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ClearCell® FX1 System

Enrichment Solution for Circulating Tumour Cells

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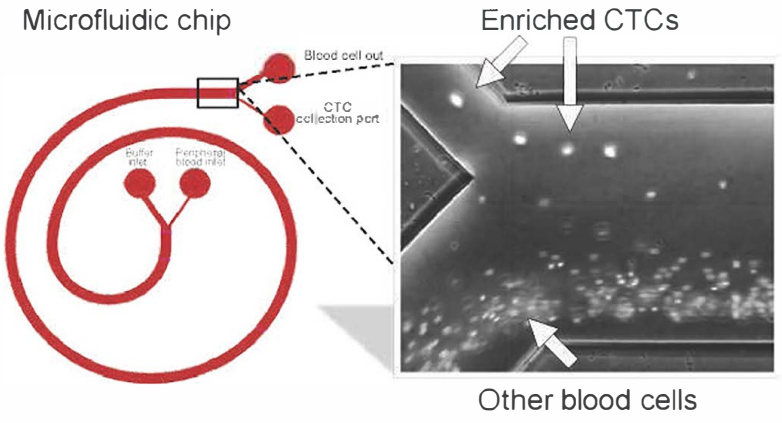
Product Introduction

ClearCell® FX is driven by the proprietary microfluidic CTChip® FR. It is one of the world's first automated system that can rapidly and efficiently enrich circulating tumour cells (CTCs) from the patients' blood. By leveraging on the process of Dean Flow Fractionation (DFF), CTCs can be isolated based on size, deformability and inertia relative to other blood components.

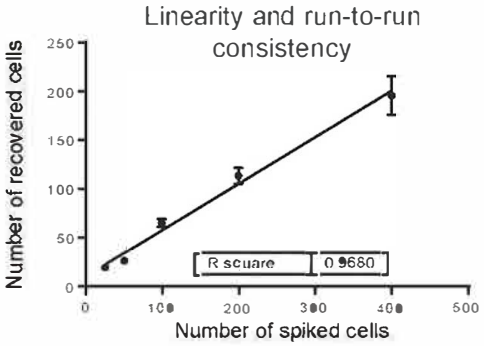
Through this process of DFF, blood cells are distributed by themselves within the channels, with the larger cells along the inner wall and the smaller cells away from the inner wall. This allows for effective and fast separation without compromising on the quality of the retrieved cells.

