

The Movember Global Action Plan 1 (GAP1): Unique Prostate Cancer Tissue Microarray Resource



Véronique Ouellet¹, Andrew Erickson^{2,3,4}; on behalf of GAP1 UTMA Contributing Investigators, Kathy Wiley⁴, Colm Morrissey⁵, Viktor Berge⁶, Carlos S. Moreno^{7,8}, Kristin Austlid Tasken^{9,10}, Dominique Trudel^{1,11}, Lawrence D. True¹², Michael S. Lewis¹³, Aud Svindland^{10,14}, Onur Ertunc^{4,15}, Igor Damasceno Vidal⁴, Adeboye O. Osunkoya^{7,8}, Tracy Jones⁴, G. Steven Bova¹⁶, Tarja Lamminen¹⁷, Ariel H. Achtman¹⁸, Mark Buzza¹⁸, Michelle M. Kouspou¹⁸, Steven A. Bigler¹⁹, Xinchun Zhou²⁰, Stephen J. Freedland^{21,22}, Anne-Marie Mes-Masson^{1,23}, Isla P. Garraway^{24,25}, Bruce J. Trock²⁶, Pekka Taimen^{27,28}, Fred Saad^{1,29}, Tuomas Mirtti^{30,31}, Beatrice S. Knudsen³², and Angelo M. De Marzo^{4,26,33}

ABSTRACT

Background: The need to better understand the molecular underpinnings of the heterogeneous outcomes of patients with prostate cancer is a pressing global problem and a key research priority for Movember. To address this, the Movember Global Action Plan 1 Unique tissue microarray (GAP1-UTMA) project constructed a set of unique and richly annotated tissue microarrays (TMA) from prostate cancer samples obtained from multiple institutions across several global locations.

Methods: Three separate TMA sets were built that differ by purpose and disease state.

Results: The intended use of TMA1 (Primary Matched LN) is to validate biomarkers that help determine which clinically localized prostate cancers with associated lymph node metastasis have a high risk of progression to lethal castration-resistant metastatic disease, and to compare molecular properties of high-risk index lesions

within the prostate to regional lymph node metastases resected at the time of prostatectomy. TMA2 (Pre vs. Post ADT) was designed to address questions regarding risk of castration-resistant prostate cancer (CRPC) and response to suppression of the androgen receptor/androgen axis, and characterization of the castration-resistant phenotype. TMA3 (CRPC Met Heterogeneity)'s intended use is to assess the heterogeneity of molecular markers across different anatomic sites in lethal prostate cancer metastases.

Conclusions: The GAP1-UTMA project has succeeded in combining a large set of tissue specimens from 501 patients with prostate cancer with rich clinical annotation.

Impact: This resource is now available to the prostate cancer community as a tool for biomarker validation to address important unanswered clinical questions around disease progression and response to treatment.

¹Centre de recherche du Centre hospitalier de l'Université de Montréal et Institut du cancer de Montréal, Montreal, Canada. ²Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom. ³Department of Pathology, Helsinki and Uusimaa Hospital District and Medicum, University of Helsinki, Helsinki, Finland. ⁴Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland. ⁵Department of Urology, University of Washington, Seattle, Washington. ⁶Department of Urology, Oslo University Hospital, Oslo, Norway. ⁷Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, Georgia. ⁸Winship Cancer Institute of Emory University, Atlanta, Georgia. ⁹Institute of Cancer Research, Oslo University Hospital, Oslo, Norway. ¹⁰Institute of Clinical Medicine, University of Oslo, Oslo, Norway. ¹¹Department of Pathology and Cellular Biology, Université de Montréal, Montreal, Canada. ¹²Department of Laboratory Medicine and Pathology, University of Washington, Seattle, Washington. ¹³West Los Angeles Veterans Affairs Medical Center and Departments of Pathology and Medicine, Cedars-Sinai Medical Center, Los Angeles, California. ¹⁴Department of Pathology, Oslo University Hospital, Oslo, Norway. ¹⁵Suleyman Demirel University, Department of Pathology, Training and Research Hospital East Campus, Isparta, Turkey. ¹⁶Faculty of Medicine and Health Technology, Prostate Cancer Research Center, Tampere University and Tays Cancer Center, Tampere, Finland. ¹⁷Institute of Biomedicine, University of Turku, Turku, Finland. ¹⁸Movember, Melbourne, Australia. ¹⁹Department of Pathology, Mississippi Baptist Medical Center, Jackson, Mississippi. ²⁰Department of Pathology, University of Mississippi Medical Center, Jackson, Mississippi. ²¹Center for Integrated Research on Cancer and Lifestyle, Cedars-Sinai Medical Center, Los Angeles, California. ²²Section of Urology, Durham VA Medical Center, Durham, North Carolina. ²³Department of Medicine, Université de Montréal, Montreal, Canada. ²⁴Department of Urology, Jonsson Comprehensive Cancer Center, David Geffen School of Medicine at University of California, Los Angeles, California. ²⁵Division

of Urology, Greater Los Angeles VA Healthcare System, Los Angeles, California. ²⁶Department of Urology and Brady Urological Institute, Johns Hopkins University School of Medicine, Baltimore, Maryland. ²⁷Institute of Biomedicine, University of Turku, Turku, Finland. ²⁸Department of Pathology, Turku University Hospital, Turku, Finland. ²⁹Department of Surgery, Université de Montréal, Montreal, Canada. ³⁰HUS Diagnostic Center, Department of Pathology, HUS Helsinki University Hospital, Helsinki, Finland. ³¹Medicum and Research Program In Systems Oncology, Faculty of Medicine, University of Helsinki, Helsinki, Finland. ³²Digital and Computational Pathology, University of Utah, Salt Lake City, Utah. ³³Department of Oncology and the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland.

