5hmC-sequencing of matched cfDNA and tissue from men with mCRPC is concordant and identifies loss of AR signaling in NEPC and DNPC

Rensheng Wan[1,2], Raunak Shrestha[1,2], Gulfem Guler[3], Yuhong Ning[3], Aishwarya Subramanian[1,2], Adam Foye[1,4], Meng Zhang[1,2], Xiaolin Zhu[1,4], Thaidy Moreno-Rodriguez[1,5], Haolong Li[1,2], Shuang G. Zhao[6,7], SU2C/PCF West Coast Prostate Cancer Dream Team, Joshi J. Alumkal[8], Rahul Aggarwal[1,4], Alexander W. Wyatt[9,10], David Quigley[1,5,11], Samuel Levy[3], Eric Small[1,4,§], Felix Feng[1,2,4,5,§], Martin Sjöström[1,2,12,13,§*]

[1]Helen Diller Family Comprehensive Cancer Center, University of California at San Francisco, San Francisco, CA, USA.
[2]Department of Radiation Oncology, University of California at San Francisco, San Francisco, CA, USA.
[3]Clearnote Health, San Diego, CA, USA.
[4]Division of Hematology and Oncology, Department of Medicine, University of California at San Francisco, San Francisco, CA, USA.
[5]Department of Urology, University of California at San Francisco, San Francisco, CA, USA.
[6]Department of Human Oncology, University of Wisconsin-Madison, Madison, WI, USA.
[7]William S. Middleton Memorial Veterans' Hospital, Madison, WI, USA.
[8]Division of Hematology and Oncology, University of Michigan Rogel Cancer Center, Ann Arbor, MI, USA.
[9]Vancouver Prostate Centre, Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada.
[10]Michael Smith Genome Sciences Centre, BC Cancer, Vancouver, BC, Canada.
[11]Department of Epidemiology and Biostatistics, University of California at San Francisco, San Francisco, CA, USA.
[12]Division of Oncology, Department of Clinical Sciences Lund, Faculty of Medicine, Lund University, Lund, Sweden.
[13]Department of Haematology, Oncology and Radiation Physics, Skåne University Hospital, Lund, Sweden
[§]Co-corresponding author
[*]Presenting author

Background: Tumor tissue biopsy is the gold standard in cancer diagnosis. However, limitations such as inaccessibility to metastatic sites and inability to capture tumor heterogeneity makes it impractical, especially for serial monitoring of metastatic castration-resistant prostate cancer (mCRPC). A liquid biopsy is easily accessible, less invasive, highly repeatable, representative of tissue biopsy and thus offers an attractive alternative; analysis of specific genetic alterations in circulating tumor (ct) cell-free DNA (cfDNA) is already in clinical use. Epigenetic assays may provide additional phenotypic tumor information and provide a platform for developing novel biomarkers. 5-hydroxymethylcytosine (5hmC) is a DNA-based epigenetic modification associated with poised and active transcription and gene regulation throughout cancer progression with promise in cancer detection and tumor classification. In this study, we aimed to study whether 5hmC-seq of cfDNA predicts gene expression in tumor tissue and can distinguish tumor subtypes defined in tissue.

Methods: We performed 5hmC-seq on cfDNA samples from 86 mCRPC patients with matched tumor tissue profiled with 5hmC-seq (N=49) and RNA-seq (N=86) and we compared cfDNA 5hmC levels with matched tissue 5hmC and gene expression. We further developed a 5hmC-seq-based ctDNA fraction classifier and assessed if gene-level and pathway-level differences between mCRPC subtypes could be detected in cfDNA using a differential 5hmC-analysis adjusting for ct-fraction.

