Prediction of ctDNA fraction for genomic biomarker testing and implementation of ctDNA based prognostication in advanced prostate cancer

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Background: Patients with advanced prostate cancer typically receive several lines of systemic therapy but there is no reliable and practical way to assess disease aggression prior to treatment initiation. Circulating tumour DNA fraction (ctDNA%) is the proportion of cell-free DNA that is tumour derived and is linked to patient prognosis in prostate cancer, but the precise relationship between ctDNA%, clinical prognostic variables and survival across treatment contexts is unclear. In addition, the inability to estimate ctDNA% adequacy for genotyping prior to blood draw remains a challenge in precision oncology. ctDNA testing is increasingly used for genomic biomarker identification but probability of an informative test result is influenced by patient ctDNA% at time of blood draw. Low ctDNA% precludes detection of clinically relevant alterations (e.g. *BRCA2* deletions). Therefore, predicting liquid biopsy success can ensure timely patient care and reduce resource waste by ensuring blood sampling is conducted when ctDNA levels are sufficient.

Methods: 738 plasma cfDNA samples from 491 clinically progressing mCRPC patients were sequenced with a 72-gene mCRPC panel. ctDNA% was estimated using validated methodology. Clinical characteristics including ECOG and serum laboratory markers were collected within 1 month prior to first-, second- or third-line therapy. OS and PSA-PFS for patients with undetectable (<2%), low (2-30%) and high (>30%) ctDNA% were compared using multivariable Cox proportional hazards models. To predict ctDNA%, an XGBoost machine learning model was trained using clinical variables

and time-matched ctDNA% from 463 first-line mCRPC patients and validated on two external clinical trial datasets.

Results: ctDNA% was strongly associated with clinical metrics of disease burden including liver metastases (median ctDNA 42% vs. 4.9% in pts with node-only disease, MWU $p<0.001$) and increased bone lesions (0% vs 10.4% in pts with 1-3 vs 10+ lesions, MWU $p<0.001$). ctDNA% was positively correlated with serum alkaline phosphatase ($\rho=0.46$), lactate dehydrogenase ($\rho=0.41$) and PSA ($\rho=0.30$). Our 8-feature XGBoost model classified a blood sample as having ctDNA% either above or below 2% with an AUC of 0.77. ctDNA>30% strongly predicted PSA progression (HR: 5.07, 95% CI: 3.68-6.98, $p<0.001$) and reduced overall survival (HR: 5.57, 95% CI: 4.10-7.55, $p<0.001$) for patients starting firstline treatment. ctDNA% remained associated with OS and PSA-PFS in the second line context and was also prognostic as a continuous variable (OS HR = 1.03, 95% CI: 1.02-1.03, $p< 0.001$; PSA-PFS HR = 1.02, 95% CI: 1.02-1.03, $p<0.001$).

Conclusions: Our ctDNA% prediction tool can help decide between ctDNA- or tissue-based mCRPC genotyping. Regardless of adequacy for genomic biomarker identification, ctDNA testing offers valuable prognostic information and should be considered for all mCRPC patients.

Funding Acknowledgements: This work was primarily funded by a Canadian Cancer Society Challenge Grant (grant #707339) to A.W.W., K.N.C., and N.M.F. N.M.F is supported by a Prostate Cancer Foundation Young Investigator Award. Other funding support was provided by the Canadian Institutes of Health Research, Prostate Cancer Canada, Movember Foundation, Jane and Aatos Erkko Foundation, Academy of Finland Center of Excellence program (project no. 312043), a Terry Fox New Frontiers Program Project Grant, the BC Cancer Foundation, and Kom op tegen Kanker (Stand Up To Cancer - Flemish Cancer Society) [grant numbers STI.VLK.2020.0006.01; STI.VLK.2022.0005.01].

Conflict of Interest Disclosure: C.M.D. reports Honoria from MSD, Bristol-Myers Squibb, Medison and Pfizer and consulting fees from Biomica LTD. E.M.K. has served in consulting or advisory roles in Astellas Pharma, Janssen, Ipsen and received honoraria from Janssen, Ipsen, Astellas Pharma, and Research Review. E.M.K. also reports research funding from Astellas Pharma (institutional) and AstraZeneca (institutional), and travel expense reimbursement from Astellas Pharma, Pfizer, Ipsen and Roche. K.N. has served on advisory boards for Abbvie, Astellas, AstraZeneca, Bayer, Bristol-Myers Squibb, Merck, Janssen and Tersera. D.L.F. has served in advisory roles or received honorarium from Janssen, Bayer, Astellas, AstraZeneca and Pfizer. M.A. is a shareholder in Fluivia Ltd. K.N.C. reports grants from Janssen, Astellas, and Sanofi during the conduct of the study. K.N.C. also reports grants and personal fees from Janssen, Astellas, AstraZeneca, and Sanofi, as well as personal fees from Constellation Pharmaceuticals, Daiichi Sankyo, Merck, Novartis, Pfizer, Point Biopharma, and Roche outside the submitted work. A.W.W. has served on advisory boards and/or received honoraria from AstraZeneca, Astellas, Bayer, EMD Serono, Janssen, Merck, and Pfizer. A.W.W.'s laboratory has a contract research agreement with ESSA Pharma. The remaining authors declare no competing interests.