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (<http://cebp.aacrjournals.org/>).

V. Ouellet and A. Erickson contributed equally to first authorship of this article.

F. Saad, T. Mirtti, B.S. Knudsen, and A.M. De Marzo contributed equally as the co-senior authors of this article.

Corresponding Author: Angelo M. De Marzo, Department of Pathology, Johns Hopkins University School of Medicine, 1550 Orleans Street, Baltimore, MD 21212. E-mail: ademarz@jhmi.edu

Cancer Epidemiol Biomarkers Prev 2022;31:715–27

doi: 10.1158/1055-9965.EPI-21-0600

This open access article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 International (CC BY-NC-ND).

©2022 The Authors; Published by the American Association for Cancer Research

Introduction

Prostate cancer is the second most common cancer and the sixth leading cause of cancer death worldwide in men (1, 2). Although some prostate cancers remain clinically indolent for many years, others progress at a variable rate to lethal metastatic castration-resistant disease and death. The death rate from prostate cancer has decreased in the last two decades, potentially as a result of better detection and treatment of clinically localized disease. However, despite this progress many men with tumors of low inherent aggressiveness are overtreated, whereas others with intermediate-risk or high-risk localized disease are undertreated (3–5). The mainstay for clinical decisions for localized prostate cancer is a biopsy followed by Gleason grading of the tumor. Gleason grading remains a powerful predictor of outcome in the extremes and those patients with Gleason Grade Group 1 (Gleason score 6) and some with low volume Grade Group 2 (3+4 = 7) are forgoing immediate treatment and increasingly enrolling in active surveillance programs. However, for those patients with clinically localized higher risk disease (high volume cancers of Grade Group 2 and higher, and any patients with Grade Groups 4–5), a significant fraction develop disease recurrence and progression after attempts at definitive local treatments (6). For these patients, additional treatments are needed, yet there are no standard adjuvant therapies for localized prostate cancer. Further, for those patients with intermediate-risk disease, the clinical course is quite variable and not well predicted by Gleason grading and clinical staging.

Presently, although a number of different molecular biomarkers have shown promise (7–9), there are no routinely employed validated biomarkers to differentiate indolent from aggressive intermediate-risk cases that can be used to enhance decision making. A subgroup of patients with clinically localized disease have prostate cancer which has spread to regional lymph nodes, which is often discovered incidentally when these lymph nodes are removed during a radical prostatectomy (RP) and examined microscopically. Interestingly, the presence of regional lymph node metastasis does not always portend an aggressive clinical course because some patients in this disease state remain stable without overt new metastatic disease and development of bone or soft tissue metastases for many years (10, 11). Indeed, some patients who are treated with androgen deprivation therapy (ADT), for either localized or metastatic disease, can have highly prolonged responses, whereas others succumb to castration-resistant prostate cancer (CRPC) quite quickly (12). Finally, when untreated, only a third of patients with biochemical recurrence (BCR) will develop clinically significant metastatic disease in a 7-year follow-up period (13), which underlines the relatively poor performance of BCR as a surrogate marker for prostate cancer survival outcomes. More precise biomarkers are needed to determine which of these locally treated cancers are likely to progress and/or develop resistance to ADT.

Another clinical problem that occurs in late-stage disease, often after several systemic therapies involving androgen signaling deprivation and/or chemotherapy, is disease heterogeneity (14–16) with some lesions responding to specific systemic agents and others not. This heterogeneity in CRPC is similar to many other types of late-stage metastatic cancer and improvements in deciphering its molecular features and mechanisms require tissue sampling from multiple metastatic sites, which is generally quite difficult outside of autopsies. The development and validation of biomarkers that can help better stratify risk in men with localized disease, to better understand who may rapidly progress on ADT, and to enhance our knowledge regarding the molecular features of late-stage disease heterogeneity was the

main rationale for initiating this Movember Global Action Plan 1 (GAP1) unique tissue microarray (TMA) project (GAP1-UTMA).

Although TMAs have been used in more than 2,000 publications in prostate cancer, the majority of these studies have been carried out by individual organizations/investigators and focused on the prognostic value of biomarkers, often using rising serum PSA as an indicator of poor outcome. Movember's Global Action Plans (GAP), launched in 2011 with the GAP1 biomarker project, were established to address critical challenges in prostate cancer research through global collaboration. Movember identified a need to create collections of valuable tissue resources with clinical annotation to support the first batch of GAP1 projects to help improve our understanding of the biology of treatment response and resistance and validating promising prostate cancer tissue-based biomarkers. In this GAP1 collaborative initiative, we assembled an international team with multidisciplinary expertise to pool diverse tissue sample resources to develop a novel set of TMAs consisting of prostate cancer tissue specimens from multiple disease states. This robust resource could not have been assembled using samples from any single institution.

