Mutational analysis of circulating tumor cells using the IsoFlux™ System and a high sensitivity TaqMan® assay (castPCR™)

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ABSTRACT

Circulating tumor cells (CTCs) are rare cells found in the blood of cancer patients with solid tumors and play a key role in cancer dissemination. There has been considerable interest in analyzing these cells as a potential source of clinically-actionable information relating to molecular profile of the patient's disease. Numerous approaches have been employed to isolate and utilize CTCs for diagnostic and discovery applications. One of the current challenges in the field is high recovery and purity of CTC and reliable detection of rare populations of CTCs in a background of leukocytes.

The IsoFlux System (Fluxion Biosciences) provides high recovery of CTCs in a format optimized for downstream analysis. Competitive Allele-Specific TaqMan® PCR (castPCR™) (Life Technologies) is a sensitive mutation detection assay designed to detecting rare mutations in a background of wild-type gDNA. When combined, these two technologies provide a sensitive detection platform for CTC mutations from a simple blood draw.

Here we present analytical and clinical validation of a KRAS mutation detection assay on CTC samples. The castPCR assay was first characterized with titered amounts of gDNA to assess sensitivity and limits of detection. Model CTC samples were prepared using spiked cell lines to further qualify the assay. For clinical validation, whole blood samples from colorectal cancer patients (N=22) were collected and analyzed using the KRAS mutation assay as well as immunofluorescence to confirm the presence of CTCs. Over 80% of the patient samples had CTC levels that exceeded the assay's limit of detection, and 36% of the patient samples had a KRAS mutation (inline with reported prevalence from tissue analysis).

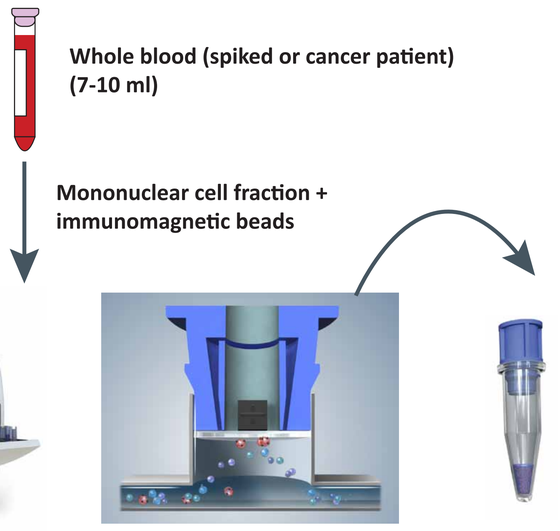
The results of this study indicate that the IsoFlux System can be combined with castPCR to provide a sensitive 'sample to answer' detection platform for DNA mutations in CTCs drawn from a routine blood sample. The assay workflow is also amenable to additional mutations relevant to clinical oncology.

METHODS AND WORKFLOW

Model CTC System - Model cancer cells (MDA-MB-231, heterozygous for KRAS G13D mutation) were spiked into fresh healthy human blood tubes (7mL, EDTA). Ficoll gradient was used to separate the mononuclear fraction. Purified gDNA from MDA-MB-231 (mutant) and Jurkat (wild-type) cells was used for analytical validation of the castPCR assay and as qPCR controls.

