Real-time HER2 Status Monitoring Using Circulating Tumour Cells

Ali Asgar Bhagat[1], Man Chun Leong[1], Tse Hui Lim[2], Yong Wei Chua[2], Garima Singh[1], Andrew Wu[1], Yifang Lee[1], Alvin Soon-Tiong Lim[2], Tony Kiat Hon Lim[2], Darren Wan-Teck Lim[3], Yoon-Sim Yap[3]

[1]Clearbridge Biomedics Pte Ltd, Singapore; [2]Department of Pathology, Singapore General Hospital, Singapore; [3]National Cancer Centre Singapore, Singapore

Background

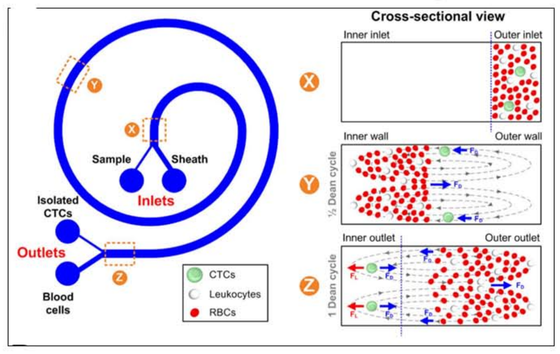
HER2 status can alter during the course of breast tumor development. While tissue sampling is invasive and cannot be available serially, liquid biopsy approach using circulating tumour cells (CTCs) provides accessible tumour material to phenotype the HER2 status of the patient to guide treatment decisions. In our study, we analyzed the HER2 status of 26 advanced stage breast cancer patient samples in Asian population and correlated the concordance between tumour tissue and CTCs.

CTC Enrichment by ClearCell® FX System

ClearCell® FX

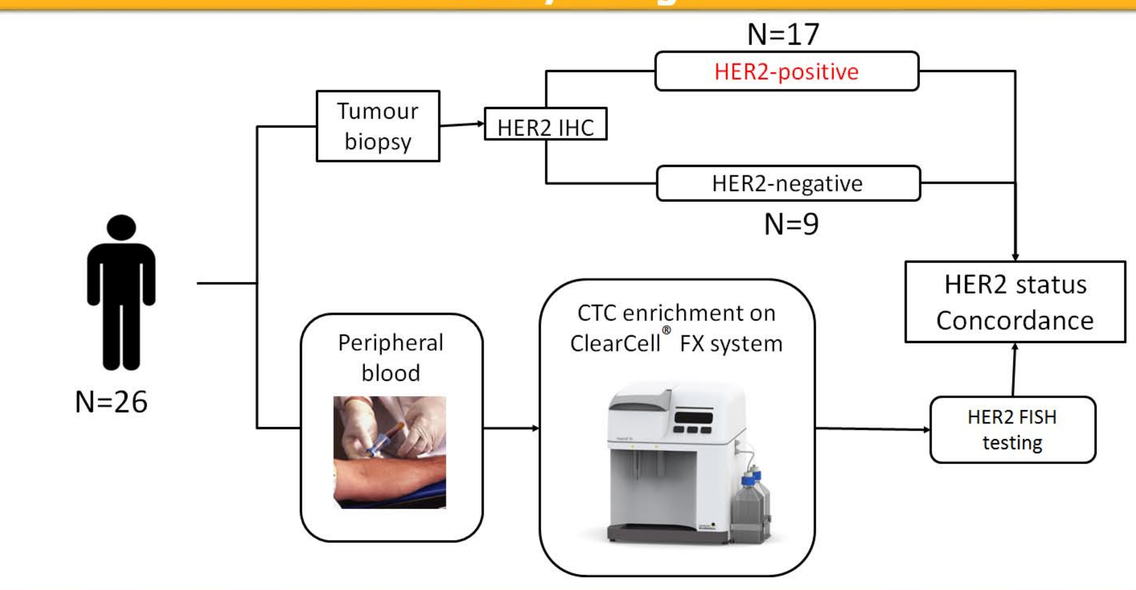
CTChip® FR1

Enrichment Principle



- Label-free isolation method
- Enrichment of CTCs based on *size & inertia*
- Retrieval of wholly *intact* and *viable* cells
- High purity, *5log$_{10}$ depletion of WBCs*
- Process high blood volume of *7.5ml*
- Fully automated CTC enrichment platform

Study Design



Materials & Methods

Patient cohort

A total of 26 late stage breast cancer patients in the Asian population setting were recruited in this study. A total of 26 samples were collected from patients diagnosed with HER2 positive (17/26) and HER2 negative (9/26) breast cancer. HER2 status was evaluated with both IHC and FISH. Serial blood draw for HER2 FISH was performed on 10/17 HER2 positive patients.