Materials and Methods

Patients and specimen collection

Formalin-fixed paraffin-embedded prostate cancer patient specimens (prostate, lymph nodes, and other metastatic sites) used for TMA construction were retrieved from each participating hospital's pathology department where patients underwent surgery, transurethral resection of the prostate (TURP) or biopsy between 1978 and 2016. These hospitals included the Centre hospitalier de l'Université de Montréal (CHUM), Johns Hopkins Hospital (JHU), Helsinki University Hospital (HUS), Institute of Biomedicine, University of Turku and Turku University Hospital (TYKS), Oslo University Hospital (OUH), University of Mississippi Baptist Medical Centre (UMBMC), Greater Los Angeles VA Healthcare System, Durham VA Health Care System, Emory University, and the University of Washington. For autopsy cases, all specimens were processed within 8 hours (University of Washington), 14 hours (JHU), or 24 hours (Oslo University Hospital) of death. Specimens were fixed in 10% neutral-buffered formalin and embedded in paraffin. Bone metastases were decalcified in 10% formic acid before embedding. The samples selected for these TMAs were chosen to represent relatively rare clinical profiles that are difficult to obtain for research purposes. Because the number of such samples at each of our institutions was still relatively small, we did not impose a statistical sampling design to select samples. Rather, these TMAs are essentially composed of convenience samples, with uniformity of clinical attributes across institutions.

All centers received ethical review board permission to use patient material and conduct this study which was conducted in accordance with ethical guidelines. The following committees approved the study: Comité d'éthique de la recherche du CHUM (CE.14.128), the Institutional Review Board of the: JHU School of Medicine's, the UMBMC, the VA Greater Los Angeles (Department of Veterans Affairs PCC #2015–040408), the Ethics Committee of Hospital District of Helsinki and Uusimaa (84/13/03/00/2014; §3 30.01.2015), the Hospital District of Southwest Finland (number T206/2014) National Supervisory Authority for Welfare and Health for HUS and TYKS (VALVIRA, 8008/06.01.03/2014), the Regional Committees for Medical and Health Research Ethics for OUH (REC 2013/1713), and Center for Healthcare Ethics for Cedar Sinai (Pro00033387 and Pro00020577). Written patient informed consents were obtained as required by individual institutional ethical review boards (CHUM CE12.216, HUS,

TYKS, and University of Washington, Cedar Sinai) or waivers were granted by the ethic review boards (JHU, OUH, both VA Health Care System and UMBMC).

Histology review

The hematoxylin and eosin (H&E) or Weigert van Gieson stained sections of the whole prostates and pelvic lymph nodes from RP for TMA1 (Primary Matched LN), biopsies, RP and TURPs for TMA2 (Pre vs. Post ADT) and prostates or metastatic lesions obtained at autopsy for TMA3 (CRPC Met Heterogeneity) were reviewed by a pathologist within each institution. For the RP specimens, the pathologists identified the index tumor (largest tumor or the highest grade nodule). The grading of the primary tumor was taken from the original diagnostic pathology reports. For other tissue types, the overall tissue quality and regions of high-tumor content with minimal necrosis were selected for inclusion. Of note, some of the metastases from TMA3 (CRPC Met Heterogeneity) have variably large regions of necrosis and some metastases have very few tumor cells, such as those from osteoblastic bones. Patient and tissue block selection were performed using specific guidelines.

TMA design, construction, and review

TMA design, construction, and review were performed according to standard operating procedures (SOP) developed at each site and reviewed centrally to assure homogeneity prior to TMA construction. To build each TMA block, CHUM and Cedars-Sinai (Greater Los Angeles VA and Durham VA Health Care System, Emory University) used the TMArrayer (Pathology Devices, Inc.), whereas HUS and TYKS used Quick Ray Manual Tissue Microarrayer Full Set (Unitma). The University of Oslo utilized a semiautomated Beecher Instrument (TMABooster OI; Alphelys). JHU, University of Washington and University of Mississippi Medical Center utilized the Estigen Tissue Science Manual Tissue Arrayer MTA-1 (Tartu; formerly Beecher instruments).

A total of three cores from prostatic tissue (0.6 or 1 mm), two to three cores from lymph nodes (0.6 or 1 mm) and other metastatic lesions (0.6 or 1 mm) were transferred to a recipient TMA block in a serial manner (nonrandomized). For some centers, adjacent benign prostate tissue cores were also arrayed on the TMA. For biopsy tissue from prostate or metastatic lesions (0.6 mm), at least two cores from each specimen were also arrayed in a serial manner (nonrandomized) on a separate recipient TMA block. For each TMA category (TMA 1–3), each institution constructed a Test TMA, which consisted of a subset of the same specimens used in the full TMAs.