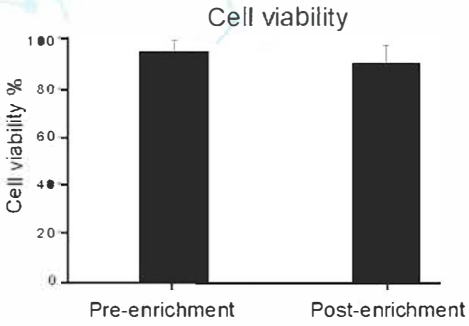


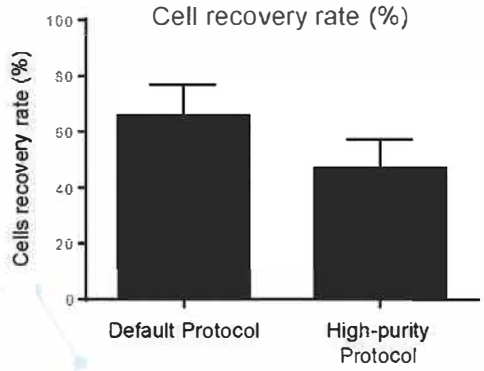
Unique Selling Proposition

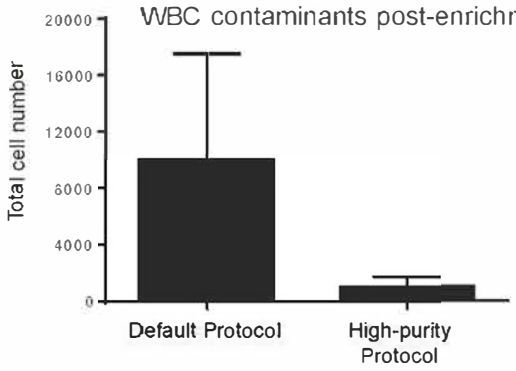
1. Fully automated CTC enrichment completed within 1 hour.
2. High CTC recovery rate - Maximum sensitivity in integrated diagnostic assay.
3. High-purity of CTCs - >99.99% removal of white blood cell.
4. High cell viability - Cell viability is about 90% after ClearCell FX® enrichment.
5. "Label-free" approach - Unbiased enrichment of heterogeneous cancer cells, from different cancer types.
6. Both cfDNA and CTC can be analyzed from a single 10 mL tube of blood.

