Correlation between HER2 and ALK status in circulating tumor Cells (CTCs) and tissue of breast and lung cancer patients

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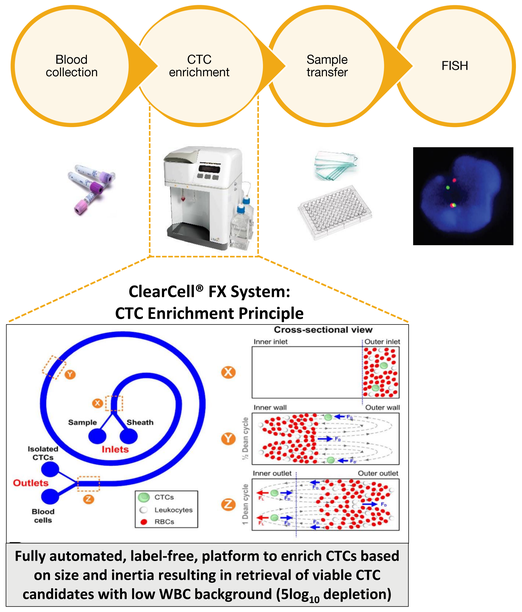
Introduction

Evaluation of CTCs offers a minimally invasive means to access tumor heterogeneity and disease evolution in response to selective pressures of targeted therapies in real-time, making them useful tools to guide treatment decisions. Here we present data on the concordance of *HER2* and *ALK* status between tissues and CTCs, and the utility of these biomarkers on CTCs to examine heterogeneity of disease and potentially prognosticate outcome in breast and NSCLC patients.

Methods

CTC Enrichment by ClearCell® FX and FISH analysis



Fully automated, label-free, platform to enrich CTCs based on size and inertia resulting in retrieval of viable CTC candidates with low WBC background ($5\log_{10}$ depletion)

Figure 1. Workflow: Whole blood from breast cancer (n=25) and NSCLC (n=27) patients was collected in EDTA tubes. 7.5ml of blood was RBC lysed, and the nucleated cells were processed for CTC enrichment using the ClearCell® FX system. The samples of enriched CTCs were fixed, cytospun onto glass slides and processed for FISH analysis.

Fluorescence *in-situ* hybridization (FISH) on CTCs: Samples were fixed with ethanol series, denatured, and hybridized with FISH probes specific for *HER2* amplification and *ALK* rearrangement. FISH scoring was performed under an epifluorescence microscope at 1000× magnification by certified cytogeneticists blinded to the previously defined *HER2* and *ALK* profiles.

ClearCell® FX system enrichment principle: Patient blood (sample) and sheath fluid enter the microfluidic chip, creating a density gradient (X). Curvature in the tube creates shear and lift forces causing cell size-dependent migration of cells across the established gradient density (Y). The larger cells (CTCs) are optimally separated from smaller blood cells at a distance of 1 dean cycle where the outlets collecting enriched CTCs (sample) and blood cells (waste) are located (Z).

Patient Demographics

Table 1. Patient Demographics of NSCLC cohort

Patient characteristics	Cases (%), *N*= 27
Age, years	32–76
Gender	
Male	16 (59.3%)
Female	11 (40.7%)
Smoking history	
Non-smoker	16 (59.3%)
Smoker	5 (18.5%)
Ex-smoker	5 (18.5%)
No info	1 (3.7%)
Clinical staging	
IB	1 (3.7%)
IIIA	1 (3.7%)
IIIB	2 (7.4%)
IV	23 (85.2%)
Histological subtype	
ALK-positive	14 (51.9%)
ALK-negative	12 (44.4%)
Therapy	
Naïve for *ALK*--targeted TKI	27/27 (100%)

Table 2. Patient Demographics of Breast Cancer cohort

Patient characteristics	Cases (%), *N*= 25
Age, years	36-66
Gender	
Male	0 (0%)
Female	25 (100%)
Clinical staging	
III	8 (32%)
IV	17 (68%)
Histological subtype	
HER2-positive	16 (64%)
HER2-negative	9 (36%)