For each TMA, we included control tissues provided by Cedars-Sinai Medical Center (CSMC; human tonsil, kidney, colon, and liver) and CHUM (xenografts containing 22rv1, PC-3, LNCaP, and DU145 prostate cancer cell lines injected into and grown in immunocompromised/nude mice). An institutional approval was provided for the use of the human control tissue specimens. Mouse xenograft experiments were performed according to institutional rules and following approbation of the protocol by the Comité institutionnel de protection des animaux. Each of the control tissues (mouse and human) was divided into eight pieces, fixed in formalin, paraffin-embedded, and sent to the individual institutions to include in each TMA block. This process of using centrally processed control cell lines and tissues provides a quality control measure of TMA slide staining to detect batch effects in staining due to tissue processing protocols (instead of underlying biology). In addition, for many IHC stains there is a wealth of prior information regarding phenotypic features of these cell lines/xenografts and the common human tissues. To demarcate the starting X and Y coordinates (e.g., the upper left corner of the

TMA block) control tissues (human kidney or liver, depending upon institutional preference) were arranged either outside of or just within the main X and Y coordinates to facilitate proper orientation of each TMA slide. Quality control of H&E-stained sections from each TMA was performed by local and central genito-urinary pathologists.

TMA data handling

Each patient was assigned a specific code, consisting of a combined unique deidentified Specimen ID and Institution-specific 3 letter ID. This information was shared with the coordinating center (JHU) and was used to enter the data into the TMAJ Database (TMAJ), an open-source software system designed to support TMA pathology data (<http://tmaj.pathology.jhmi.edu/>; refs. 17, 18). The TMAJ database tracks the specimen ID, and institution and information for each FFPE block (e.g., anatomic site of origin) that is used for TMA punching and provides an export of a TMA map that was used for all sites for quality control. Clinical data associated with specimens were collected in a spreadsheet containing predefined data elements (Supplementary Table S1) with specific definitions to promote harmonization of data between sites. No HIPAA-defined protected health information is included in the data. These data were sent to the central repository where they were reviewed for consistency and when inconsistencies were identified, they were rectified by communication with the initiating site and modified if needed. Revised clinical data from each site were collated into a unique SAS database. These data are stored on a secure server in the central repository and are linked to the specimen data in the TMAs using the same unique identifier (Specimen ID and institutional ID) mentioned above. The combined clinical data from all sites are only accessible to the study biostatistician and research coordinator at the Coordinating Center. Each TMA slide from each TMA block is assigned a unique ID in the TMAJ database, and data about which stain was performed, including the site, antibody, and experimental details are recorded.

Storage of TMA blocks, unstained slides, and whole slide images

Each TMA block is kept at room temperature within its respective institution due to legal and ethical restrictions. TMA blocks were subjected to sectioning ($N = 20$ slides) at each respective institution and the H&E section as well as the remaining 19 unstained slides of each TMA were sent to the coordinating center. Following reception, the H&E slides were subjected to whole slide scanning using a Hamamatsu Photonics NanoZoomer XR, SN 510076 instrument and whole slide scan image files were uploaded to a web-based slide image management system (Concentriq from PROSCIA) and shared with the other sites. The remaining unstained TMA slides are stored in a plastic bag in a monitored -20°C freezer according to PCBN program SOPs (18), until requested by approved investigators.

IHC and scoring

Each Test TMA slide was stained for the following: recombinant anti-PSMA (Abcam, clone EPR 6253, rabbit monoclonal, dilution 1:300), PTEN (Cell Signaling Technology, clone D4.3 XP, rabbit monoclonal, dilution 1:100), anti-ERG (Roche, clone EPR3864, rabbit monoclonal, 23 $\mu\text{g}/\text{mL}$), AR (androgen receptor, Cell Signaling Technology, clone D6F11, rabbit monoclonal, dilution 1:400), prostate-specific antigen (PSA, clone ER-PR8, monoclonal anti-mouse, dilution 1:50), and NKX3.1 [rabbit polyclonal (19), dilution 1:1,000]. The slides were stained using the Ventana automated platform (Ventana Discovery Ultra HQ-HRP Hapten Detection, Ventana Medical System). The stained TMA sections were scanned as above for H&E slides using the Hamamatsu NanoZoomer, stored on Concentriq (PROSCIA), and

shared across all sites. For scoring the IHC staining, pathologists with expertise in prostate cancer reviewed the scanned slides and scored each spot. For every marker, staining was considered positive or negative. Staining was considered negative if there was a complete

absence of staining in the tumor cells. Positive staining in tumor cells was categorized as homogeneous or heterogeneous, with heterogeneity meaning either a difference in the fraction of positive tumor cells, the intensity of the staining across the tumor cells or both. For scoring of

Table 1. Unique tissue microarray description.