System Performance Parameters



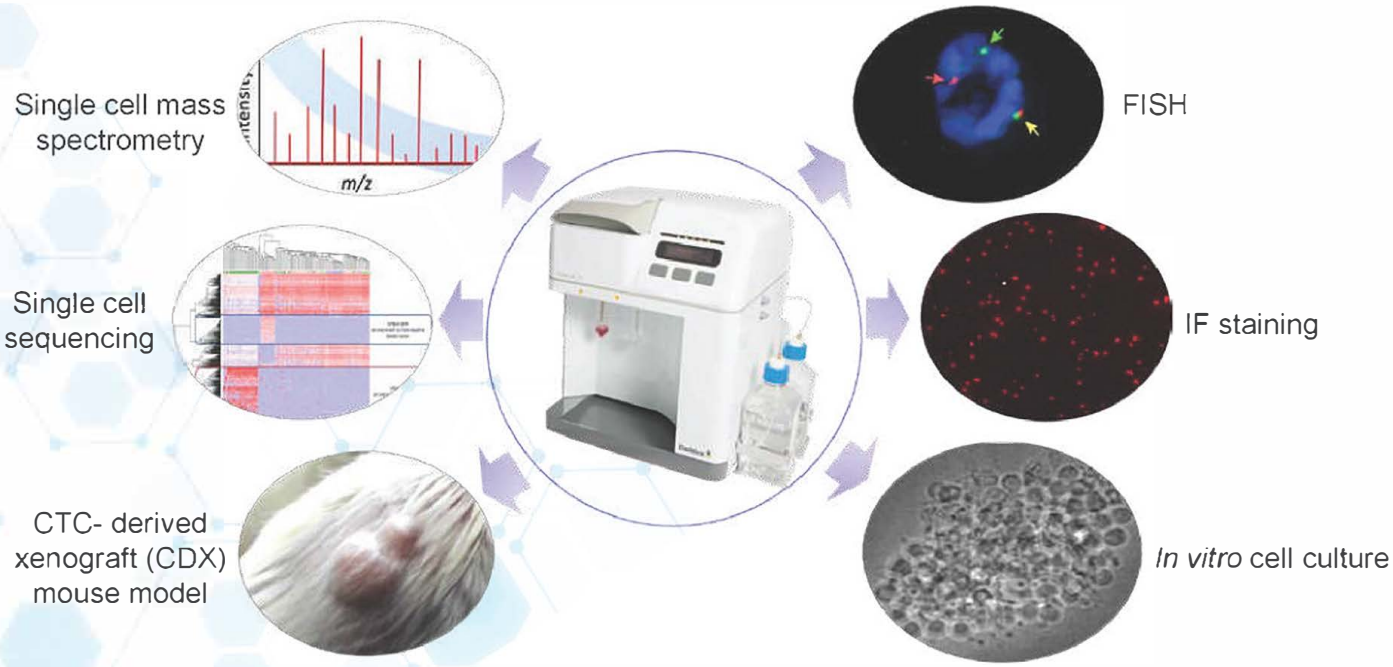






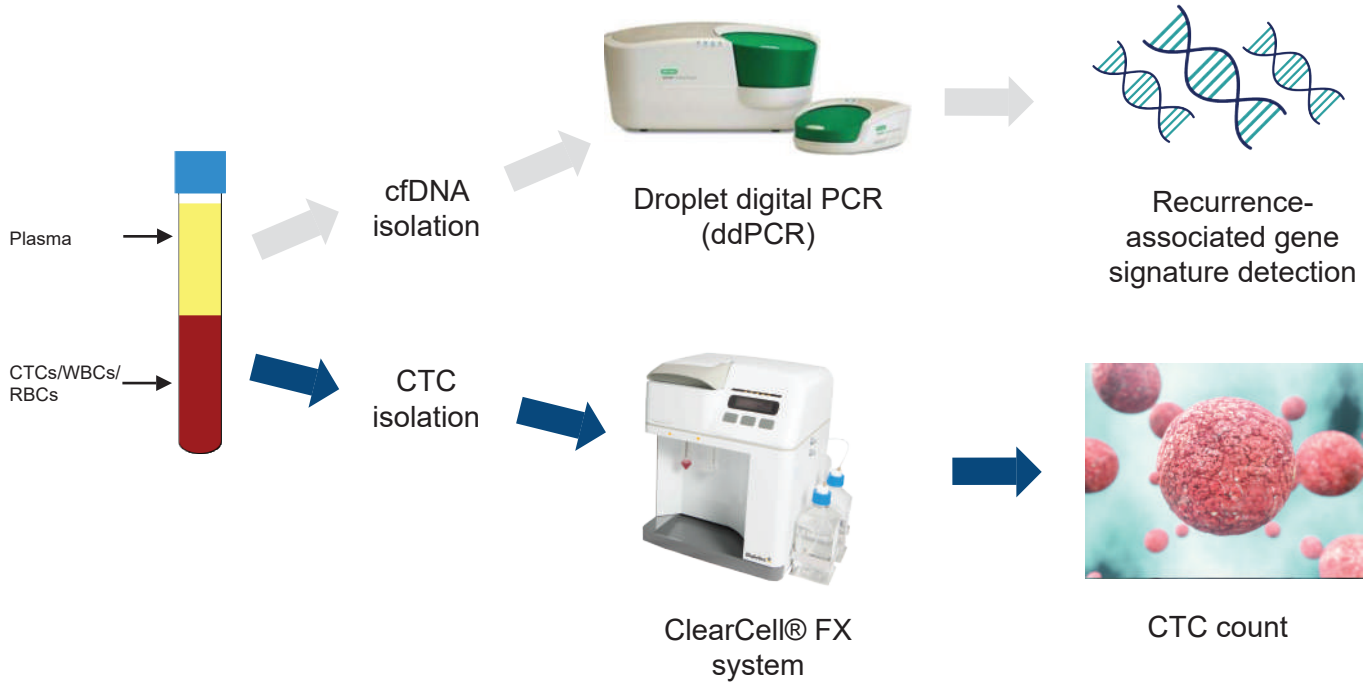
Wide Range of Applications

CTCs isolated by ClearCell® FX system are label-free, intact and viable, which enables cancer discovery as well as better patient management.

