Investigation of circulating tumor cells using RarePlex® Developer assays for custom biomarkers and validation of the RarePlex AR-V7 assay

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Background

There is considerable interest in investigating circulating tumor cells (CTCs) for biomarkers that provide information on drug target expression, response to therapy, and metastatic potential. Currently, options for investigating biomarkers on CTCs are limited due to challenges of developing multiplexed assays. The RareCyte platform combines CTC sample preparation, multiparameter fluorescence staining, imaging, and single cell retrieval. RarePlex Developer kit technology enables the simple addition of biomarkers of interest to a base epithelial CTC detection assay.

Methods

Blood samples spiked with positive and negative cell lines for each investigative biomarker were processed using AccuCyte® Sample Preparation System. Slides were auto-stained by immunofluorescence with the RarePlex® CTC Staining Kit utilizing a three-channel CTC detection base: a nuclear dye, anti-CD45 antibody to exclude white blood cells, and cocktailed antibodies to cytokeratin (CK) and epithelial cell adhesion molecule (EpCAM). Two additional RarePlex Developer fluorescence channels were used for detection of various investigational markers, including AR, AR-V7, PSMA, Her2, ER, PR, EGFR, Vimentin and Ki67 under optimized conditions. Stained slides were imaged with the CyteFinder® Imaging System. CTCs were identified using machine learning-based algorithms and confirmed by user review in CyteHub® software. Biomarker analysis was performed by visual observation and mean fluorescence intensity (MFI) measurements on confirmed CTCs. Analytic validation studies of the AR-V7 CTC assay were performed using 22RV1 (high), LNCaP (low) and BT-474 (negative) cell lines. Developer assays were applied to clinical samples from lung, prostate, and breast cancer patients.

Results

Developer technology successfully tested a broad range of biomarkers on cell line control samples with default antigen retrieval and fixation conditions. For each biomarker, MFI thresholds that segregated negative and positive cell lines were statistically defined. The AR-V7 threshold was optimized for statistical accuracy and identified ~90% of 22RV1 cells, ~30% of LNCaP cells and ~3% of BT-474 cells. When applied to clinical samples of appropriate type, staining with proper localization was observed.

Conclusions

CTC assays for the biomarker characterization of breast, prostate, and lung cancers were created using RareCyte Developer technology. This flexible format provides a platform for custom CTC assay development, enabling characterization of phenotypic heterogeneity. Analytic validation of the AR-V7 CTC assay was successfully performed.

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