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Exosomal RNA based liquid biopsy detection of androgen receptor variant 7 in plasma from prostate cancer patients.

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Introduction:

Androgenic signaling is a critical part in the growth and survival of prostate cancer (PCa), especially in advanced, metastatic or recurrent disease. Consequently, the androgen receptor (AR) is a target for hormone therapies. Full-length androgen receptor mRNA is derived from 8 exons and encodes an N-terminal transactivation domain (exons 1-2), a DNA-binding domain (exons 2-3) and C-terminal ligand binding domain (exons 4-8). However, AR variants have been identified in which the ligand binding domain is removed by splicing between exons 1-3 and intronic, cryptic exons. One of these variants, ARv7, when expressed in PCa patients and cultured PCa cells, confers resistance to hormone therapy treatment. To avoid ineffective procedures, the development of a liquid biopsy test for ARv7 expression would be a useful and non-invasive means of determining treatment strategies for PCa patients.

Methods:

The ARv7 assay leverages Exosome Diagnostics' proprietary spin column-based extraction technology. The assay comprises a column-based isolation protocol extracting total exosomal RNA from 0.5-4 mls of Pca patient plasma, followed by detection of both full-length AR and the AR v7 variants, using qPCR. Assay quality is monitored by inclusion of internal and external controls.

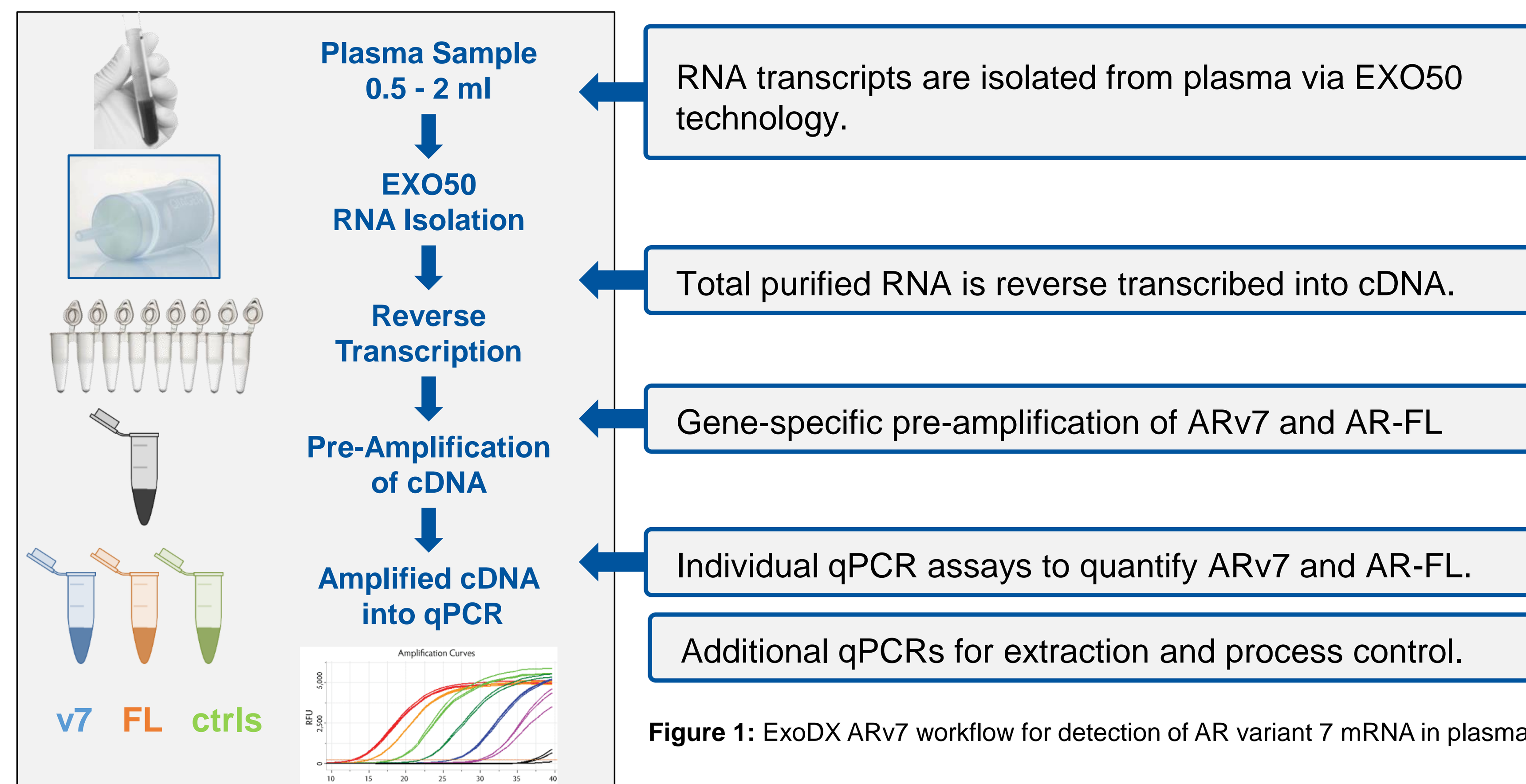

Figure 1: ExoDX ARv7 workflow for detection of AR variant 7 mRNA in plasma

Assay Performance

Assay performance was evaluated by spiking exoRNA, extracted from healthy patient plasma, with synthetic reference RNA for AR variant 7. The RNA samples were then processed through ExoDx workflow outlined in figure 1. Limit of detection (LOD) was determined to be about 4 copies per reaction. Efficiency was approximately 96% (data not shown)

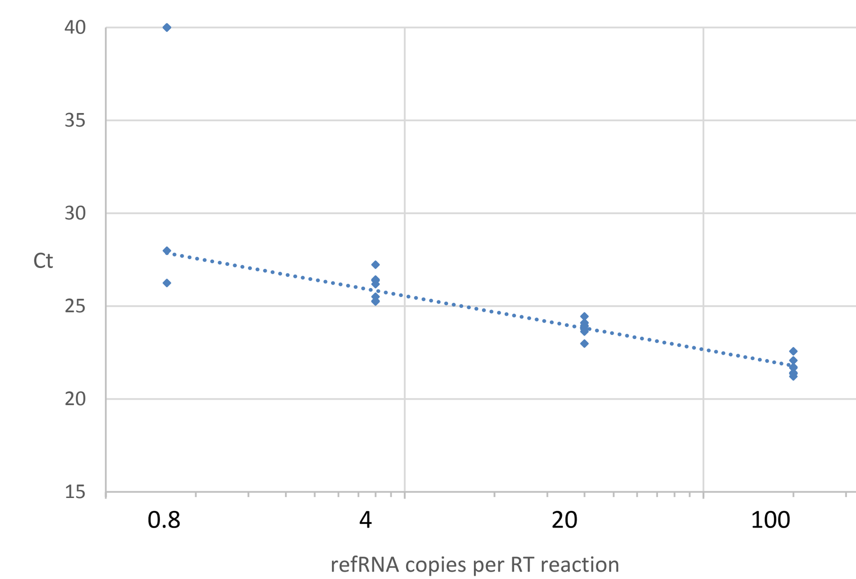

Figure 2: Standard curve for the detection of AR variant 7 mRNA

Cell-line verification

Assay performance was also evaluated with RNA extracted from cell line cultures. Two-step RT-qPCR was performed on 2.5ug RNA from 7 cell lines (pre-amplification was unnecessary for these samples). The presence of AR variant 7 and full length AR (figure 3) is consistent with data presented in the literature.