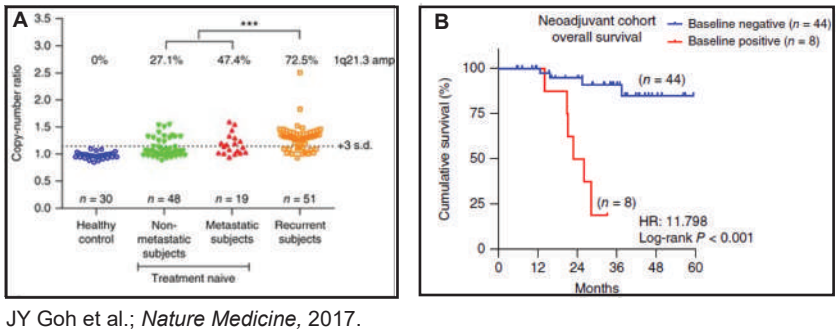


2. Breast Cancer Remission Monitoring

Workflow for combined CTC and cfDNA analysis

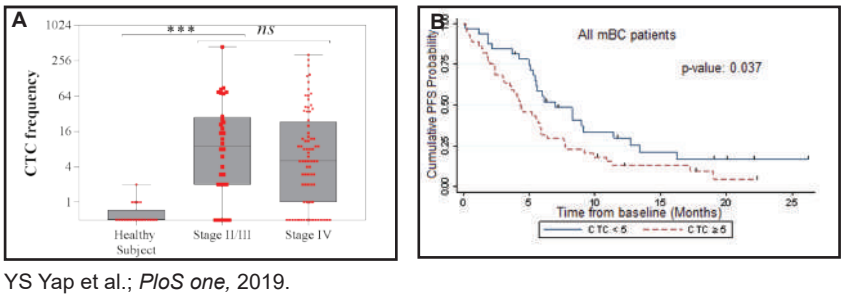


Clinical Evidence



JY Goh et al.; *Nature Medicine,* 2017.

Fig. 1: 1q21.3 detected in cfDNA is a strong prognostic biomarker for recurrence in breast cancer
(A) Copy number ratio of 1q21.3 is significantly higher in recurrent patients as compared to non-recurrent patients
(B) Patients with 1q21.3 amplification is associated with poor overall survival



YS Yap et al.; *PloS one,* 2019.

Fig. 2: CTC frequency is associated with poor survival outcome in breast cancer patients
(A) CTCs are detected in breast cancer patients from Stage II- IV
(B) Metastatic breast cancer (mBC) patients with 5 or more CTCs / 7.5 mL of blood are associated with poor progression-free survival (PFS)

Selected Publications

1. Single-cell gene expression profiling of patient-derived CTCs for recurrence prediction

Addressing cellular heterogeneity in tumor and circulation for refined prognostication. PNAS (2019)

- Specific gene signatures with distinct gene expression profiles in CTCs from patients with differing metastatic potential were identified by single cell CTC analysis (Fig. 1A)
- The use of CTC-derived gene expression signature further refines a prognostic risk model in predicting recurrence in non-small cell lung cancer that takes into account intratumor heterogeneity (ITH) (Fig. 1B)

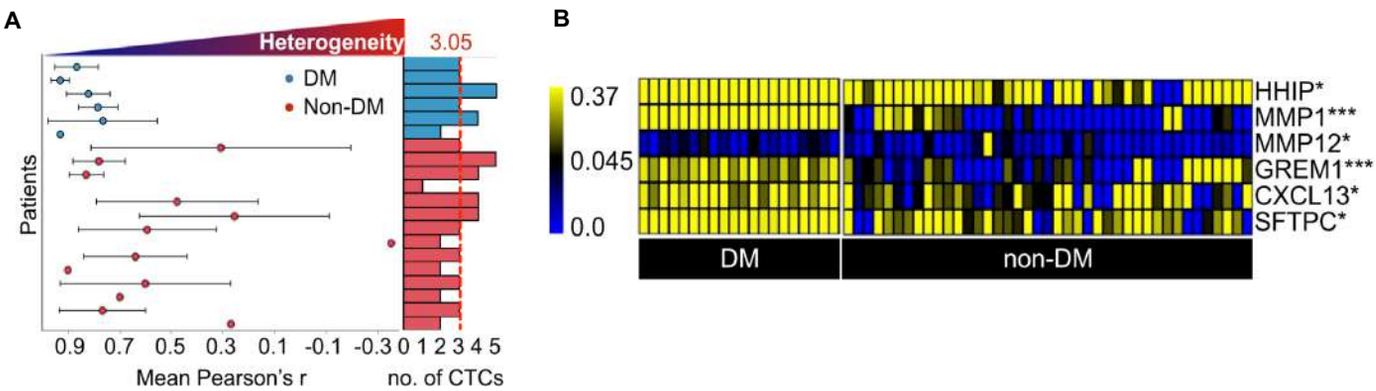


Fig. 1: A. Heterogeneity in 15-gene matrisome expression across all CTCs detected within the same patient with (blue) or without (red) distant metastases (DM) **B.** Distinct signature gene expression profiles in patients with (DM) and without (non-DM) distant metastases

