Analytic validation of an assay to detect androgen receptor splice variant ARv7 protein expression on circulating tumor cells from prostate cancer patients

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BACKGROUND

Circulating tumor cells (CTCs) can provide information on drug target expression, response to therapy, and disease prognosis from a non-invasive blood draw. Presence of the androgen receptor splice variant ARv7 in prostate cancer cells is associated with resistance to second generation anti-androgen therapies. We report here the analytical validation of an immunofluorescence assay for characterization of ARv7 protein expression on CTCs using the RareCyte platform.

METHODS

Blood samples from healthy normal donors spiked with positive and negative cell lines for ARv7 expression were processed using the AccuCyte® Sample Preparation System. Slides were stained by immunofluorescence using an automated slide staining system and the RarePlex® ARv7 CTC Panel Kit comprised of a nuclear dye, anti-CD45 antibody to exclude white blood cells, cocktailed antibodies to cytokeratin (CK) and epithelial cell adhesion molecule (EpCAM), and an ARv7 antibody. Stained slides were imaged with the CyteFinder® Instrument. CTCs were identified using machine learning-based algorithms and confirmed by user review. Mean fluorescence intensity (MFI) measurements were used as a metric for ARv7 expression on confirmed CTCs. Analytic validation studies of the ARv7 CTC assay were performed using 22RV1 (ARv7-high), LNCaP (ARv7-low), and BT-474 (ARv7-negative) cell lines. Performance studies included accuracy, sensitivity, specificity, repeatability, and inter-stainer run coefficient of variation. Performance metrics for CTC recovery were calculated on spike-in and clinical samples. For a gold standard comparison, the number of CTCs found in the ARv7 assay was compared to the number of CTCs found with the CTC detection assay.

RESULTS

An ARv7 MFI threshold that segregated negative and positive cell lines was statistically defined. This threshold identified 83% of 22RV1 cells as positive for ARv7, 91% of BT-474 cells as negative, with an overall accuracy of 90%. When the assay was applied to clinical prostate cancer samples, staining with proper nuclear localization was observed. CTC recovery was at least as high with the ARv7 assay as with the base CTC detection assay.

CONCLUSIONS

An ARv7 assay was developed and validated which shows good sensitivity, specificity, accuracy, and repeatability in discerning between ARv7 positive and negative cell lines when spiked into healthy normal blood. CTC detection was comparable to a gold standard assay when applied to mCTC studies and clinical samples. ARv7 positive and negative CTCs were identified from clinical samples based upon the statistical cutoffs determined via mCTC studies. While ARv7 positive CTCs were detected in this clinical comparison, a larger clinical study will be required to determine the clinical cut off for ARv7. ARv7 presence has been found to predict resistance to anti-androgen therapies. Staining ARv7 on CTCs may enable the use of a liquid biopsy assay to predict drug response. The ARv7 Staining Kit is also compatible with RarePlex 488 Developer Kit for addition of a second biomarker.