Novel Blood Based Circulating Tumor Cell Biomarker For Breast Cancer Detection

Chun-Yu Liu[1234], Yi-Fang Tsai[345], Feng-Ming Lin[6], Jen-Chia Wu[6], Yen-Shu Lin[35], Ta-Chung Chao[134], Kuang-Liang King[35], Pei-Ju Lien[37], Jane Wang[348], Yung-Hui Lin[38], Yi-Chen Lai[38], Huangpin Hsieh[6], Anjay Saklecha[6], Jr-Ming Lai[6], Shih-En Chang[6], Mana Javey[6], Drew Watson[6], Rui Mei[6], Ling-Ming Tseng[3459]*

1. Division of Medical Oncology, Department of Oncology, Taipei Veterans General Hospital, Taipei, Taiwan
2. Division of Transfusion Medicine, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan
3. Comprehensive Breast Health Center, Taipei Veterans General Hospital, Taipei, Taiwan
4. School of Medicine, National Yang-Ming University, Taipei, Taiwan
5. Division of General Surgery, Department of Surgery, Taipei Veterans General Hospital, Taipei, Taiwan
6. CellMax Life U.S., 1271 Oakmead Parkway, Sunnyvale, CA 94085
7. Department of Nursing, Taipei Veterans General Hospital, Taipei, Taiwan
8. Department of Radiology, Taipei Veterans General Hospital, Taipei, Taiwan
9. Division of Experimental Surgery, Department of Surgery, Taipei Veterans General Hospital, Taipei, Taiwan

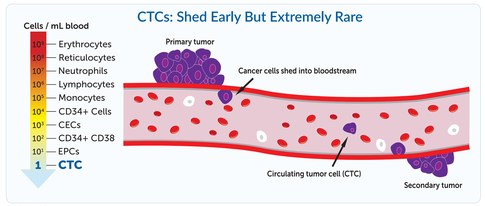
* **Corresponding author: lmtseng@vghtpe.gov.tw**

ESMO 2019 PRESENTATION NUMBER 256P

BACKGROUND AND PURPOSE

The goal of mammogram screening (and other breast-cancer screening tests) is to detect breast cancer earlier than it would otherwise manifest clinically, when it is more likely to be localized. Data clearly show that detection of breast cancers at smaller sizes and earlier stages is associated with better patient outcomes, lower morbidity, and reduced breast cancer deaths.[1,2]

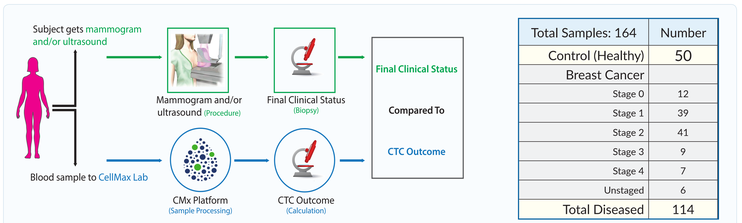
There is an unmet need for a blood test to detect breast cancer in women with dense breast tissue or clinically aggressive subtypes that may be missed by mammograms. Cell-free DNA in blood has shown 15-58% sensitivity for breast cancer.[3] In this study, we evaluated the performance of a circulating tumor cell (CTC) assay as a complimentary biomarker for detecting breast cancer in an Asian population, which has high incidence of dense breast tissue.



[Fig.1] One of the new developments in the study of cancer over the last decade has been the role of circulating tumor cells (CTCs). The technological challenge lies in finding these extremely rare cells in the blood and to keep them viable for further analysis.

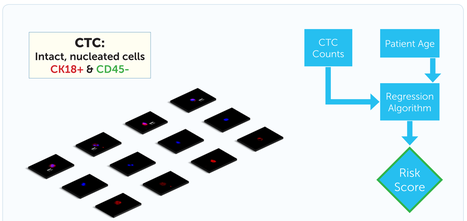
METHODS AND STUDY DESIGN

A single-center, IRB-approved, prospective and blinded clinical study was conducted on 114 Taiwanese females with biopsy-confirmed breast cancer, and 50 healthy controls confirmed by ultrasound or mammogram **[Fig.2]**. Four milliliters of blood was collected prior to imaging and processed using the CellMax biomimetic platform (CMx Platform™) which enumerates CTCs utilizing selection criteria based on a set of markers (cytokeratin 18, mammaglobin, CD45), cell morphometry (size, N/C ratio) and nucleus morphology **[Fig.3]**. Logistic regression models for CMx CTC counts and patient age were used to assess the classification performance of the CMx test **[Fig.4]**.