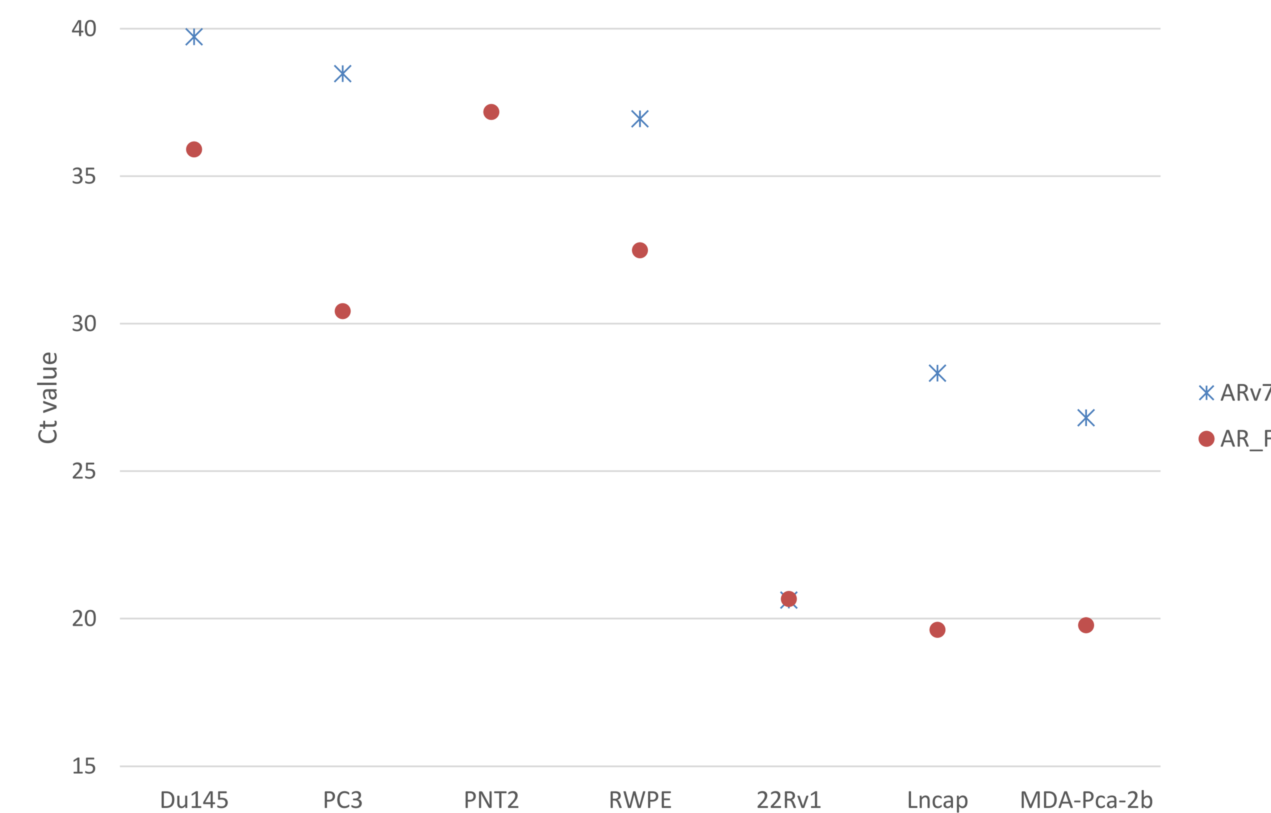

Figure 3: Detection of AR variant 7 and full-length AR in cell lines

Patient Data

A cohort of 6 Pca patients with advanced prostate cancer were screened using this ARv7 assay. Plasma volumes were in the range of 0.5-1.8 mls. AR and ARv7 status was efficiently determined in all patient samples.