Results: Patients with androgen receptor (AR)-positive prostate cancer (ARPC) exhibited lower ct-fraction, whereas patients with AR-negative tumors displayed higher ct-fraction ($p=0.04$). Nearly 50% of protein-coding genes showed significant concordance between cfDNA 5hmC and tissue 5hmC, and 30% were significantly concordant with RNA-seq, after adjusting for ct-fraction (adjusted $p<0.05$). Genes with highest correlation between cfDNA 5hmC and tissue RNA were enriched in the androgen response, EGFR and ERBB

signaling pathways. Compared to ARPC, neuroendocrine (NE) prostate cancer (NEPC) displayed upregulated 5hmC enrichment in NE-related genes, such as SRRM4, CEACAM5, and PROX1, and downregulated androgen response and MYC target pathways in cfDNA. As expected, an NE pathway score calculated from cfDNA 5hmC was significantly different between tissue-confirmed ARPC and NEPC ($p=0.03$). Double-negative prostate cancer (DNPC) on the other hand showed downregulated androgen response and MYC targets and upregulated epithelial cell pathways compared to ARPC in cfDNA.

Conclusions: We created a cohort of 86 matched tissue and cfDNA samples and demonstrated concordance for most transcribed protein-coding genes. Expected biological differences previously seen in tissue between NEPC and ARPC were readily detectible in cfDNA. Furthermore, DNPC showed a clear downregulation of androgen response signaling in cfDNA, indicating the possibility to identify a group of patients in addition to classical NEPC that may have reduced response to standard androgen-targeting agents. Future work will aim at independent validation and to develop single-sample subtype classifiers and prognostic as well as treatment-predictive 5hmC-seq biomarkers for men with advanced prostate cancer.

Funding Acknowledgements
M. Sjöström was supported by the Swedish Research Council (Vetenskapsrådet) with grant number 2018-00382, the Swedish Society of Medicine (Svenska Läkaresällskapet), a Prostate Cancer Foundation Young Investigator Award and a Swedish Cancer Society Junior Clinical Investigator Award. R. Shrestha was funded by a Prostate Cancer Foundation Young Investigator Award. Xiaolin Zhu was funded by a Prostate Cancer Foundation Young Investigator Award. S.G. Zhao was funded by a Prostate Cancer Foundation Young Investigator Award, DOD W81XWH2110205, NIH 1DP2CA271832-01, and is a UW Carbone Cancer Center Ride Scholar. H. Li was supported by a Prostate Cancer Foundation Young Investigator Award. D.A. Quigley was funded by Prostate Cancer Foundation Challenge Awards and the UCSF Benioff Initiative for Prostate Cancer Research. F.Y. Feng was funded by Prostate Cancer Foundation Challenge Awards. Additional funding was provided by a UCSF Benioff Initiative for Prostate Cancer Research award. F.Y. Feng was supported by NIH/NCI 1R01CA230516-01, 1R01CA227025, and by NIH P50CA186786. F.Y. Feng was supported by Department of Defense grant W81XWH-19-1-0682.

Conflicts of Interest Disclosure Statement
A. Foye reports personal fees from Varian Medical Systems outside this work. S.G. Zhao has patent applications with Veracyte on molecular signatures in prostate cancer unrelated to this work and has a family member employed by Artera. S. Levy holds stock as an employee of Clearnote Health. Y. Ning is an employee of Clearnote Health. G. Guler is an employe of Clearnote Health. R. Aggarwal reports grants from Janssen, Amgen, Zenith Epigenetics, and Xynomic Pharmaceuticals; grants and personal fees from AstraZeneca, Merck, and Novartis; personal fees from Dendreon, Elsevier, Exelixis, Jubilant Therapeutics, Bayer, Pfizer, and Alessa Therapeutics outside the submitted work. E.J. Small reports other support from Fortis, Harpoon, Teon, Janssen, Johnson & Johnson, and Ultragenyx during the conduct of the study; other support from Fortis, Harpoon, Teon, Janssen, Johnson & Johnson, and Ultragenyx outside the submitted work. A.W. Wyatt reports personal fees from AstraZeneca, Merck, and Janssen, and grants from ESSA Pharma outside the submitted work. F.Y. Feng reports personal fees from Janssen Oncology, Bayer, PFS Genomics, Myovant Sciences, Roivant Sciences, Astellas Pharma, Foundation Medicine, Varian, Bristol Myers Squibb (BMS), Exact Sciences, Clearnote Health, Novartis, and Tempus; other support from Serimmune and Artera outside the submitted work. M. Sjöström reports personal speaker fees from Astellas and personal consulting fees from Veracyte and Adelphi Targis outside the submitted work.