2. Next Generation sequencing (NGS)

Succinct workflows for circulating tumor cells after enrichment: From systematic counting to mutational profiling. PLOS ONE (2017)

- Immunofluorescence-based CTC enumeration workflow established with 80.4% sensitivity and 85.7% specificity in 56 cancer patients and 21 healthy donors (Fig. 2)
- Next-generation sequencing (NGS) workflow integrated to detect somatic mutations in CTCs (Table 1)

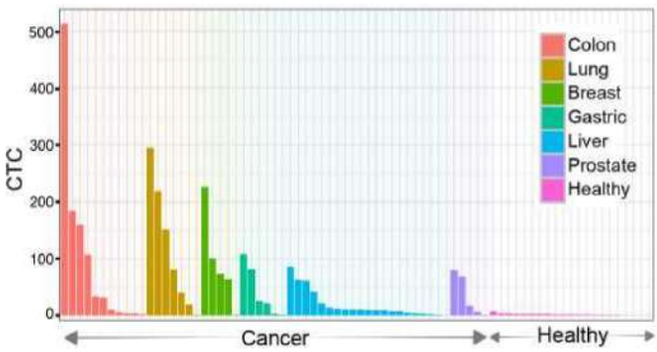


Fig. 2: No. of CTCs per 8mL of blood detected in individual cancer patient and healthy donor

Sample	CTC number	Genomic coordinate	Mutation	Somatic *p* value in technical repeat (coverage)
Patient-12	10	Chr17: 7573937	A364S	***Run 1***
				$p = 1.8 \times 10^{-7}$ (448952)
				Run 2
				$p = 1.9 \times 10^{-3}$ (335959)
Patient-19	45	Chr6: 152419988	S559A	***Run 1***
				$p = 7.9 \times 10^{-4}$ (57352)
				Run 2
				$p = 0.023$ (62598)

Table 1. Sequencing results of CTCs from patients

Selected Publications

3. Single cell metabolic profiling for biomarkers discovery

Live single cell mass spectrometry reveals cancer- specific metabolic profiles of circulating tumor cells. Cancer Science (2019)

- Metabolomic profile of single CTCs obtained from gastric cancer (GC) and colorectal cancer (CRC) patients were analysed using live single cell mass spectrometry
- Cancer origin-specific biomarkers and metabolic profiles were elucidated in gastric cancer and colorectal cancer CTCs (Fig. 3A & 3B)

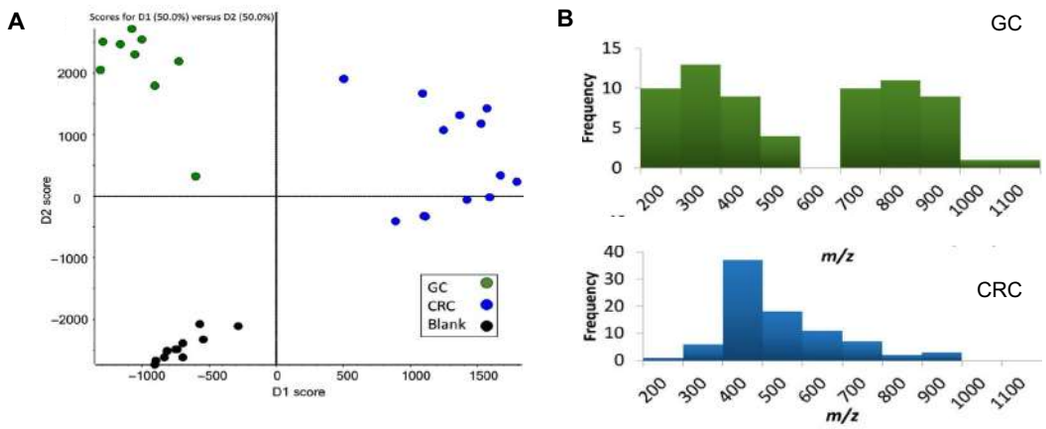


Fig. 3: A. Principle component analysis (PCA) clustering of single cell profiles of CTCs from gastric cancer (GC) and colorectal cancer (CRC) **B.** Histogram of the frequency of peak distribution across m/z scale in GC and CRC