Results

Detection of *HER2* amplification and *ALK* rearrangement by FISH in CTCs enriched using ClearCell® FX system

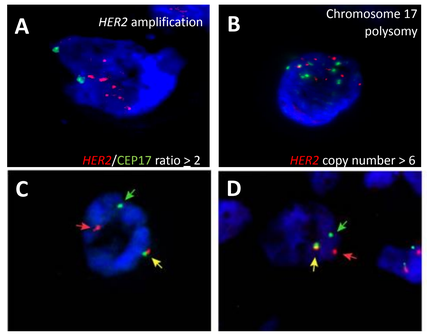


Figure 2. Representative images of *HER2*+ CTCs defined as **(A)** *HER2* amplification (*HER2*/CEP17 ratio >2) or **(B)** chromosome 17 polysomy (>6 copies) from breast cancer patient samples. *ALK* rearrangement was accessed using the Vysis ALK Break Apart FISH Probe Kit (Abbot Molecular) and a representative *ALK*+ **(C)** cell in tumor tissue and **(D)** CTC are shown. Cells were defined to have *ALK* rearrangement (*ALK*+) when the 5' (**green**) and 3' (**red**) labels are separated by ≥ 2 signal diameters. *ALK* rearrangement patterns observed in tissue and CTCs include 1F1R1G, 2F1R, 2F2R, 1F1R and 1F2R with 1F1R1G being most frequently observed (as shown, C and D).

Strong correlation between *HER2* and *ALK* status between tissue and CTCs

A.

ALK		Tumor tissue	
		ALK (+) †	ALK (-)
CTC	ALK (+)*	12	0
	ALK (-)	0	12

100% Sensitivity/Specificity (Based on ROC curve)

† *ALK*+ tissue cut point >15% cell positivity

* *ALK*+ CTC based on empirical cut-off >2 CTCs/~2 ml blood

B.

HER2		Tumor tissue	
		HER2 (+) †	HER2 (-)
CTC	HER2 (+) *	10	1
	HER2 (-)	6	8

62.5% Sensitivity/ 88.9% Specificity (Based on ROC curve)

† *HER2*+ tissue cut point IHC 3+ and IHC2+ FISH confirmation

* *HER2*+ CTC empirical cut-off of > 3 cells/7.5 ml blood

Figure 3. (A) Patients were defined as *ALK*(+) using >15% of tumor cells in FFPE tissue and >2 CTCs positive for *ALK* rearrangement in tissue and CTCs, respectively. *ALK* rearrangements in CTCs were identified in 12/12 (100%) *ALK*(+) patients and 0/12 (0%) of *ALK*(-) patients. Based on ROC curve analysis, sensitivity = 100% and specificity = 100%. **(B)** Cells identified with *HER2* gene amplification and CEP17 polysomy are scored as "positive cells". *HER2*(+) CTCs were identified in 10/16 (62.5%) *HER2*(+) patients. *HER2* gene-amplified CTCs are identified in 7/16 (43.8%) *HER2*(+) patients, range (0-12 cells/7.5ml blood, N=16 patients). 0/9 (0%) *HER2*(-) patients were identified with *HER2* gene-amplified CTCs, although CEP17 polysomy cells are observed in 5/9 (55.6%) patients. Based on ROC curve analysis, 62.5% sensitivity and 88.9% specificity.

Association between number of *HER2*-positive CTCs at baseline and trastuzumab response

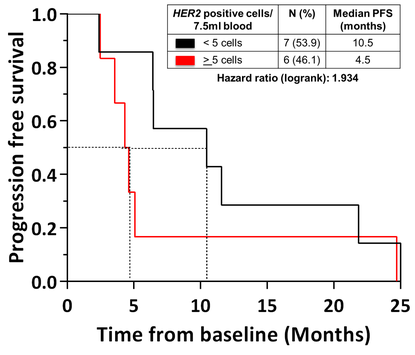


Figure 4. Kaplan-Meier survival curve of *HER2*-positive tumor patients undergoing trastuzumab treatment. High *HER2*-positive cell cut-off defined as ≥5 cells from 7.5 ml analyzed blood. Median PFS for *HER2* high (≥5 cells) vs *HER2* low (<5 cells) is 10.5 vs 4.5 months; HR 1.934.

