Clinical impact of high-risk genomic mutations and prostate cancer lineage state identified through high purity circulating tumor cell RNA sequencing in patients with metastatic prostate cancer

Marina N Sharifi[1,2], Jamie M. Sperger[1,2], Amy K. Taylor[1,2], Katharine Tippins[1], Shannon R Reese[1], Viridiana Carreno[1,2], Alex H Chang[1,2], Kyle T. Helzer[3], Matthew L. Bootsma[3], Grace C. Blitzer[3], John Floberg[3], Christos Kyriakopoulos[1,2], Hamid Emamekhoo[1,2], Scott T. Tagawa[4], MD, Atish D. Choudhury[5], Andrew J. Armstrong[6], Dana E. Rathkopf[7], Himisha Beltran[5], Peter S. Nelson[8], Scott M. Dehm[9], David Kosoff[1,2,10], Rana R. McKay[11], Xiao Wei[5], Martin Sjöström[12], Shuang G. Zhao[3], Joshua M. Lang[1,2]

[1]Department of Medicine, [2]University of Wisconsin Carbone Cancer Center, and [3]Department of Human Oncology, University of Wisconsin, Madison, [4]Weill Cornell Medical College, New York, NY, [5]Dana-Farber Cancer Institute, Boston, MA, [6]Duke Cancer Institute Center for Prostate and Urologic Cancer, Duke University, Durham, NC, [7]Department of Medicine, Division of Solid Tumor Oncology, Genitourinary Oncology Service, Memorial Sloan Kettering Cancer Center, New York, NY, [8]Division of Human Biology, Fred Hutchinson Cancer Center, Seattle, WA and Department of Urology, University of Washington, Seattle, WA, [9]Masonic Cancer Center, University of Minnesota, Minneapolis, MN, [10]William S Middleton Memorial Veterans Hospital, Madison, Wisconsin, [11]Department of Medicine, University of California, San Diego, [12]Department of Clinical Sciences Lund, Division of Oncology, Lund University, Lund, Sweden and Department of Hematology, Oncology and Radiation Physics, Skåne University Hospital, Lund, Sweden.

Introduction: Specific classes of genomic mutations, including *RB1*, *AR*, *PTEN*, *TP53* and homologous recombination repair (HRR) genes, are associated with poor prognosis and treatment resistance in metastatic prostate cancer (mPC). Lineage state transitions from adenocarcinomas to neuroendocrine prostate cancer (NEPC) can also drive treatment resistance and poor survival. Identifying the timing and association of diverse genomic mutations with lineage state transitions has been limited by the challenges of obtaining serial tumor biopsies. Here we report an integrated analysis of clinical next-generation sequencing (NGS) data, circulating tumor DNA, and mPC lineage state phenotypes with a novel high purity circulating tumor cell (CTC) RNA sequencing method. **Methods:** We collected 273 CTC samples from 117 patients with mPC in a prospective biomarker trial. 69 patients had clinical NGS from a contemporary tissue biopsy. CTCs were purified via immunomagnetic capture on a microfluidic platform and analyzed via RNA-seq. We compared CTC lineage state phenotypes, somatic mutation status and median overall survival (mOS). **Results:** Gene expression analysis of high CTC purity samples identified four CTC phenotypes: luminal A-like (LumA), luminal B-like (LumB), low proliferation (LP), and neuroendocrine (NE). Compared to patients with low CTC burden/purity (mOS NR), patients with LumA and LP phenotypes had similar survival ((LumA: mOS 13mo, p=ns, LP: mOS: 11.8mo, p=ns), while patients with LumB and NE phenotypes had shorter survival (LumB: mOS 6mo, HR 9.1[3.8-21.8], p<0.0001, NE: mOS 3.7mo, HR 11.8[2.4-57.2]), p=0.0019). mOS for patients with high-risk genomic mutations (*AR*, *RB1, PTEN, TP53*) and a LumA or LP lineage state was 12.7mo versus 4mo for patients with high-risk mutations and a LumB or NE lineage state (HR 3.59 [1.51-8.51], p=0.004). mOS was not reached for patients without high-risk mutations and a LumA or LP lineage state vs 8mo for patients without high-risk mutations but a LumB or NE lineage state (HR 19.7 [1.99-1.95], p<0.0001). Among patients treated with 177Lu-PSMA-617 in a prospective substudy (n=37), no patients had pretreatment CTC NE phenotype, but pre-treatment CTC LumB phenotype was associated with decreased rPFS (3.5mo vs 11.7mo, HR 4.8 [95% CI 1.4-16.1], p<0.005) and OS (7.6mo vs NR, HR 9.3 [95% CI 2.3-37.8], p<0.0005) compared to pretreatment LumA/LP/Low CTC burden phenotypes. **Conclusions:** Lineage state transitions detected by high purity CTC RNAseq are prognostic for poor OS and decreased benefit from 177Lu-PSMA-617. The presence of high-risk genetic mutations including *RB1* is also prognostic for poor OS and combined evaluation of high-risk genetic mutation status with lineage state phenotype identifies patients with the worst clinical outcomes that would benefit from treatment intensification and early disease monitoring for these more aggressive subtypes of mCRPC.