4. Patient-derived xenograft mouse model for drug resistance analysis

Xenograft tumors derived from malignant pleural effusion of the patients with non-small-cell lung cancer as models to explore drug resistance. Cancer Communications (2018)

- Malignant tumor cells were isolated from the pleural fluid of two non-small cell lung cancer (NSCLC) patients using the ClearCell® FX system and subcutaneously inoculated into female CB17-SCID mice to generate xenograft tumor model (Fig. 4)
- Drug-resistant (crizotinib or osimertinib) xenografts were generated by prolonged treatment with the drugs
- Whole exome sequencing (WES) showed that while the genotypes of xenograft and patient tumor are similar, acquired somatic mutations can be identified in drug-resistant xenografts (Table 2)

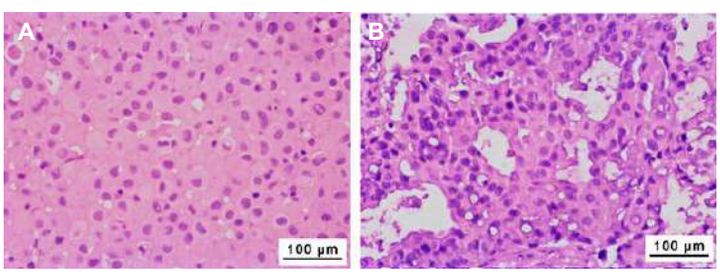


Fig. 4: H&E staining of the tumor biopsy from **A.** patient and **B.** xenograft tumor with matched histology

Sample	Key mutations
Biopsy from patient CTC15035$_{EML-4-ALK}$	EML4–ALK fusion (EML4 exon18–ALK exon 20)
Crizotinib-6 xenograft	EML4–ALK fusion (EML4 exon18–ALK exon 20) ALK: E1210K (9%)
Biopsy from patient CTC15063$_{EGFR, L858R,T790M}$	EGFR: L858R (85.7%) T790M (71.5%)
Osimertinib-3 xenograft	EGFR: L858R (53.6%) , T790M (41.7%); PIK3C2A: R86fs (11%); BRAF: G7V (11.5%)

Table 2. Sequenced mutations in patient tumor and drug-resistant xenografts. Drug-resistance associated mutations are shown in red

Selected Publications

5. *In vitro* drug sensitivity testing

Detection of CTCs in portal vein was associated with intrahepatic metastases and prognosis in patients with advanced pancreatic cancer. Journal of Cancer (2018)

- The portal vein or peripheral blood samples from 29 patients with advanced pancreatic cancer were processed using ClearCell® FX system. CTCs counts in the portal vein were significantly higher as compared to peripheral blood
- The overall survival was significantly shorter in patients with portal vein CTCs over 150 per 7.5 mL of blood than those portal vein CTCs less than 150 per 7.5 mL of blood (Fig. 5A)
- *In vitro* drug sensitivity testing showed that CTCs derived from portal vein blood were highly resistant to several chemotherapy regimens (Fig. 5B)

