Clinical and functional characterization of the *HOXB13 X285K* germline mutation specific to men of African-ancestry

Mayuko Kanayama[1], Yidong Chen[2,3], Emeline Colomba[4], Yusra Shao[5], Fabiana Montenegro[1], Changxue Lu[1], Sarah M. Nielsen[6], Emily M. Russell[6], Edward D. Esplin[6], Hao Wang[7], John C. Henegan[8], Elisabeth I. Heath[3], William B. Isaacs[1,5], Régine Marlin[4], Emmanuel S. Antonarakis[9], Jun Luo[1]

[1]Department of Urology, James Buchanan Brady Urological Institute, Johns Hopkins University School of Medicine, Baltimore, MD, U.S.A. [2]Greehey Children's Cancer Research Institute, [3]Department of Population Health Sciences, the University of Texas Health San Antonio, San Antonio, TX, USA. [4]Department of Molecular Cancer Genetic, University Hospital of Martinique, Fort de France, France. [5]Karmanos Cancer Institute, Department of Oncology, Wayne State University School of Medicine, Detroit, MI, USA. [6]Invitae Corporation, San Francisco, CA, USA. [7]Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD, U.S.A. [8]Department of Medicine, University of Mississippi Medical Center, Jackson, MS, USA.[9]Department of Medicine, Masonic Cancer Center, University of Minnesota, Minneapolis, MN, U.S.A.

Background Heritable genetic risk factors play a significant role in prostate cancer etiology and clinical practice. Recent reports have uncovered a stop-loss *HOXB13* variant c.853delT (referred to as X285K) predisposing men of West African ancestry to aggressive prostate cancer. Our studies seek to determine the clinical relevance of *HOXB13* (X285K) in comparison with *HOXB13* (G84E) and *BRCA2* pathogenic/likely pathogenic (P/LP) variants to elucidate the oncogenic mechanisms of the X285K protein and to characterize the therapeutic implications of X285K.
Methods To determine the clinical relevance of *HOXB13* (X285K) in comparison with *HOXB13* (G84E) and *BRCA2* P/LP variants, real-world data from 21,393 men with prostate cancer undergoing the Detect Hereditary Prostate Cancer (DHPC) sponsored testing program from 2019-2022 (Invitae Corporation, San Francisco, CA) was analyzed. Genetic testing results were compared among patient groups according to self-reported race/ethnicity, Gleason scores, and AJCC stages using exact test. For evaluation of oncogenic functions associated with the X285K protein, cell-line models were subjected to RNA sequencing, ChIP sequencing, ATAC sequencing, and Western blot analyses. To characterize the therapeutic implications of X285K, six mHSPC X285K-carrier patients with treatment outcome data were identified through a multi-institutional effort.
Results *HOXB13* (X285K) was significantly enriched in self-reported Black (1.01%) versus White (0.01%) patients. We observed a trend of more aggressive disease in the *HOXB13* (X285K) and *BRCA2* P/LP carriers than in the *HOXB13* (G84E) carriers. In *in vitro* functional analysis, replacement of the wild-type (WT) HOXB13 protein with the X285K protein resulted in a gain of an E2F/MYC signature, validated by the elevated expression of Cyclin B1 and c-Myc, without affecting the androgen response signature. Elevated expression of Cyclin B1 and c-Myc was explained by enhanced binding of the X285K protein to the promoters and enhancers of these genes, which resulted in increased chromatin accessibility. Detailed retrospective chart reviews revealed that X285K carriers may be sensitive to AR-targeted therapies.
Conclusions *HOXB13* X285K is significantly enriched in self-reported Black patients and X285K carriers detected in the real-world clinical setting have aggressive prostate cancer features similar to the *BRCA2* carriers. Functional studies revealed a unique gain-of-function oncogenic mechanism of the X285K protein in regulating E2F/MYC signatures. Our studies have resulted in the inclusion of the variant in genetic testing reports. Retrospective chart reviews indicate that X285K carriers generally benefit from longer responses to AR-targeted therapies. Together, our findings underscore the clinical utility of germline genetic testing and provide new information to facilitate clinical interpretation of genetic testing findings in a population disproportionately affected by prostate cancer.

Funding Acknowledgements
The generous support from the Patrick C Walsh Hereditary Prostate Cancer Program and the Ambrose Monell Foundation are gratefully acknowledged. The study was also supported by a Prostate Cancer

Foundation Young Investigator Award (to M.K.). Next-generation sequencing data were generated at the Genome Sequencing Facility/Mays Cancer Center Next-generation Sequencing Shared Resource which is supported by NIH-NCI P30 CA054174, N.I.H. Shared Instrument grant 1S10OD021805-01 (S10 grant), and Cancer Prevention and Research Institute of Texas (CPRIT) Core Facility Award (RP160732).

Conflicts of Interest Disclosure Statement

J.L. has served as a paid consultant/advisor for Sun Pharma, has received research funding to his institution from Calibr, Cardiff Oncology, and is the lead inventor of AR-V7-related technologies owned by Johns Hopkins University and licensed to Qiagen, and A&G.
W.B.I. is a co-inventor on a patent (no. 9593380; Johns Hopkins Univ.) related to the discovery of HOXB13 as a prostate cancer susceptibility gene.
S.M.N., E.M.R., and E.D.E. are employees and stockholders of the Invitae Corporation.
E.D.E. is a scientific advisory board member and stockholder of Taproot Health.
E.S.A. has served as a paid consultant/advisor for Sanofi, Dendreon, Janssen Biotech, ESSA, Merck, AstraZeneca, Clovis Oncology, Lilly, Bayer, and received an honorarium from Sanofi, Dendreon, Medivation, Janssen Biotech, ESSA, Astellas Pharma, Merck, AstraZeneca, Clovis Oncology. E.S.A. received research funding from Janssen Biotech, Johnson & Johnson, Sanofi, Dendreon, Aragon Pharmaceuticals, Exelixis, Millennium, Genentech, Novartis, Astellas Pharma, Tokai Pharmaceuticals, Merck, AstraZeneca, Clovis Oncology, Constellation Pharmaceuticals, as well as travel accommodations from Sanofi, Dendreon, Medivation. E.S.A. is a co-inventor of a technology owned by Johns Hopkins University and licensed to Qiagen.