

A liquid biopsy-based targeted proteomic assay to identify actionable targets for late-stage prostate cancer

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Background: Anti-cancer drugs primarily target proteins such as kinases or cell surface proteins. Although clinical trials confirm target protein presence at the patient's enrollment, many fail due to inadequate efficacy, lack of response or off-target effects. Patient-specific protein profiles could reduce harm and improve trial design by selecting patients most likely to benefit. Hence, we developed a liquid biopsy-based proteomic assay to identify druggable targets from patient-derived circulating tumor cells (CTCs) and plasma.

Methods: A total of 34 blood samples were collected from 27 pretreated metastatic castration resistant prostate cancer (mCRPC) patients. Matched CTC and white blood cell (WBC) fractions were obtained from 27 samples, while plasma was obtained from 24 samples, 17 of which overlapped with the CTC and WBC samples. Samples were processed using Astrin Bioscience's AI-empowered proprietary label-free holographic imaging and in-flow protein marker expression to enrich CTCs. Patient matched WBCs and plasma were also collected. Samples were processed to generate peptides for analysis on a FAIMS equipped Stellar instrument. Heavy labeled peptide standards were used for internal normalization.

Results: CTCs were successfully isolated from 27 samples, with a mean CTC count of 3.20 cells/mL and a median of 1.70 CTCs/mL. Patients had a median age of 67; 56% had Gleason grade group 4-5 histology, 12% had visceral metastases, 81% had received three or more prior systemic therapies, and median PSA level was 15 ng/mL. 28 prostate or cancer-specific target proteins were selected for targeted proteomic analysis. We assessed 10 different proteins in our preliminary study. Proteomic analysis of 27 pairs of matched CTC and WBC samples revealed diverse protein profiles. Several clinically relevant mCRPC proteins were detected in CTCs: AR (100% of subjects), B7-H3 (96%), PD-L1 (96%), PSMA (56%) and TROP2 (7%), with minimal or no detection in WBCs. Neuroendocrine markers were also detected: chromogranin-A (15%), DLL3 (7%) and UCHL1 (15%). Moreover, a patient sampled at two time points showed changes in the abundance of target proteins over time. Analysis of plasma from 17 samples with matched CTC and WBC fractions revealed strong concordance with the CTC protein profiles: AR (94%), PD-L1 (94%) and B7-H3 (100%). In addition, we detected the target proteins over time in 7 patients who had blood collected at two or three time points.

Conclusions: Distinct protein expression between CTCs and WBCs, along with detection of expected cancer-related markers, confirm successful CTC isolation. The assay's ability to detect and quantify key protein targets in both CTCs and plasma makes it broadly applicable. This first-of-its-kind proteomic assay can guide precision oncology by profiling multiple druggable proteins from individual patients, ultimately minimizing harm and improving targeted therapy selection.

Funding Acknowledgements:

No external financial support or grants were received directly related to this project.

Conflicts of Interest Disclosure Statement:

SYB, AH, KJK, NRB, KM, ARH, GS, ZI, CGG, MA, BRK, JH and JMD are full-time employees of Astrin Biosciences and report stock ownership in Astrin Biosciences. ESA reports grants and personal fees from Janssen, Sanofi, Bayer, Bristol Myers Squibb, Curium, MacroGenics, Merck, Pfizer, AstraZeneca, and Clovis; personal fees from Aadi Bioscience, Aikido Pharma, Astellas, Amgen, Blue Earth, Boundless Bio, Corcept Therapeutics, Exact Sciences, Hookipa Pharma, Invitae, Eli Lilly, Foundation Medicine, Menarini-Silicon Biosystems, Tango Therapeutics, Tempus and Z-alpha; grants from Novartis, Celgene, and Orion; and has a patent for an AR-V7 biomarker technology that has been licensed to Qiagen.