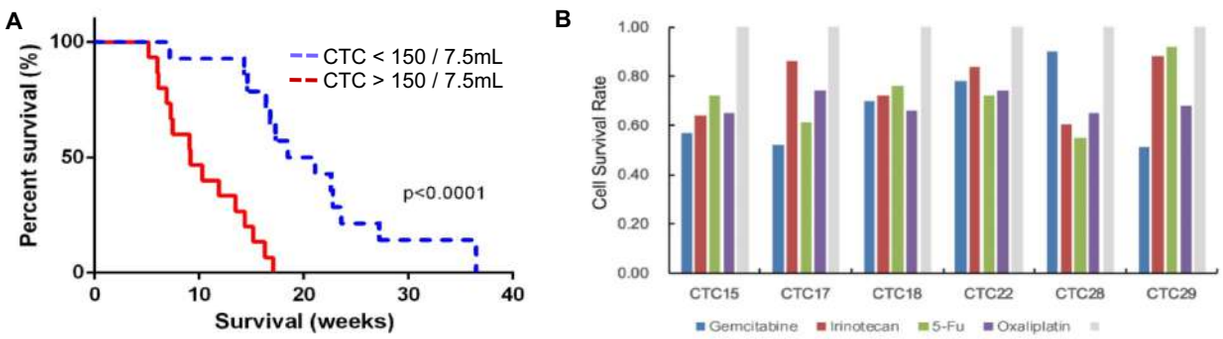


Fig. 5: A. Overall survival of patients with portal vein CTCs above or below 150 CTCs/7.5mL **B.** Drug sensitivity of portal vein CTCs

6. Fluorescent *in-situ* hybridization (FISH) for personlised treatment and monitoring

Concordance of Anaplastic Lymphoma Kinase (ALK) Gene Rearrangements Between Circulating Tumor Cells and Tumor In Non-small Cell Lung Cancer. Oncotarget (2016)

- Anaplastic lymphoma kinase (ALK) gene rearrangement in CTCs from non-small cell lung cancer (NSCLC) was evaluated by fluorescent *in-situ* hybridization (FISH) hybridization
- Over 90% concordance rate in ALK rearrangement pattern was observed between CTCs and primary tumor tissues
- An index case suggests that ALK-positive rearranged CTCs can dynamically monitor efficacy of crizotinib treatment and disease progression

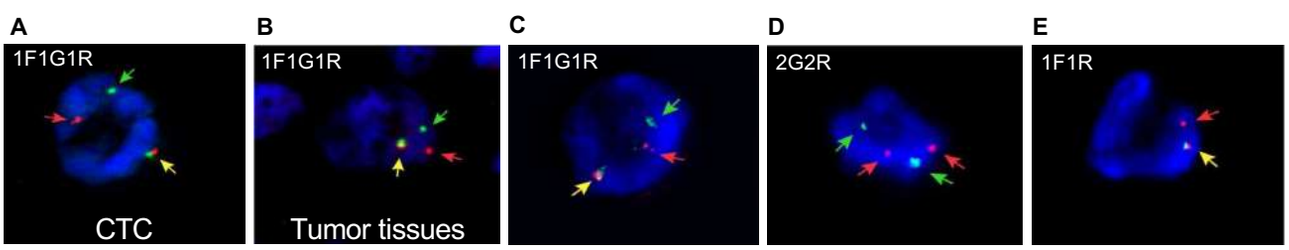


Fig. 6: Representative FISH images showing concordant ALK F1G1R rearrangement in **A.** CTC and **B.** tumor tissue. **C.**, **D.** & **E.** show representative FISH images of multiple ALK rearrangement patterns in a patient with progression after crizotinib treatment.

Publications

Breast Cancer

YS Yap et al. **"Detection and prognostic relevance of circulating tumour cells (CTCs) in Asian breast cancers using a label-free microfluidic platform."** *PloS one,* vol. 14, no. 9, 2019. doi: 10.1371/journal.pone.0221305.

Qian, Chen, et al. **"Impeding Circulating Tumor Cell Reseeding Decelerates Metastatic Progression and Potentiates Chemotherapy."** *Molecular Cancer Research: MCR,* vol. 16, no. 12, 2018. doi: 10.1158/1541-7786.

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Lung Cancer

SB Lim et al. **"Addressing cellular heterogeneity in tumor and circulation for refined prognostication."** *PNAS,* vol. 116, no. 36, 2019. doi: 10.6084/m9.figshare.9202241.v1.