(A) Design of the TMA series								
Test TMA	TMA1 (primary matched LN)	TMA2 (pre vs. post ADT)	TMA3 (CRPC met heterogeneity)					
Sample type	• Subset of tissue included in the corresponding TMA (1,2,3)	• Paired untreated primary PC tissue and LN metastasis	• Pre- and post-ADT CRPC PC primary tissue and metastasis	• Matched multiple metastasis sites				
Subseries	• Test TMA1 • Test TMA2 • Test TMA3	• TMA1a (>5 years follow-up data) • TMA1b	• TMA2a • TMA2b: biopsy specimens					
Purpose	• To preserve the full TMA resource from wasteful use • To evaluate if biomarkers show consistent and adequate signal-to-noise staining • To identify potential site-specific bias due to internal tissue-processing protocols	• To compare protein expression in primary PC and concurrent LN metastases. • Provide biological insights into the mechanism of LN metastasis. • Determine expression patterns of biomarkers in the pre-metastatic setting and select biomarkers of aggressive disease • Identify biomarkers that are associated with further spread of the cancer (TMA1a).	• To profile biomarker expression in CRPC and comparison to pre-treatment.	• To study the heterogeneity of metastatic disease in patients with lethal PC. • To determine differences between bone and soft tissue metastases in the same patient.				
Inclusion criteria	• The same as the corresponding TMA series	• Tissue samples consist of matched untreated PC primary RP specimen and untreated LN metastasis	• Patients who developed CRPC • Matched tissue samples (pre to post ADT) or CRPC specimen • Pre-ADT specimen being TURP or RP • CRPC specimen TURP or metastatic site • Follow-up data available	• Specimen from multiple metastatic sites from a single patient • Available cancer treatment data • Treatment-naïve prostate, LN, and metastatic tissue from autopsy • Metastasis to pelvic LN from patients with advanced metastatic disease				
Exclusion criteria	• The same as the corresponding TMA series • Biopsy specimens	• Specimen from PC patients who have received systemic treatment • Patient without 5-year follow-up data (TMA1a) • Patient with 5-year follow-up data	• Patients who received ADT prior to RP as they do not have CRPC • Patients with PSA recurrence without tissue specimen • Tissue from a TURP chip that shows extensive cautery	• Tissue from autopsies performed after 24 hours post-mortem				
(B) TMA series composition								
	Test TMA			TMA1		TMA2		TMA3
	1	2	3	1a	1b	2a	2b	
Number of TMA blocks	5	4	3	6	8	4	3	7
Number of centers	6	5	3	4	5	4	3	3
Specimen	RP: 40 LN: 22	Pre-ADT RP: 4 TURP: 2	Prostate:4 LN: 19 Bone: 25 Liver: 6 Lung: 7 Other: 1	RP: 107 LN: 107	RP: 200 LN: 200	Pre-ADT RP: 12 TURP: 10 Met: 1	Pre-ADT Pros Bx: 36 Post-ADT Pros Bx: 4	Prostate: 45 ^a LN: 72 Bone: 116 Lung: 20 Liver: 36 Other: 29
		CRPC RP: 2 TURP: 24				Post-ADT RP: 3 TURP: 104 Met: 4		

^aFive prostate specimens are those obtained from radical prostatectomy and the remaining from rapid autopsy.

Table 2. Demographic and clinical variables of the Unique Tissue Microarray series.

(A) TMA 1 (primary matched LN)		
Variable	TMA1a	TMA1b
Number of patients	107	200
Year of diagnosis, median (IQR)	2010 (2004–2013)	2012 (2009–2013)
Follow-up time, median (IQR, months)	32.8 (12.4–60) ^a	30.5 (12–51) ^a
Age at diagnosis, median (IQR, years)	63 (59–67)	63 (58–68) ^b
Race, <i>n</i> (%)		
White	98 (92)	157 (79)
Black	4 (4)	27 (14)
Asian	0 (0)	2 (1)
Hispanic	2 (2)	2 (1)
Other	3 (3)	3 (2)
Missing	0 (0)	9 (5)
PSA (ng/mL) at diagnosis, median (IQR)	15.0 (8.5–32) ^c	12.0 (7.6–22.7) ^c
Clinical stage, <i>n</i> (%)		
T1	30 (28)	58 (29)
T2	17 (16)	44 (22)
T3	16 (15)	13 (7)
Tx	44 (41)	4 (2)
Missing	0 (0)	81 (41)
Biopsy Gleason score, <i>n</i> (%)		
6	8 (7)	11 (6)
3+4	15 (14)	34 (17)
4+3	25 (23)	49 (25)
8	21 (20)	34 (17)
9–10	17 (16)	62 (31)
Missing	21 (20)	10 (5)
RP Gleason score, <i>n</i> (%)		
≤6 ^d	0 (0)	2 (1)
3+4	14 (13)	15 (8)
4+3	29 (27)	63 (32)
8	14 (13)	24 (12)
9–10	50 (47)	90 (45)
Missing	0 (0)	6 (3)
Surgical margins, <i>n</i> (%)		
Negative	38 (36)	87 (44)
Positive	66 (62)	106 (53)
Missing	3 (3)	7 (4)
Pathological findings, <i>n</i> (%) ^e		
EPE	83 (78)	153 (77)
SVI	73 (68)	135 (68)
LNI	107 (100)	195 (98)
Number of positive LN, <i>n</i> (%)		
1	53 (50)	64 (32)
2	20 (19)	38 (19)
≥3	18 (17)	38 (19)
Missing	16 (15)	60 (30)
Treatment received post-RP, <i>n</i> (%)		
No treatment	35 (33)	71 (36)
RT	8 (7)	13 (7)
ADT	13 (12)	67 (34)
RT+ADT	29 (27)	47 (24)
Chemo	0 (0)	0 (0)
ADT+Chemo	5 (5)	2 (1)
ADT+RT+Chemo	9 (8)	0 (0)
Recurrence, <i>n</i> (%)		
No recurrence	23 (22)	69 (35)
Biochemical	47 (44)	85 (43)
Local	6 (6)	1 (1)
Missing	31 (29)	45 (23)
Metastasis, <i>n</i> (%)		
No	48 (45)	136 (68)
Yes	27 (25)	20 (10)
Missing	32 (30)	44 (22)

