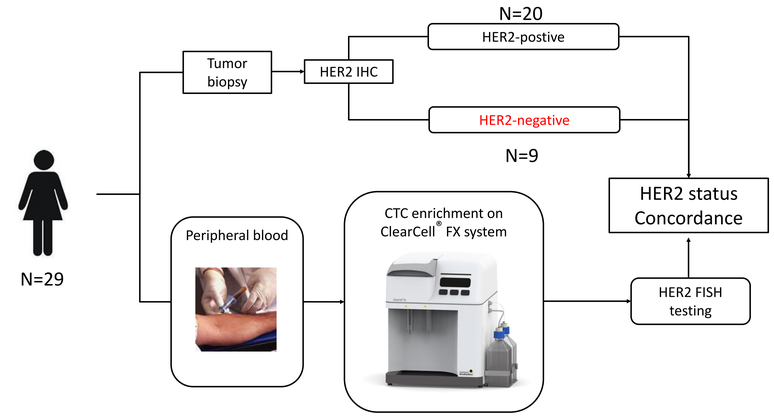
Elucidating *HER2* molecular heterogeneity of circulating tumor cells among breast cancer patients

Man Chun Leong[1], Tse Hui Lim[2], Yong Wei Chua[2], Elaine Hsuen Lim[3], Kiley Wei Jen Loh[3], Guek Eng Lee[3], Rebecca Dent[3], Raymond Chee Hui Ng[3], Andrew Wu[1], Wan-Teck Lim[3], Alvin Soon-Tiong Lim[2], Yoon-Sim Yap[3]. [1]Clearbridge Biomedics, Singapore, Singapore; [2]Department of Pathology, Singapore General Hospital, Singapore, Singapore; [3]National Cancer Centre Singapore, Singapore, Singapore

Introduction

HER2-positive tumors are often associated with poor prognosis, chemo-resistance and some patients eventually develop refractory disease during HER2 targeted therapy. While different mechanisms of trastuzumab resistance are being identified in recent years, the contribution of confounding factors such as inherent genomic heterogeneity, equivocal HER2 amplifications and apparent chromosome 17 (CEP17) polysomy have been less understood thus far. In this pilot study, we aim to examine HER2 heterogeneity in CTCs obtained from breast cancer patients in the Asian population setting.

Workflow



Materials and methods

Patient cohort

A total of **40** samples from **29** advanced stage (Stage 3 and 4) breast cancer patients in the Asian population setting were analyzed in this study. **20/29 (69%)** of the patients recruited in this study had HER2-positive tumor, while **9/29 (31%)** of the patients have HER2-negative tumor. HER2 status on tumor samples was predominately evaluated with IHC and equivocal IHC results were further confirmed with HER2 FISH. **21** patients had blood samples collected at treatment baseline. **8/20 (40)** of the HER2-positive patients cohort had serial blood draws for HER2 FISH analysis.

CTC enrichment

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

HER2 Fluorescent *in-situ* hybridization (FISH)

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

Results

Detection of HER2 amplification and chromosome 17 polysomy on circulating tumor cells

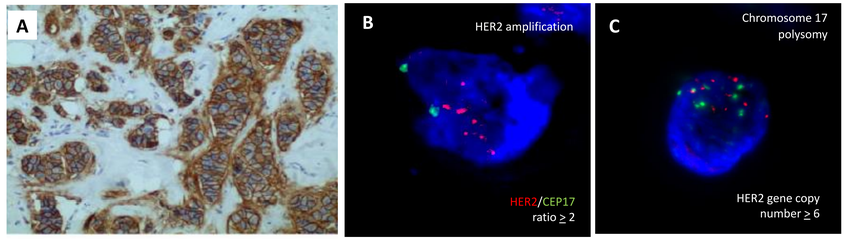


Figure 1. Representative images of HER2 positive tissues and HER2-positive CTCs. (A) Positive HER2 immunohistochemical staining on breast cancer tissue. (B) CTCs with HER2 gene amplifications and (C) chromosome 17 polysomy

- Both HER2 IHC and HER2 FISH are approved methodologies in selecting patients who may benefit from trastuzumab therapy.
- **HER2 amplification** (HER2 gene/chromosome 17 ratio ≥ 2) and **chromosome 17 polysomy with HER2 gene copy number ≥ 6** are interpreted as HER2-positive under the current ASCO/CAP guideline (2013).