HER2 status in CTCs: Analysis of heterogeneity and disease evolution in patients on trastuzumab treatment

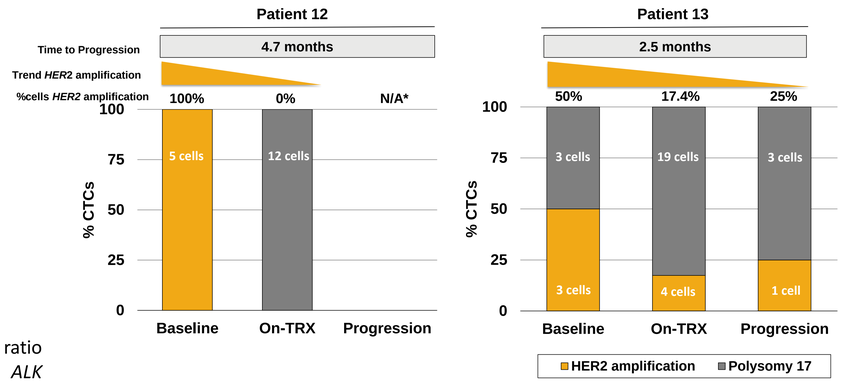


Figure 5. Representative case studies where *HER2* status in CTCs was evaluated at baseline, on trastuzumab treatment (on-TRX) and at progression for patients 12 (left) and 13 (right) are shown. CTCs defined as *HER2* amplification (**gold**) and polysomy 17 (**grey**) are represented as percent of total CTCs detected and actual cell numbers are indicated. The trend in ratio of *HER2* amplified CTCs is summarized. * Sample at progression for Patient 12 was not available.

The frequency and ratio of CTCs with *HER2* amplification changes during treatment, highlighting the evolution of disease under selective pressures of targeted therapy. CTCs may provide a useful tool to evaluate these changes, capture heterogeneity of disease during treatment and potentially help guide treatment decisions.

Clinical response to Crizotinib correlates with *ALK* positive CTCs

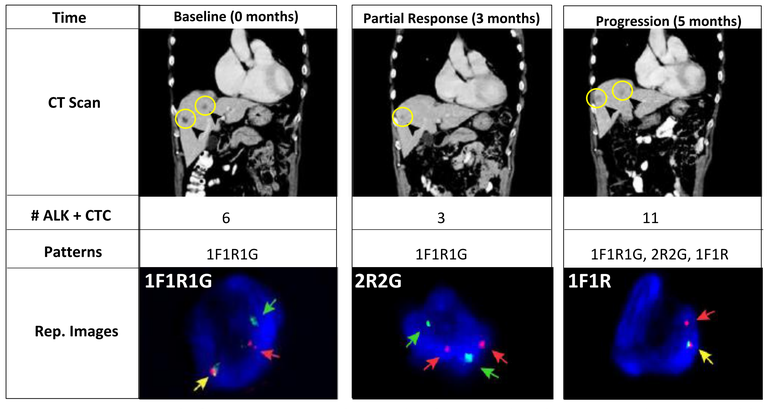


Figure 6. A case study from a never smoker, male, diagnosed with NSCLC and no accessible tissue for *ALK* FISH testing is shown. CT Scans and blood draws to examine *ALK* rearrangement patterns in CTCs was performed at baseline (0 months), partial response (3 months) and progression (5 months). Shown are the CT scans illustrating presence of metastatic tumor(s) on the liver (yellow circles), number of *ALK*+ CTCs, rearrangement patterns detected and representative FISH images for the respective time points. The rearrangement pattern in the image is indicated.

Conclusions

The results presented contribute to the growing body of evidence regarding the utility of using CTCs as tools to access the heterogeneity and evolution of cancer when under selective pressures of targeted therapy. The strong concordance of *HER2* and *ALK* status between tissue and CTCs in breast and NSCLC warrants further investigation into using CTCs enriched using the ClearCell® FX system to potentially help guide treatment decisions.