J Garcia et al. **"Semi-automatic PD-L1 characterization and enumeration of circulating tumor cells from non-small cell lung cancer patients by immunofluorescence."** *Jove,* vol. 14, no. 150, 2019. doi: 10.3791/59873.

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Y Xu et al. **"Xenograft tumors derived from malignant pleural effusion of the patients with non-small-cell lung cancer as models to explore drug resistance."** *Cancer Communications,* vol. 38, no. 1, 2018. doi: 10.1186/s40880-018-0284-1.

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Other Cancers

Y Abouleila et al. **"Live single cell mass spectrometry reveals cancer-specific metabolic profiles of circulating tumor cells."** *Cancer Science,* vol. 110, no. 2, 2018. doi: 10.1111/cas.13915.

X Lu et al. **"Detection of CTCs in portal vein was associated with intrahepatic metastases and prognosis in patients with advanced pancreatic cancer."** *Journal of Cancer,* vol. 9, no. 11, 2018. doi: 10.7150/jca.23989.

K Onidani et al. **"Monitoring of cancer patients via next-generation sequencing of patient-derived circulating tumor cells and tumor DNA."** *Cancer Science,* vol. 110, no. 8, 2019. doi: 10.1111/cas.14092.

Selena Y. Lin et al**. "Prospective Molecular Profiling of Circulating Tumor Cells from Patients with Melanoma Receiving Combinatorial Immunotherapy."** *Clinical Chemistry,* vol. 65, no. 11, 2019. doi: 10.1373/clinchem.2019.307140.

VCL Wong et al. **"Succinct workflows for circulating tumor cells after enrichment: From systematic counting to mutational profiling."** *PloS one,* vol. 12, no. 5, 2017. https://doi.org/10.1371/journal.pone.0177276.

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CT Lim and DSB Hoon. "**Circulating tumor cells: Cancer's deadly couriers."** *Physics Today,* vol. 67, no. 2, 2014. https://doi.org/10.1063/PT.3.2275.

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Breast Cancer

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Head and Neck Cancer

A Kulasinghe et al. **"Circulating tumour cells: the tumour trail left in the blood."** *AACR,* vol. 78, no. 13, 2018. doi: 10.1158/1538-7445.AM2018-5572.

H Shoji et al. **"Next-generation sequencing of circulating tumor cells isolated from peripheral blood of patients with head and neck or gastrointestinal cancer."** *ESMO Asia*, vol. 27, no. 6, 2016. doi: 10.1093/annonc/mdw392.

Posters

Melanoma

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Method

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YF Lee et al. **“Highly accurate genetic profiling of circulating tumor cells using a label-free inertial microfluidic approach coupled with droplet PCR-based next-generation sequencing.”** *AACR,* vol. 76, no. 14, 2016. doi: 10.1158/1538-7445.AM2016-3953.

YF Lee et al. **“Genetic profiling of circulating tumor cells using a label- free inertial microfluidic approach coupled with downstream next generation sequencing.”** *AACR,* 2015. https://www.biolidics.com/publications-and-posters.

About Biolidics Limited

Incorporated in 2009, Biolidics (formerly known as Clearbridge Biomedics Pte Ltd) is a Singapore-based medical technology company focusing on the development of cell enrichment systems which, when combined with other analytical tests, have a wide range of applications for cancer diagnosis, prognosis, treatment selection and treatment monitoring.

Biolidics has developed the ClearCell® FX1 System, a fully automated device which relies on a novel patented technology to separate and enrich cancer cells from blood.

Biolidics' ClearCell® FX1 System allows users of the system to perform liquid biopsies to test for the presence of cancer cells (specifically circulating runour cells, or CTCs) in blood samples or perform further analysis on cancer cells.

Liquid biopsies (i.e. analysis of the circulating tumour cells in blood samples) have many applications throughout the various stages of a patient's cancer journey, from cancer screening and staging to personalised treatment, and post-cancer monitoring.

Ordering Information

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