Prospective Clinical Study of Circulating Tumor Cells for Colorectal Cancer Screening

Results From a Multi-Year, 620 Sample Study

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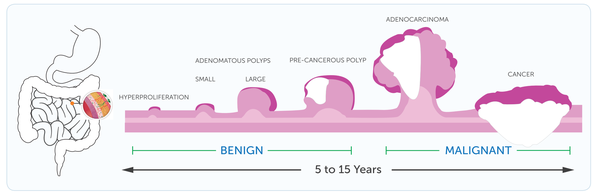
BACKGROUND AND PURPOSE

Colorectal cancer (CRC) is among the most preventable cancers when precancerous lesions are detected at an early stage because of its slow progression **[Fig.1]**. However, almost **two-thirds of CRC cases are diagnosed at a late stage** when treatment is difficult. The 5-year survival rate for Stage 1 colorectal cancer is 91%, but falls to 14% for Stage 4 patients [1, 2]. This is why it is so important to undergo regular yearly screening to ensure that there are no abnormalities developing inside the colon.



[Fig.1] Due to its slow progression, early detection can be effective in decreasing both the incidence and mortality rate of CRC.

Current screening methods for CRC require bowel prep or stool-based testing that are inconvenient, resulting in low compliance **[Fig.2]**. Stool based tests also have limited sensitivity for the detection of precancerous lesions. We have conducted a prospective clinical study over a period of >3 years to assess a novel assay to **detect and enumerate circulating tumor cells (CTCs)** in a blood sample for **early CRC detection [Fig.3]**.

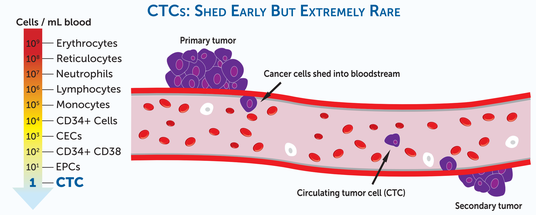
Guideline-Recommended Screening Tests

Test	Specimen Type
Colonoscopy	Invasive (needs bowel prep)
gFOBT	Stool (3 samples)
FIT	Stool
Stool DNA + FIT	Stool

- NCCRT Goal For Screening Compliance "80% by 2018"
- 1/3 of Americans have never been screened [3]
- 87% of non-compliant individuals preferred blood tests to stool-based testing [4]

[Fig.2] Despite its importance, participation in CRC screening is still low. Characteristics of current screening tests may limit compliance; many of these tests are time-consuming and invasive.

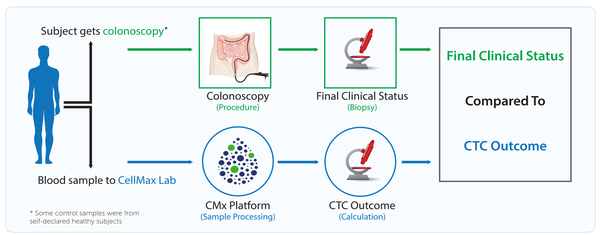
CTCs: Shed Early But Extremely Rare



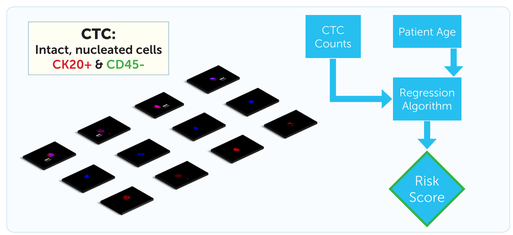
[Fig.3] One of the new developments in the study of cancer over the last decade has been the role of circulating tumor cells (CTCs). The technological challenge lies in finding these extremely rare cells in the blood and to keep them viable for further analysis.