Funding: We would like to acknowledge funding from the National Institutes of Health [grant numbers DP2 OD030734 to SGZ, 1UH2CA260389 to AJA, DER, JML, R01CA247479 to JML, R01CA276269 to SMD, JML, P50CA269011 to JML, SGZ; P50CA097186 to PSN; R01CA234715-01 to PSN; R01CA266452 to PSN; R01CA280056 to PSN, R50CA283815 to JLS, R50CA293840 to JMS], Department of Defense [grant numbers PC190039 to SGZ, PC200334 to SGZ, JML, PC180469 to JML, PC220240 to MNS, PC230533 AKT], Prostate Cancer Foundation (2024 TACTICAL award to FYF, PSN, JML, Movember Foundation—PCF Challenge Award to JML, 2023 Debbie & Mark Attanasio – PCF Young Investigator Award to AKT, 2022 Point Biopharma Young VAlor Investigator Award to MNS, 2021 Michael and Patricia Berns-PCF Young Investigator Award to MS), the University of Wisconsin Office of the Vice Chancellor for Research and Graduate Education (PICI award to SGZ). Shared research services at the UWCCC Core are supported by Cancer Center Support Grant [grant number P30 CA014520]. We would like to thank all patients who participated in this study. We are also grateful for the help of Katie Kovacich for project management, and the UWCCC biospecimen team, especially Laura Ruelle, Hannah Ranous and Ashley Rohm. We thank the University of Wisconsin Carbone Cancer Center Circulating Biomarker Core, supported by P30 CA014520, for use of its facilities and services.

Conflicts of interest: KTH has a family member who is an employee of Epic Systems. MLB has a family member who is an employee of Luminex – A DiaSorin Company. SGZ reports unrelated patents licensed to Veracyte, and that a family member is an employee of Artera and holds stock in Exact Sciences. SMD reports consulting relationships with BMS, Oncternal therapeutics, Janssen R&D/J&J and a grant from Pfizer/Astellas/Medivation (the grant was submitted to Medivation, ultimately funded by Astellas and then moved to Pfizer). FYF reports personal fees from Janssen Oncology, Bayer, PFS Genomics, Myovant Sciences, Roivant Sciences, Astellas Pharma, Foundation Medicine, Varian, Bristol Myers Squibb (BMS), Exact Sciences, BlueStar Genomics, Novartis, and Tempus; other support from Serimmune and Artera outside the submitted work. MS reports speaker fees from Astellas, Scientific Advisory board for Veracyte/Adelphi Targis. MNS reports research support from Novartis unrelated to the submitted work. J.M.L. holds equity in Salus Discovery LLC, which has licensed some of the technology used in this manuscript. JML is a consultant/AdvisoryBoard Member for Janssen, Pfizer, Astellas, Immunomedics; receives institutional research funding from Immunomedics. CEK holds stock in EPIC Systems and Biogen, reports institutional research support from Sanofi-Aventis, AstraZeneca, Bristol Meyers Squibb, ESSA Pharma, Gilead Sciences, Incyte Corporation, Merck, Madison Vaccines, EMD Serono, and Pionyr Immunopharma outside the submitted work and consulting fees from Sanofi-Aventis, AVEO Pharmaceuticals, EMD Serono, Exelixis, Janssen Pharmaceuticals, Pfizer. RRM reports consulting fees from Ambrx, Arcus, AstraZeneca, Aveo, Bayer, Bristol-Myers Squibb, Calithera, Caris, Dendreon, Exelixis, Johnson & Johnson, Lilly, Merck, Myovant, NeoMorph, Novartis, Pfizer, Sanofi, SeaGen, Sorrento Therapeutics, Telix, Tempus and institutional research support from ArteraAI, AstraZeneca, Exelixis, BMS, Oncternal, Bayer, Tempus outside the submitted work. XXW reports consulting fees from Dendreon, Macrogenics, Novartis and research support from BMS outside the submitted work. PSN reports personal fees from Janssen, Bristol Myers Squibb, Pfizer and research support from Janssen and Oncternal for work unrelated to the present study.