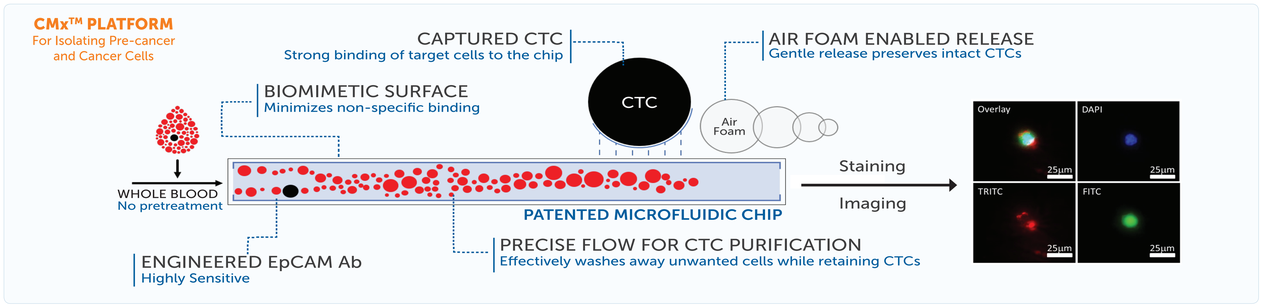


Total Samples: 164	Number
Control (Healthy)	50
Breast Cancer	
Stage 0	12
Stage 1	39
Stage 2	41
Stage 3	9
Stage 4	7
Unstaged	6
Total Diseased	114

[Fig.2] Prospective study design to evaluate the performance of the CMx test for early and pre-cancer detection.



[Fig.4] CTCs which are intact, nucleated, and EpCAM+CK18+/CD45- are enumerated and combined with patient ages using a regression algorithm to derive a patient's risk score.



[Fig.3] This study uses a patented microfluidic platform that processes 2mL of patient whole blood. It is particularly sensitive due to a number of innovations, including a high affinity EpCAM antibody, a biomimetic surface coating that rejects most blood cells and ability to gently release captured CTCs via a proprietary air-foam release mechanism. CTCs are stained and confirmed with MGB and CK18 antibody that is more specific for the epithelial cells derived from the breast tissue.

RESULTS

Of the 114 cancer cases (80% were stage 0~2), the subtypes were confirmed for 102 (62% ER/PR+ HER2-, 22% HER2+, 16% TNBC). CTC count was a significant predictor of cancer status (Likelihood Ratio P-value = 0.0001). At 90% specificity (exact 95% CI: 78.2%, 95.6%) sensitivity was 56.3% (95% CI: 43.3%, 68.6%) for the most common subtype ER/PR+HER2-, 36.4% (17.2%-59.3%) for HER2+, 43.8% (19.8%- 70.1%) for TNBC, and 46.5% (37.1%- 56.1%) overall.

Sensitivity was 62.5% (35.4%- 84.8%) for late stage (Stage III/IV cancer) and 43.5% (33.2%-54.2%) for early stage (Stage 0, I or II cancer) patients. In the subset of 41 individuals with an indeterminate classification of BIRADS 3 (likely benign) or BIRADS 4 (likely malignant) sensitivity was 90% and specificity was 47.6% (95% CI: 25.7%, 70.2%).

Study Results: Test Performance for Various Clinical Statuses

Subject Category	Sensitivity (95% CI)
BIRADS 3/4	90% (25.7%, 70.2%)
Stage III/IV cancer	62.5% (35.4%, 84.8%)
Stage 0/I/II cancer	43.5% (33.2%, 54.2%)

CONCLUSIONS

Circulating tumor cells as a blood-based biomarker have great potential for use in the management of breast cancers. Testing for CTCs is efficient, non-invasive, and can improve the current standard of care. In this initial study, our CTC assay was shown to be a significant predictor of cancer by demonstrating robust performance in distinguishing breast cancer patients from healthy controls. The CTC assay can easily be combined with cfDNA to enhance detection rates. Proof-of-concept data also suggests potential for a rule out test to avoid unnecessary follow-up/biopsies in BIRADS 3/4 patients. This assay is rapid, inexpensive, and easy to implement in most clinical labs. Given its broad applicability, this technology has the potential to have a substantive impact on the diagnosis and treatment of many cancers.

ACKNOWLEDGEMENTS

The authors sincerely appreciate the help and contributions from staff at TVGH and National Yang-Ming University and all patients who contributed samples to this study.

DISCLOSURE

The authors declare no conflicts of interest.

REFERENCES:

1. Berry, D.A., Cronin, K.A., Plevritis, S.K., Fryback, D.G., Clarke, L., Zelen, M., Mandelblatt, J.S., Yakovlev, A.Y., Habbema, J.D.F. and Feuer, E.J., 2005. Effect of screening and adjuvant therapy on mortality from breast cancer. New England Journal of Medicine, 353(17), pp.1784-1792.
2. Bleyer, A. and Welch, H.G., 2012. Effect of three decades of screening mammography on breast-cancer incidence. New England Journal of Medicine, 367(21), pp.1998-2005.
3. Liu, M.C., Maddala, T., Aravanis, A., Hubbell, E., Beausang, J.F., Filippova, D., Gross, S., Jamshidi, A., Kurtzman, K., Shen, L. and Valouev, A., 2018. Breast cancer cell-free DNA (cfDNA) profiles reflect underlying tumor biology: The Circulating Cell-Free Genome Atlas (CCGA) study. J Clin Oncol, 36, p.12021.