Prospective Clinical Study of a Prostate Cancer (PCa) Rule-Out Blood Test for PSA Gray Zone Patients Using a Sensitive Circulating Tumor Cell Assay

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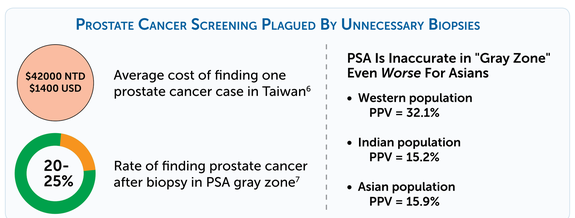
BACKGROUND AND PURPOSE

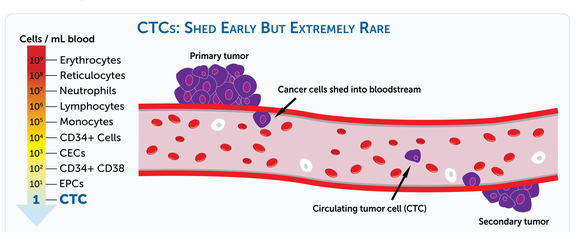
Prostate cancer has nearly a 100% survival rate if diagnosed early.[1] Though recognized worldwide as the standard prostate cancer screening test, PSA testing is tissue-specific, not cancer-specific, resulting in **more chances of a false positive** due to non-cancerous conditions such as prostate enlargement, prostatitis, or urinary tract disease - especially with **"gray zone" results between 4-10 ng/mL**.[2, 3, 4]

The diagnostic confirmation of prostate cancer in patients with a PSA in the gray zone is controversial, often leading to **unnecessary biopsies**. With no consensus guidelines for patient management in the gray zone, **nearly 80%** of prostate biopsies performed are negative for cancer,[5] unnecessarily subjecting thousands of men to harmful side effects of overtreatment, including impotence and incontinence.

We developed a new **circulating-tumor-cell (CTC)** **[Fig.2]** assay for detection of prostate cancer in patients in the PSA gray zone, with the goal to **decrease the number of unnecessary prostate biopsies**.



[Fig.1] Prostate screening programs in countries such as Taiwan are not cost-effective. With no consensus guidelines for patient management in the 4-10ng/ml "gray zone", thousands of men are subjected unnecessarily to the harmful side effects of overtreatment.

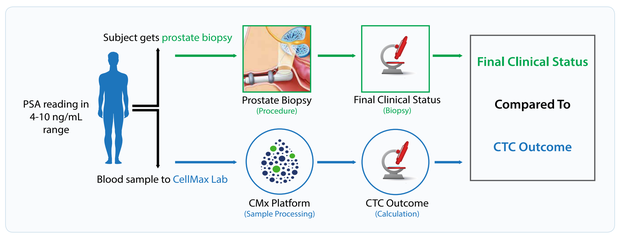


[Fig.2] One of the new developments in the study of cancer over the last decade has been the role of circulating tumor cells (CTCs). The technological challenge lies in finding these extremely rare cells in the blood and to keep them viable for further analysis.

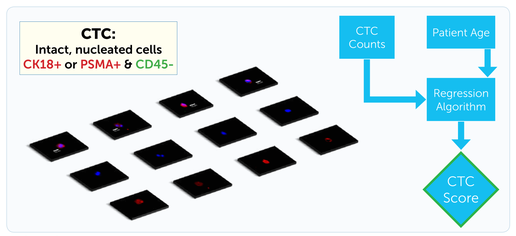
METHODS AND STUDY DESIGN

A prospective clinical study was conducted in 200 high-risk subjects. All subjects underwent routine prostate screening including PSA testing and digital rectal exam (DRE). 4 mL of blood was drawn and processed for CTC analysis using the CellMax biomimetic platform (CMx) **[Fig.4]**.

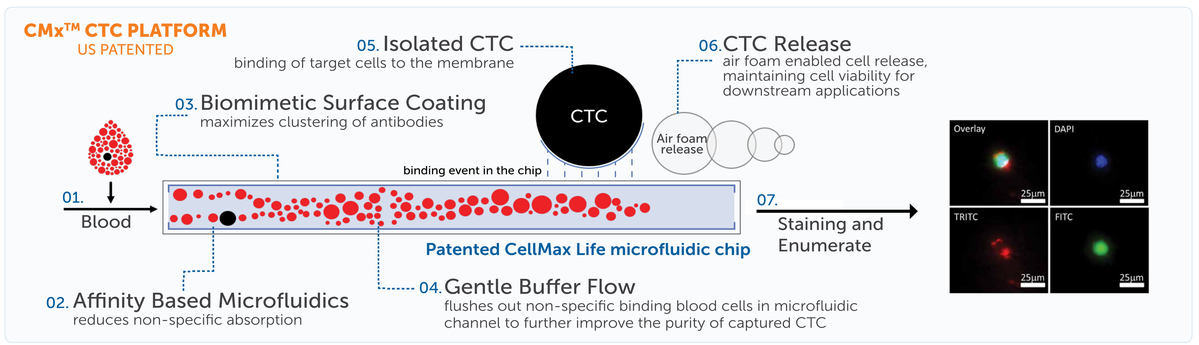
A subset of 84 subjects with PSA levels in the gray zone (4-10 ng/ml) and those diagnosed as "diseased" based on PSA and DRE results also underwent a biopsy for comparison with blinded CTC test results **[Fig.3]**. The CellMax CTC Prostate Test uses a proprietary microfluidic biochip that accurately captures and enumerates CTCs with antibodies to EpCAM, CK18 and PSMA **[Fig.5]**. Multivariate regression models incorporating CTC Prostate Test results were utilized to derive age-adjusted CTC scores predictive of clinical outcomes.



