General Biologicals Corporation

7th ACTC
Advances in Circulating Tumor Cells
"Liquid Biopsy: From Bench to Bedside"

Isolation of CTCs and CTMs using the Size-Based CellBio™ a2000 system in Non-Small Cell Lung Cancer (NSCLC)

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Abstract

The circulating tumor cells (CTCs) and circulating tumor microemboli (CTMs) are key targets for tracing tumors in the blood circulation. The size-based label free isolation of CTCs and CTMs offers the advantages of capturing target cells with higher efficiency while maintaining their native structure. In this study, we have demonstrated the use of CellBio a2000 platform for isolating and identifying the CTCs and CTMs in 35 patients diagnosed with Non-Small Cell Lung Cancer (NSCLC); Among the selected cohort, 7 patients were newly diagnosed and underwent CTC screening using the CellBio system prior to starting treatment, while 28 patients were at varying stages of treatment. The dual-staining strategy was analyzed to identify the targets and demonstrate its advantages for process standardization. The Papanicolaou staining gives the morphological structure details while subsequent immunostaining provides the molecular analysis of the CTCs and CTMs. CTCs/CTMs was detected in 26 of 35 (74%) patients by the CellBio system. The CTC was identifiable in all the 7/7 (100%) untreated patients and the 19/28 (68%) patients undergoing different stages of treatment. CTMs were identified in 7 patients, indicating a high risk of metastasis.

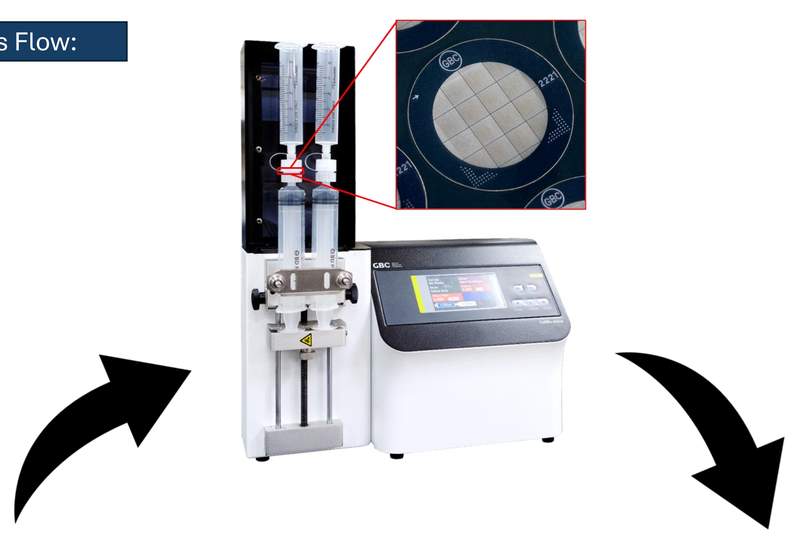
Introduction

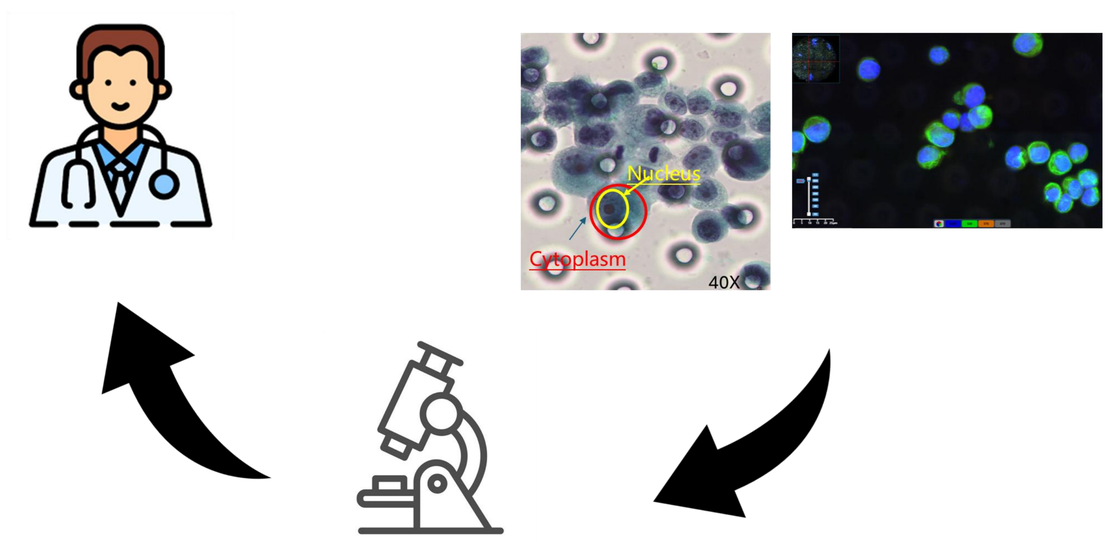
Lung cancer is the leading cause of cancer-related deaths worldwide. The major reason behind the high mortality rate is the lack of early diagnosis. Tissue biopsy is the gold standard for diagnosis but poses challenges in sampling small and poorly located lesions. To overcome these challenges, CTCs have been defined as early indicators of metastasis and can be used for non-invasive cancer monitoring, whereas CTMs reflect the aggressiveness of tumor cells and predict poor prognosis [1,2]. The CellBio system was designed as a Bench-Top platform for reliable isolation of CTC & CTM from the blood sample.