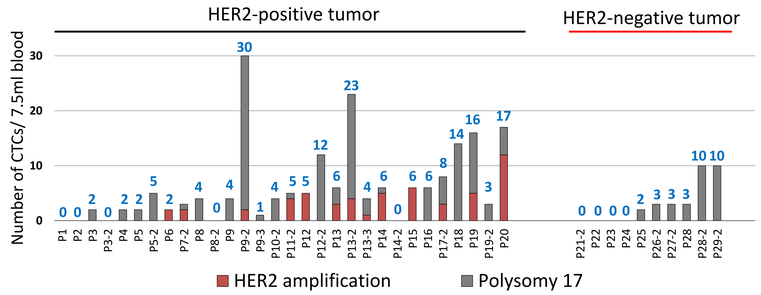


Figure 2. Frequency of CTCs with HER2 amplification and Chromosome 17 polysomy

HER2-positive tumor cohort

- HER2-positive CTCs were successfully identified in 18/20 (90%) of the HER2-positive tumor cohort.
- HER2 gene amplification and chromosome 17 polysomy were detected among 10/20 (50%) and 16/20 (80%) patients respectively.
- Overall, the frequency of HER2-positive CTC ranged from 2 to 30 cells per 7.5ml blood (median: 4 HER2+ CTCs/7.5ml).

HER2-negative tumor cohort

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

HER2-positive tumors are associated with higher frequency of HER2-positive CTCs

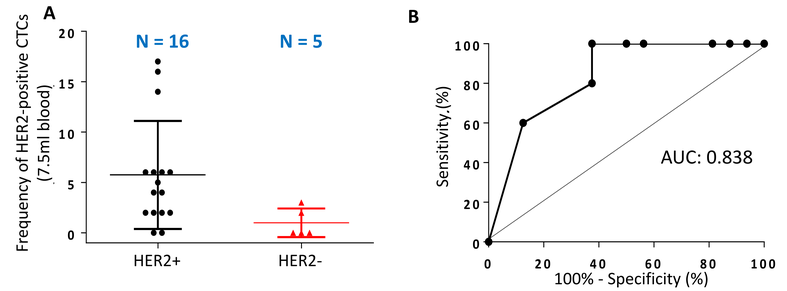


Figure 3. (A) Frequency of HER2-positive CTCs among patients with HER2-positive and HER2-negative tumors and (B) ROC curve showing sensitivity

- Frequency of HER2-positive CTCs among patients with HER2-positive tumor is much higher than those with HER2-negative tumor at treatment baseline (p-value = 0.021).
- A "false positive" cut-off of more than 2 cells/7.5ml blood was established using receiver operating characteristic (ROC) curve analysis to account for pericentromeric 17 repeats rather than true "polysomy 17"
- Overall concordance rate of ~70% between paired tumor tissue and CTC was observed.

Association of HER2-positive CTC counts with traszutumab treatment response

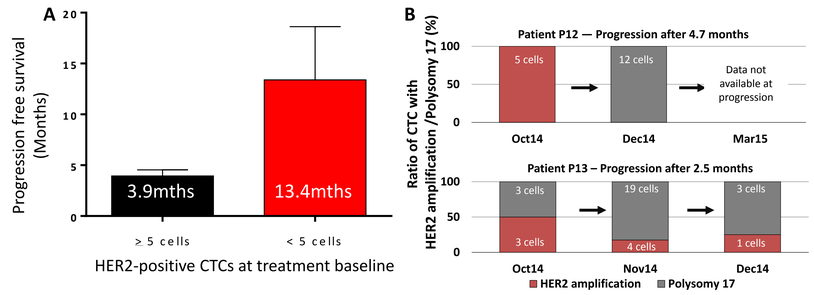


Figure 4. (A) Progression free survival among HER2-positive tumor patients undergoing traszutumab treatment (B) Representative index cases showing the ratio of HER2-amplified and polysomy 17 CTCs throughout the course of treatment

- Patients with higher HER2-positive CTC counts (≥5 HER2-positive CTCs) at treatment baseline has a relatively shorter progression free survival time.
- The frequency and ratio of CTCs with HER2 amplification and polysomy 17 varies during the course of traszutumab treatment, which may suggest genetic heterogeneity and differential sensitivity of HER2-positive tumor cells to traszutumab.

Conclusions

- CTCs capture the heterogeneity of breast cancer, and could potentially overcome limitations of tissue biopsy which are invasive and site specific.
- HER2-negative patients, as confirmed by tissue biopsy, with HER2-positive CTCs pose interesting questions while determining treatment regime and may have clinical implications and warrant further investigation.