[Fig.3] Prospective study design to evaluate the performance of the CellMax CTC Prostate Test for prostate cancer detection in patients with a PSA reading in the 4-10 ng/ml range.



[Fig.5] CTCs which are intact, nucleated, and EpCAM+ / CK18+ or PSMA+ / CD45- are enumerated and combined with patient ages using a regression algorithm to derive a patient's risk score.



[Fig.4] 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 CK18 and PSMA antibodies that are more specific for the epithelial cells derived from the prostate.

RESULTS

84 subjects with PSA levels in the gray zone (4- 10 ng/ml) were included in this study. Prostate biopsy results were available for a subset of 42 patients; 10 had confirmed cancer. A CTC score was calculated as a nonlinear weighted combination of the captured CTCs identified with CK18 and PSMA antibodies. After adjustment for age and PSA, the **CTC score remained a significant predictor of clinical outcome in the PSA gray zone** (likelihood ratio p-value = .013) whereas PSA was not **[Fig.6-8]**. The **sensitivity and specificity of the CTC score were 80.0% (95% CI: 44.4%, 97.5%) and 93.8% (95% CI: 79.2%, 99.2%).** Negative % agreement and Positive % agreement was 93.8% (95% CI: 79.2%, 99.2%) and 80.0% (95% CI: 44.4%, 97.5%) **[Fig.9]**. Given the observed odds ratio for CTC score in the study, approximately 0.90 (95% CI 0.79, 0.98), the study is appropriately powered.

Patient Disposition

Sample Type	Number
Total samples in subset	42
Confirmed Cancer	10
Confirmed Non-Cancer	32
Disease Prevalence	24%

Source	Likelihood Ratio χ^2	df	Prob (> χ^2)
Age	11.4	1	0.00074
CTC score	5.93	1	0.015

[Fig.7] After adjustment for age, CTC score was a significant (p=0.015) predictor of cancer in the PSA gray zone of 4-10 ng/ml.

Source	Likelihood Ratio χ^2	df	Prob (> χ^2)
Age	7.93	1	0.0049
iPSA	0.94	1	0.33

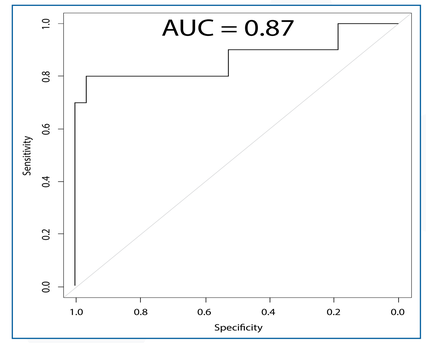
[Fig.6] After adjustment for age, iPSA was not a significant (p=0.33) predictor of cancer in the PSA gray zone of 4-10 ng/ml.

Source	Likelihood Ratio χ^2	df	Prob (> χ^2)
Age	10.2	1	0.0014
iPSA	1.24	1	0.27
CTC score	6.23	1	0.013

[Fig.8] After adjustment for age and iPSA, CTC score is a significant (p=0.013) predictor of cancer in the PSA "gray zone" of 4-10ng/ml. However, iPSA was not a significant (p=0.27) predictor of cancer after adjustment for age and CTC score.

Performance Results

	Value
Sensitivity	80.0%
Specificity	93.8%
PPV	80.0%
NPV	93.8%

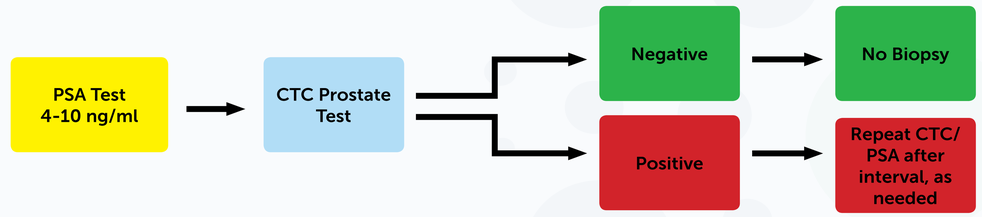


[Fig.9] CTC Prostate Test performance summary and Receiver Operating Curve for CTC score adjusted for age

CONCLUSIONS

This study demonstrates the CTC Prostate Test as a valuable new biomarker in prostate cancer, and proves its clinical utility in the PSA gray zone by helping physicians stratify patients who do not need a prostate biopsy. The test has the potential to reduce unnecessary biopsies in gray zone patients by up to 90%. This is one of the first clinical studies to show the utility of CTCs for accurate prostate cancer detection in the PSA gray zone.

CTC Prostate Test Is A Useful Additional Marker In The Clinical Pathway For PSA Gray Zone Management



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