(Continued on the following page)

Table 2. Demographic and clinical variables of the Unique Tissue Microarray series. (Cont'd)

(A) TMA 1 (primary matched LN)		
Variable	TMA1a	TMA1b
Vital status, <i>n</i> (%)		
Alive	62 (58)	139 (70)
PCa death	10 (9)	10 (5)
Other cause of death	4 (4)	10 (5)
Dead unknown cause	1 (1)	0 (0)
Missing	30 (28)	41 (21)
(B) TMA2 (pre vs. post ADT)		
Number of patients	114	
Year of diagnosis	1998 (1993–2002)	
Follow-up months, <i>m</i> (IQR)	90 (50–128)	
Age at diagnosis, median (IQR, years)	67 (60–75)	
Race, <i>n</i> (%)		
White	96 (84)	
Black	14 (12)	
Hispanic	2 (2)	
Other	1 (1)	
Missing	1 (1)	
PSA at diagnosis, ng/mL, median (IQR) ^f	29.3 (13.0–73.0) ^f	
PSA pre-ADT, ng/mL, median (IQR)	33.0 (13.0–91.0) ^f	
PSA post-ADT (CRPC), ng/mL, median (IQR)	31.5 (7.5–102.3) ^f	
Pre-ADT Specimen Gleason Sum ^g		
≤6 ^d	11 (10)	
7	32 (28)	
8	17 (15)	
9	22 (19)	
10	2 (2)	
Missing	30 (26)	
First-line treatment, <i>n</i> (%)		
ADT ^h	77 (68)	
ADT+RT	11 (10)	
RT	7 (6)	
RP	13 (11)	
Neoadjuvant ADT- RP	0 (0)	
Other	6 (5)	
Subsequent treatment, <i>n</i> (%)		
No subsequent treatment	52 (46)	
ADT	56 (49)	
RT	1 (1)	
ADT+RT	5 (4)	
Recurrence, <i>n</i> (%)		
Biochemical	82 (72)	
Local	14 (12)	
Distant	18 (16)	
Metastasis, <i>n</i> (%)		
No	15 (13)	
Yes	99 (87)	
Metastasis site, <i>n</i> (%)		
None	17 (15)	
Lymph node	3 (3)	
Bone	43 (38)	
Visceral	15 (13)	
Lymph node and bone	8 (7)	
Bone and visceral	21 (18)	
Lymph node, bone, and visceral ⁱ	7 (6)	
CRPC treatment, <i>n</i> (%)		
No ADT	77 (68)	
ADT one line	9 (8)	
ADT 2 lines	8 (7)	
Missing	20 (18)	
Vital status, <i>n</i> (%)		
Alive	15 (13)	
PCa death	87 (76)	
Other cause death	8 (7)	
Missing	4 (3)	

(Continued on the following page)

Table 2. Demographic and clinical variables of the Unique Tissue Microarray series. (Cont'd)

(C) TMA3 (CRPC met heterogeneity)	
Number of patients	81
Age at diagnosis, median (IQR)	64 (60–73) ^j
Year of diagnosis, median (IQR)	1997 (1992–2002) ^j
Follow-up time months, median (IQR)	79 (39–135) ^j
Race, <i>n</i> (%)	
White	57 (70)
Other	7 (9)
Missing	17 (21)
PSA at Dx, ng/mL, median (IQR)	12 (7.0–64.6) ^j
Biopsy Gleason score, <i>n</i> (%)	
≤6 ^d	3 (4)
7	14 (17)
8	10 (12)
9	15 (19)
10	2 (2)
Missing	37 (46)
Primary treatment, <i>n</i> (%)	
None	6 (7)
RP	15 (19)
RT	1 (1)
ADT	38 (47)
Other	6 (7)
Missing	15 (19)
Metastatic treatments, <i>n</i> (%)	
None	7 (10)
1	10 (12)
2	5 (6)
3	6 (7)
4	10 (12)
≥5	28 (35)
Missing	15 (19)
Metastatic treatment type, <i>n</i> (%) ^e	
ADT	59 (73)
RT	25 (31)
Ketaconazole	28 (35)
Taxotere	37 (46)
Taxol	17 (21)
Carboplatin	17 (21)
Estramustine (Emcyt)	10 (12)
Mitoxantrone	16 (20)
Cytoxin+5FU	1 (1)
Abiraterone	2 (2)
Enzalutamide	1 (1)
Bisphosphonate	45 (56)
Missing	15 (19)
CRPC, <i>n</i> (%)	
No	7 (9)
Yes	59 (73)
Missing	15 (19)
Months from Dx to CRPC, median (IQR)	54 (21–91) ^j

Abbreviations: ADT, androgen deprivation therapy; EPE, extraprostatic extension; LNI, lymph node involvement; n/a, not applicable; OC, organ confined; PCa, prostate cancer; RP, radical prostatectomy; SVI, seminal vesicle involvement; RT, radiation.