CTC enrichment

Blood from breast cancer patients was collected in EDTA tubes. 7.5ml of blood was RBC lysed, and enriched for CTCs on the ClearCell® FX system. Enriched CTCs were fixed and cytospun onto glass slides.

HER2 Fluorescence *in-situ* hybridization (FISH)

Sample were fixed with ethanol series, denatured, and hybridised with FISH probes. HER2 FISH scoring were performed under an epifluorescence microscope at 1000× magnification, by certified cytogeneticists, blinded to the tumour HER2 profile. The HER2 FISH data on the CTCs profile was compared against the HER2 status on tumour tissue.

Results

Detection of HER2 in CTCs

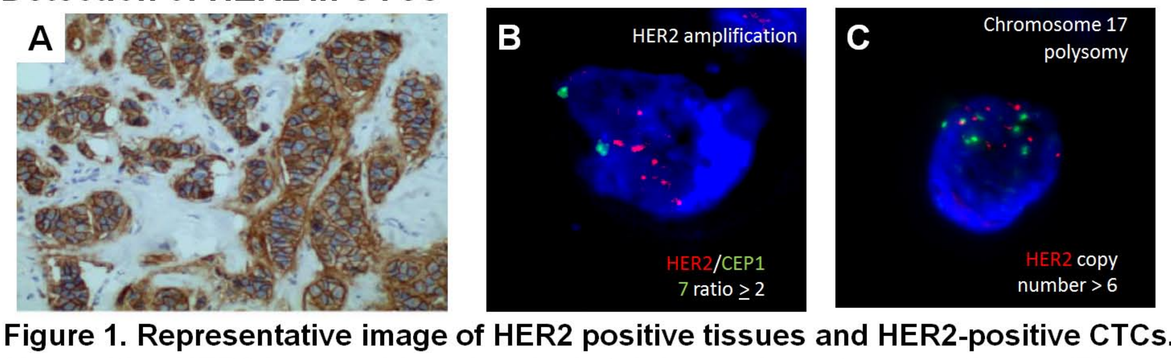


Figure 1. Representative image of HER2 positive tissues and HER2-positive CTCs. (A) positive HER2 immunohistochemical staining on breast cancer tissue. (B) CTCs with HER2 gene amplifications and (C) chromosome 17 polysomy

Results

Frequency of HER2 positive CTCs in patients

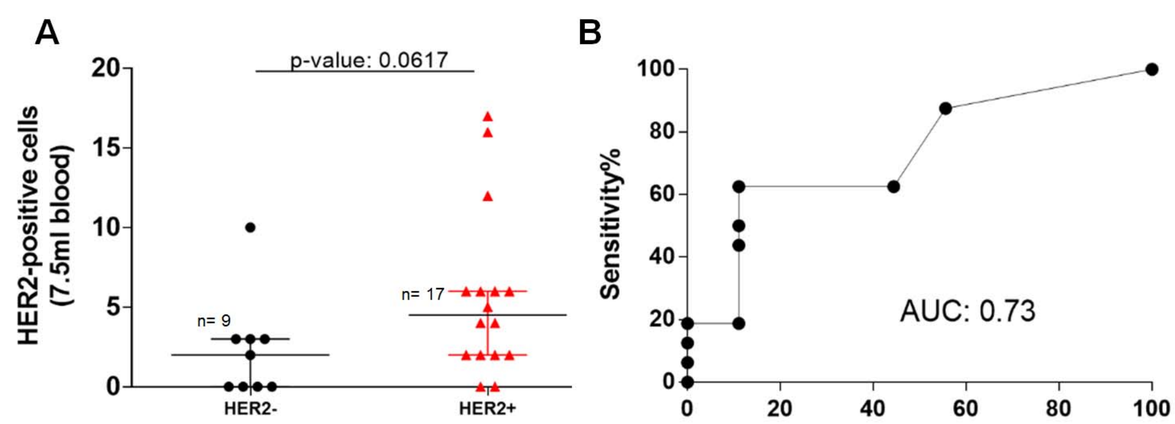


Figure 2. (A) Frequency of CTCs with HER2 mutations and (B) Receiver operating characteristic (ROC curve) showing classification performance

- Frequency of HER2-positive CTCs is higher in HER2-positive breast cancer patients than HER2-negative cohort (p-value = 0.0617).
- Detected HER2-positive CTC counts ranged from 2 to 30 cells from 7.5ml blood (median: 4 HER2+ CTCs/7.5ml).
- A "false positive" cut-off of more than 2 cells/7.5ml blood were established using receiver operating characteristic (ROC) curve analysis.
- Overall concordance rate of ~70% between paired tumour tissue and CTC among the 26 patients.