METHODS AND STUDY DESIGN

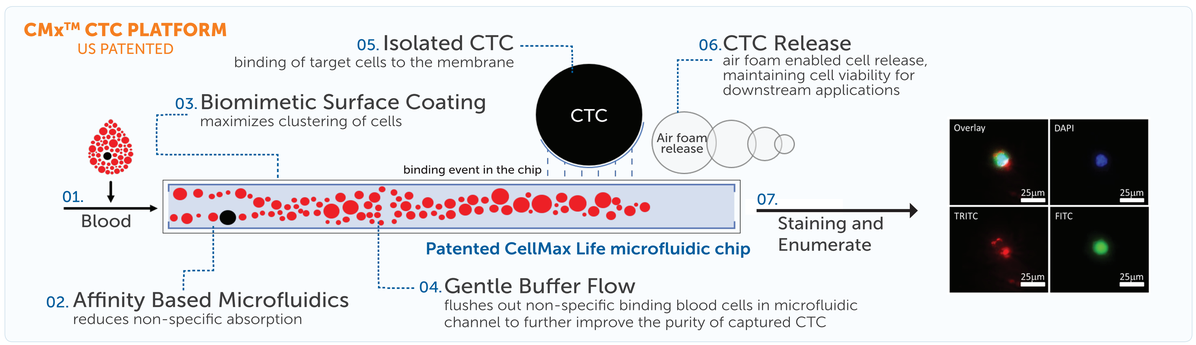
A prospective clinical study was conducted with 620 samples including 438 with adenoma, polyps or stage 1-4 CRC, and 182 healthy controls **[Fig.4]**. For each subject, 2mL peripheral whole blood collected through a routine blood draw was processed using the **CellMax biomimetic platform (CMx)**. The CMx test is uses a **proprietary microfluidic biochip that minimizes non-specific binding and accurately captures and enumerates CTCs [Fig.5]**. Diagnosis was confirmed by colonoscopy and biopsy (when attainable). A multivariate analysis was performed to assess the clinical performance characteristics of the CMx test **[Fig.6]**.



[Fig.4] Prospective study design to evaluate the performance of the CMx test for early and pre-cancer detection.



[Fig.6] CTCs which are intact, nucleated, and EpCAM+CK20+/CD45- are enumerated and combined with patient ages using a regression algorithm to derive a patient's risk score.



[Fig.5] 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 CK20 antibody that is more specific for the epithelial cells derived from the lower digestive track such as colon and rectum.

RESULTS

Disease status was evaluated by a standard clinical protocol which included colonoscopy and biopsy results when attainable. Probability of CRC risk was assessed by an age-adjusted regression model which correlated CTCs to clinical status. **The CMx test's overall accuracy was 88% for all stages of colorectal illness, including precancerous lesions**.

Stratification Of Individuals Based On High Or Low Risk

Predicted Risk Factor [1]	Healthy	Pre-cancer	Cancer (327)				
			Unstaged	Stage 1	Stage 2	Stage 3	Stage 4
High Risk	6	84	23	50	72	100	40
Low Risk	176	27	3	6	12	18	3
Total	**182**	**111**	**26**	**56**	**84**	**118**	**43**

Patient Disposition

Total Samples: 620	Number	Age
Control (Healthy)	182	20 - 80
Pre-cancer (Adenoma/ Advanced Adenoma/Stage 0)	111	20 - 81
CRC	327	31 - 87
Stage 1	56	
Stage 2	84	
Stage 3	118	
Stage 4	43	
Un-staged	26	
Total Diseased	438	

Test Performance

	Diseased			Healthy	Total
	Cancer	Pre-Cancer	Total		
Test +ve	285	84	369 (True Positive)	6 (False Positive)	375
Test -ve	42	27	69 (False Negative)	176 (True Negative)	245
Total	**327**	**111**	**438**	**182**	**620**

Study Results

Tested Samples	Sensitivity	Specificity	AUC
All (620)	**84%**	**97%**	**0.87**
Pre-cancerous lesions (111)	**77%**	**97%**	**0.84**
Cancer (327)	**87%**	**97%**	**0.88**

CONCLUSIONS

The study has demonstrated high accuracy for the detection of CRC using a novel CTC assay. **It is the first such study to show high sensitivity in the detection of precancerous colorectal lesions**. The simple blood draw required can be easily integrated into a patient's routine physical, increasing test compliance.

Comparison: Guideline-Recommended Screening Tests

Test	Sensitivity for CRC	Sensitivity for Pre-cancer
CMx™	87%	77%
gFOBT [5]	62-79%	2-10%
FIT [6]	73-88%	24%
Stool DNA + FIT [6]	92%	42%
Colonoscopy [5]	75-93%	76-94%

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† Includes indeterminate risk