^aBased on 78 “1a” patients, 158 “1b” patients.

^bBased on 191 “1b” patients.

^cBased on 101 “1a” patients, 192 “1b” patients.

^dBased on initial pathology report.

^eCategories are not mutually exclusive, so total is more than 100%.

^fBased on 90 patients with PSA at diagnosis data, 97 with pre-ADT PSA data, and 112 with post-ADT PSA data.

^gThe pre-ADT Gleason score was based on prostatectomy Gleason score unless only biopsy Gleason score was available. One patient for TMA2 has the variable “preadt_gs” = missing, but also had the variable “rp_gs” = 7, so this patient was included as Gleason 7, not missing.

^hThis included 26 patients with primary orchiectomy and 4 patients with adjuvant orchiectomy.

ⁱThese patients were coded “4,5” which had been assigned as a separate category, so BT created a category that combined category 4 (lymph node and bone) and category 5 (visceral and bone).

^jAge at diagnosis and year of diagnosis both based on 66 patients; follow-up time based on 48 patients; PSA at diagnosis based on 42 patients; months from Dx to CRPC based on 47 patients.

PTEN and ERG, if there was a complete absence of staining in tumor cells and surrounding stromal cells, then that given TMA spot was considered inadequate for scoring and was not included. PTEN staining was considered ambiguous and was not scored when negative staining in the tumor was associated with only very weak positive, or negative staining in surrounding stromal cells. Similar methods for PTEN staining and scoring have been published previously (20, 21). TMA data were recorded at each site by the study pathologist for each marker by using a google sheet with pull-down menus for diagnoses and scoring. For each test-TMA stained slide, the data was then consolidated into a larger excel spreadsheet for initial data tabulation in SAS and separately in STATA 15.

Statistical analyses

In this study we performed IHC staining for six different biomarkers in the Test TMAs, primarily for quality control and proof of concept purposes. For each Test TMA, we obtained descriptive summary statistics of the proportion of cases staining negatively, and positively (homogeneous and heterogeneous) for well characterized biomarkers. In addition, for all three Test TMAs we compared the percentage of patients with PTEN loss between those with ERG positive versus ERG negative tumors. All statistical analyses were carried out using SAS v9.4 (SAS Institute).

Although the current report focuses primarily on the Test TMAs, the full TMAs are powered to detect differences in biomarker propor-

tions of 15 to 20 percentage points. Power calculations were performed with PASS v. 21 (NCSS Software, Inc.).

Data availability statement

The data generated in this study are available upon request from the corresponding author.

Results

Brief descriptions of TMAs and demographics

The main objective of the GAPI Unique TMAs was to create a TMA-based resource to address three outstanding questions. The purpose of each TMAs and their intent of use as well as the details regarding the inclusion and exclusion criteria are presented in **Table 1A**. The TMA composition including number of TMA blocks, specimens, tissue type specification, and tissue core numbers are described in **Table 1B**. Demographic and clinico-pathologic data for each TMA series are presented in **Table 2**.

Landmark PC markers

In this study, as proof of concept, we used the Test TMAs that will be provided, as a first assay control, to all researchers with approved TMA requests. These TMAs consist of a subset of cases included in the full TMAs. Test TMAs of each series were stained at a single institution for six IHC-based biomarkers. These markers were chosen because they