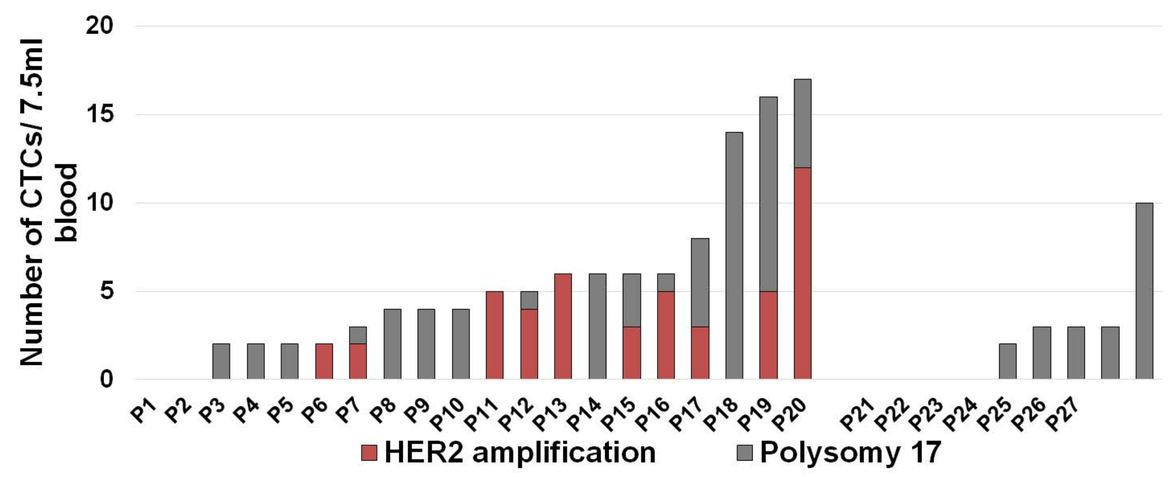


Figure 3. Frequency of CTCs with HER2 amplification and Chromosome 17 polysomy at treatment baseline

HER2-positive tumour cohort

- HER2-positive CTCs were successfully identified in 14 out of 17 HER2+ patients (82.4%)
- HER2 gene amplification and chromosome 17 polysomy were observed in 10 (58.8%) and 13 (76.5%) patients respectively.

HER2-negative tumour cohort

- HER2 amplification was not observed in any of the 9 patients with HER2-negative tumours
- CTCs with chromosome 17 polysomy was detected in 5/9 (55.6%) patients in this cohort (median: 2 HER2+ CTCs/7.5ml).

Survival association of HER2+ CTCs

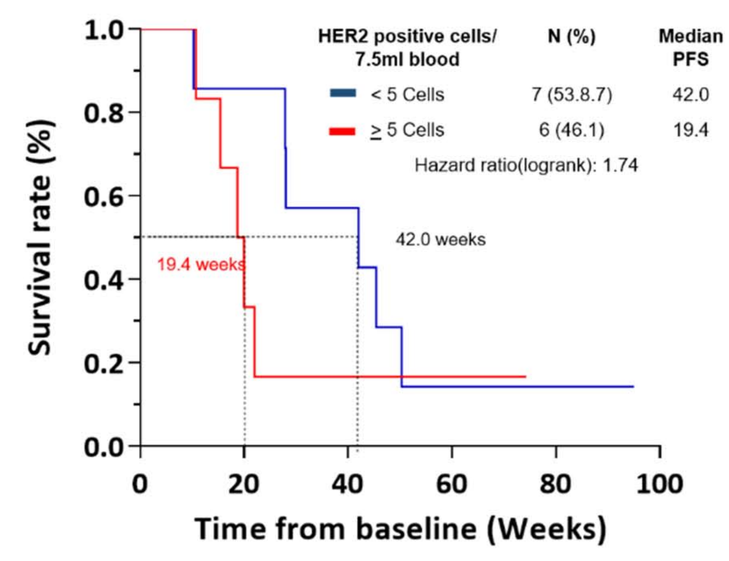


Figure 4. Kaplan-Meier survival analysis of high HER2 positive cells versus low HER2 positive cells in blood.

- High HER2 positive cell count cut-off defined at ≥5.
- Hazard ratio of 1.74 for patients with high HER2 positive cell count.

Conclusion

There is a strong concordance of HER2 status in CTCs and primary tumour. CTCs can provide real- time snapshots of HER2 status for tumor monitoring that may help informed treatment decisions.