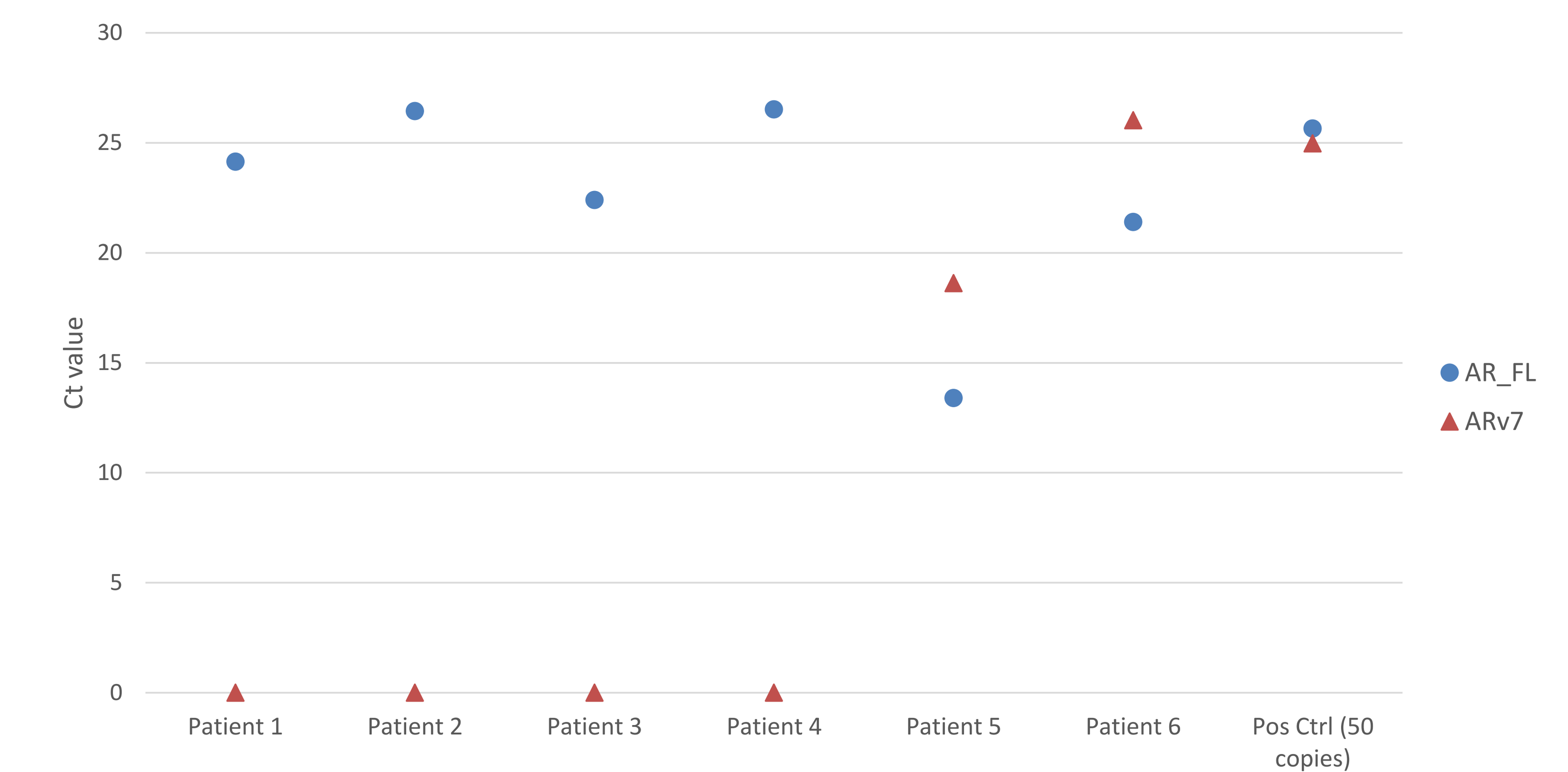

Figure 4: Detection of AR variants in PCa patient plasma

Conclusions:

Liquid biopsies represent a low-risk, non-invasive and viable approach to testing for biomarkers in prostate cancer patients. Here, we demonstrate the capability of our diagnostic test to determine the presence of AR alternate transcripts in plasma. Since this analyte is the result of alternative splicing, it would not be detected in cell-free DNA alone. Monitoring plasma levels of ARv7 transcripts could enable effective personalized treatment for patients with advanced prostate cancer and has clear clinical application.

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