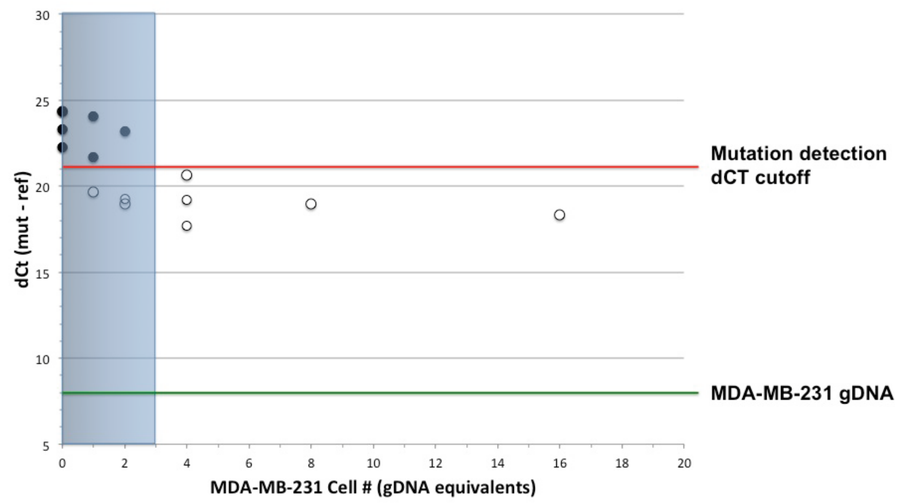
IsoFlux CTC Isolation - Samples were processed on the IsoFlux System using anti-EpCAM immunomagnetic beads. Enriched CTCs were eluted in less than 10µL and saved for either enumeration or mutation detection. Enumeration samples were counted using fluorescence microscopy (CK+ / CD45- / nucleated / intact) .

castPCR mutation detection - gDNA was isolated from enriched CTC samples and processed using a StepOne Plus qPCR instrument (Life Technologies). A panel of 7 clinically relevant KRAS mutations were used alongside a KRAS reference assay. Mutations were called when the dCt (mutant Ct - reference Ct) fell below the established cutoff.

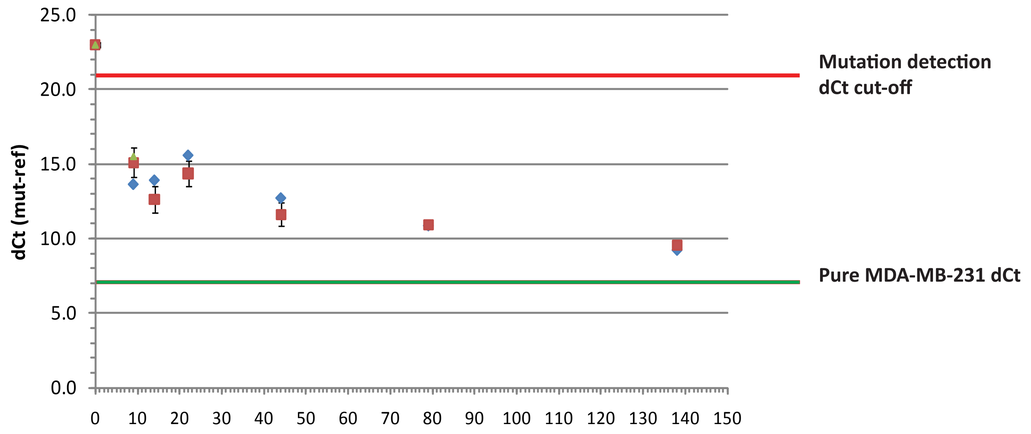


RESULTS

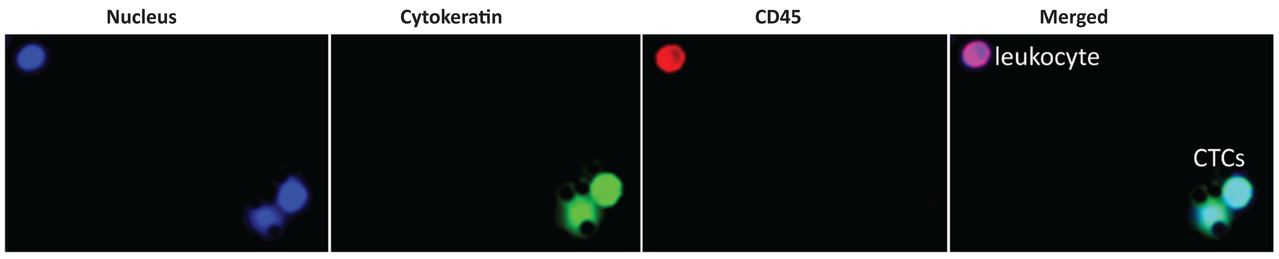
castPCR analytical validation
Assay sensitivity and linearity of KRAS G13D castPCR mutation assay was analyzed by titration studies using purified gDNA from wild-type Jurkat cells and KRAS mutant MDA-MB-231 cells (heterozygous G13D KRAS mutation). castPCR showed high sensitivity of detecting G13D mutation in a background of wild type gDNA, down to 4 MDA-MB-231 cells in 10,000 Jurkat cells equivalence.



