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.

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). Fixol 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 10s, 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 gDNA Isolation

RESULTS

castPCR analytical validation

Assay sensitivity and linearity of KRAS G13D castPCR mutation assay was analyzed through testing 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 cell equivalents.

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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