Material & Methods

Samples from 35 NSCLC patients were enrolled to identify CTCs and CTMs in 7.5 ml of blood. The population of enrolled people includes untreated as well as undergoing treatment. The samples were processed using the optimized procedure on the CellBio platform for isolation and staining of targets on the iFiltration™ filter. The two different stain procedures (Papanicolaou & immunofluorescent stains) were used to compare the efficiency of the different staining methods in detecting the CTCs. The filters analyzed by lab cytologists before review and confirmation by the pathologist and doctor to enumerate the CTCs and CTMs observed in the sample. The study was approved by the Research Ethic Committee of the National Taiwan University Hospital (202407098DIPA)

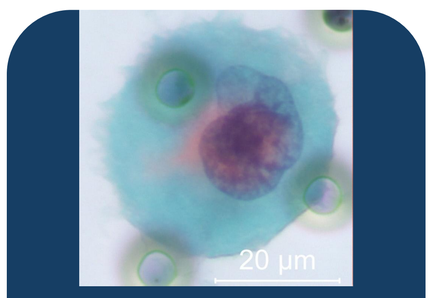
Process Flow:



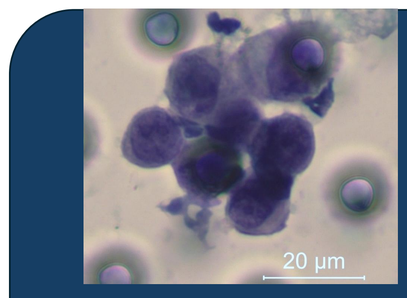


Results

Papanicolaou Stain:

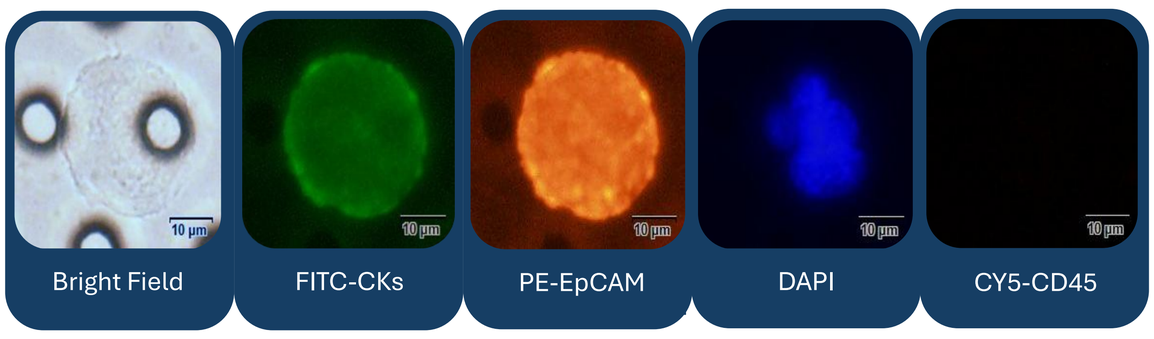


A typical CTC observed in the patient sample



A typical CTM observed in the patient sample

Fluorescence Stain:



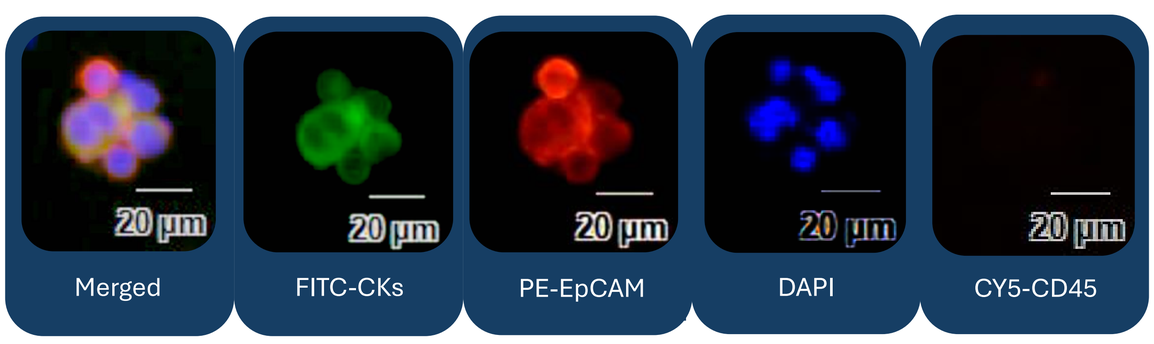


Table: clinical performance of CellBio system for isolating the CTC/CTM in NSCLC patients.

	No of patient (CTC+)	No. of patient (CTC-)	Total cases	Sensitivity (%)	Specificity (%)
Untreated					
Combine two stain	7	0	7	100	
Fluorescence stain	6	1	7	86	
PAP stain	3	4	7	43	
Undergoing Treatment					
Combine two stain	19	9	28	68	
PAP stain	18	10	28	64	
Fluorescence stain	11	17	28	39	
Total					
Combine two stain	26	9	35	74	100
PAP stain	21	14	35	60	100
Fluorescence stain	17	18	35	49	100
Healthy patient	0	60	60		100

Conclusion

The data presented here indicate that both CTCs and CTMs are identifiable in NSCLC patients using the CellBio a2000™ system. The number of CTC or presence of CTM varies during the different stages of cancer as well as physiological conditions of patient. The dual-staining procedure demonstrated that Papanicolaou and immunofluorescence stains provide complementary information on tumor cells, thereby improving the sensitivity and reliability of CTC screening.

References

1. Ren, F., Fei, Q., Qiu, K. *et al.* Liquid biopsy techniques and lung cancer: diagnosis, monitoring and evaluation. *J Exp Clin Cancer Res* **43**, 96 (2024).
2. Liao, M.Y., Hao, Y.J., Luo, C.S. et al. Development and validation of a novel combinational index of liquid biopsy biomarker for longitudinal lung cancer patient management. *The Journal of Liquid Biopsy*, 6, p.100167 (2024).