients, consecutively enrolled; their clinical-pathological characteristics are summarized in Table 1 (patients' data). Sequential enrollment was based on the availability of both a baseline CTC count at metastatic disease diagnosis (at least 50 CTCs/7.5 ml PB) and an informed consent for an additional blood draw to perform the xenograft assay. After baseline assessment, the disease status was evaluated depending on the type and schedule of treatment.

Human CTC before injection



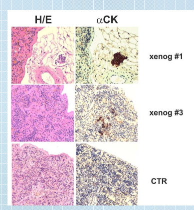
We detected live and apoptotic CTCs by integrating the CTC assay with an anti-M30 mAb, that binds a neoepitope disclosed by caspase cleavage of cytokeratin 18 (CK18) in early apoptosis. Before CTC injection the great majority of the CTCs was live.

Human CTC and DTC in mice



The presence of human CTCs in murine peripheral blood (PB) and of human disseminated tumor cells (DTCs) in murine bone marrow (BM) was assessed by CellSearch, adapting the standard procedure to small volumes. Quantitative results were expressed per 0.75 ml of blood for both CTCs and DTCs.

Neoplastic cells within the spleen of xeno-transplanted mice



The picture shows Haematoxylin and Eosin (H&E) staining (left panel) and pan-cytokeratin immunostaining (right panel) of murine spleens; original magnification, x20.

Xenog #1: small neoplastic emboli found into perisplenic small vessels (pt. #1 in Table 1). Xenog #3: pan-cytokeratin immunostaining disclosed small groups of anti-human cytokeratin positive cells (undetectable at the H&E staining) into the spleen red pulp (pt. #3 in Table 1).

CTR: Spleen from control mice does not display any morphological (left picture) or immunohystochemical (right picture) evidence of anti-human cytokeratin positive cells.

Conclusions

The procedure for enrichment and injection of CTCs appears to be highly efficient. We found human CTCs in muPB, muBM and spleen samples but we did not find signs of tumor in any inspected organ or at the injection site. These findings provide evidences that in our xenograft assay the EpCAM-positive fraction of CTCs rescued from prostate cancer patients and breast cancer patient retains as a peculiar characteristic the impressive migratory capacity, and long-survive capacity. We cannot exclude that the higher efficiency of our xenograft assay may be simply due to the use of an automated platform to enrich EpCAM-positive CTCs from PB.

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