IsoFlux System and castPCR mutation detection analytical validation
MDA-MB-231 cells were spiked into 7ml of fresh healthy human donor whole blood. Samples were processed on the IsoFlux System using anti-EpCAM immunomagnetic beads. One set of the IsoFlux-enriched samples was enumerated to determine number of recovered cancer cells and total number of nucleated cells (i.e. background). gDNA was isolated from the other 2-3 sets of matched IsoFlux-enriched samples. castPCR was used to detect G13D mutations in these samples. Assay sensitivity to positively call mutations is down to 9 target cells or lower.

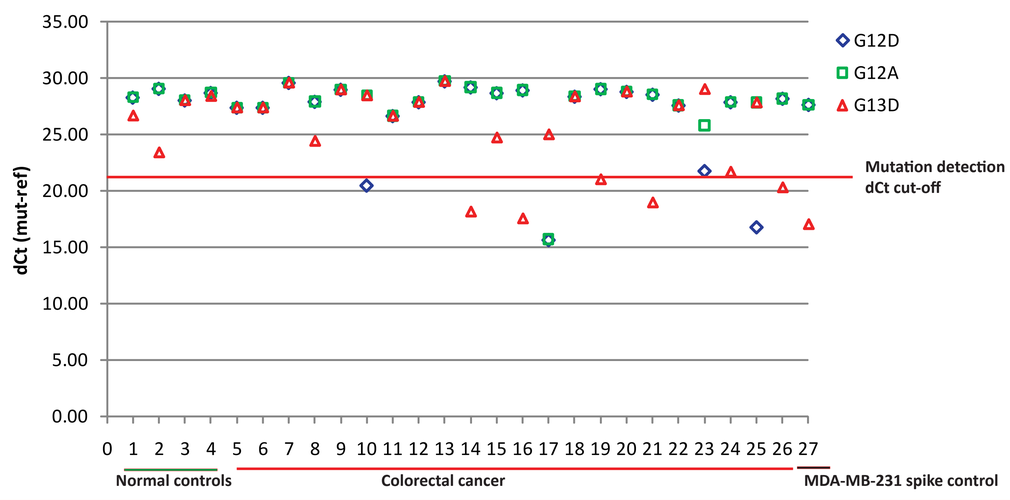


Clinical evaluation
Blood samples (N=22) were acquired from late stage metastatic colorectal cancer patients. One tube of blood (7-10mL, EDTA) from each patient sample was enriched by IsoFlux and enumerated for CTCs (CK+/CD45-/nucleus). A matched sample from each patient was processed by IsoFlux and tested for a panel of KRAS and BRAF mutations using castPCR. Four healthy blood control samples were run in parallel to establish mutation detection baseline. 82% of samples had CTCs above the assay's limit of detection, indicating this test could be practically applied in clinical use. 36% of samples has a KRAS mutation detected, a value that is in line with KRAS mutation prevalence known from primary tissue analysis.



Assay Type	KRAS Mutation Assays							BRAF Mutant Assay
Life Technologies Assay ID	516 c.34G>T	517 c.34G>A	518 c.34G>C	520 c.35G>T	521 c.35G>A	522 c.35G>C	532 c.38G>A	476 c.1799>A

*KRAS and BRAF references assays run accordingly to generate dCt.



Patient samples	22
% CTC positive	82%
% mutation positive	36%

CONCLUSIONS

- The IsoFlux System delivers high recovery of circulating tumor cells in an optimal format for downstream analysis (high CTC recovery and viability, low elution volume, low background)
- castPCR mutation detection assays demonstrated high sensitivity in detecting rare mutations in the presence of wild-type background
- Capturing CTCs with the IsoFlux System followed by castPCR mutation detection provides a complete 'sample-to-answer' workflow for real-time information on tumor mutation status