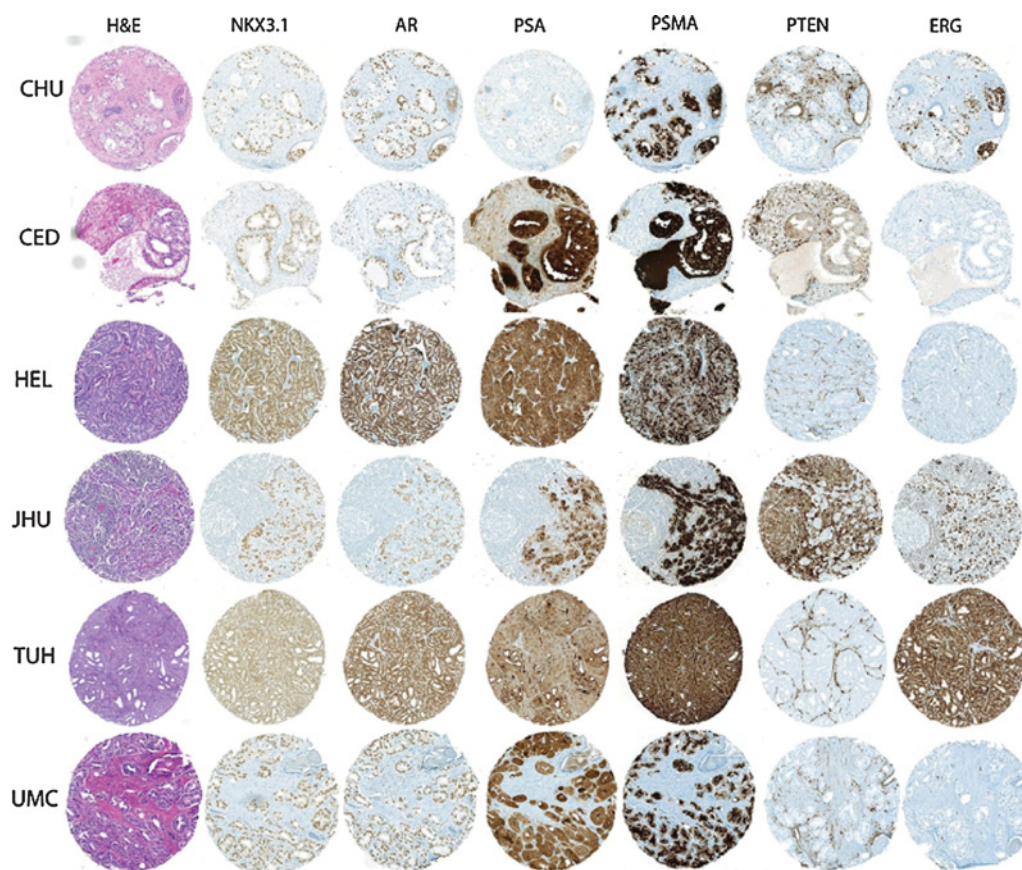


Figure 1.

Representative low-power view of staining of TMA cores from Test TMA1 (Primary Matched LN). Each row shows staining from a single adjacent TMA core chosen from either a primary tumor or lymph node metastatic site from the indicated institutions.

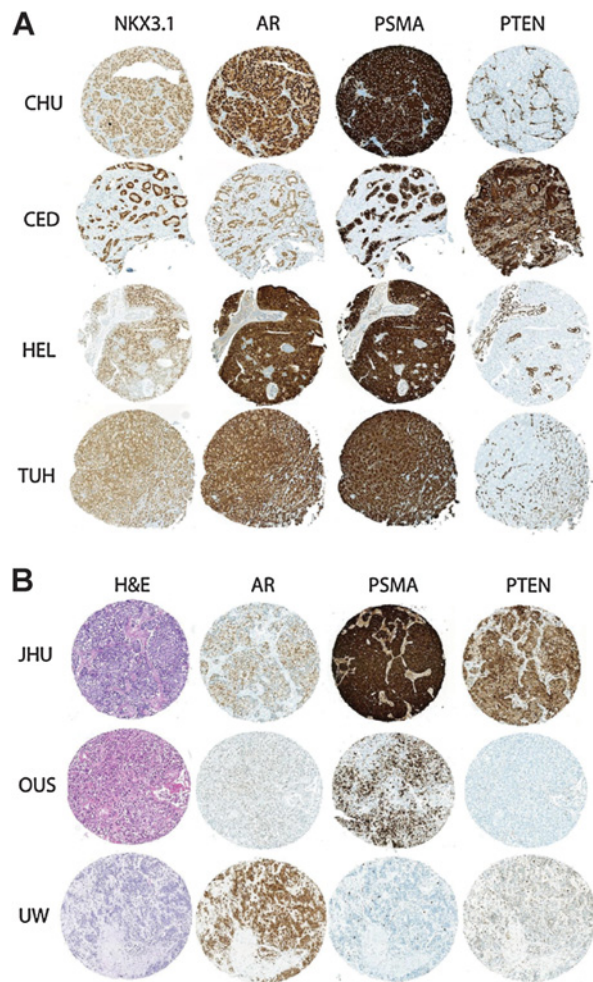


Figure 2. Representative images of histologic stainings from Test TMA2 (Pre vs. Post ADT) and TMA3 (CRPC Met Heterogeneity). Low-power images from (A) Test TMA2 (Pre vs. Post ADT) and (B) Test TMA 3 (CRPC Met Heterogeneity). Each row shows staining from a single adjacent TMA core from the indicated institutions.

are either selectively expressed in prostate cancer compared with most other cancer types (e.g., PSA, NKX3.1, PSMA, ERG, and AR), are known to be associated with disease progression (e.g., PTEN), or are a prostate cancer drug target (AR; refs. 22–24). Furthermore, well-validated assays exist that can be performed using automated IHC staining, which are generally robust when considering variables relevant to this study such as wide variability in tissue block age, tissue fixation extent, tissue handling, processing, and storage (e.g., for PTEN, see ref. 21). **Figure 1** shows an example of IHC and H&E staining for each of the six IHC markers across Test TMA1 (Primary Matched LN). Representative images from Test TMAs 2 and 3 are shown in **Fig. 2A** and **B**. All xenograft tissues stained as expected for each marker (Supplementary Table S2), providing excellent quality assurance for the IHC staining. For example, LNCaP and CWR22rv1 were positive for AR, NKX3.1, and PSMA and PC-3 and DU145 were negative for these markers. All xenografts were negative for ERG,

LNCa and PC-3 were negative for PTEN, and DU145 and 22Rv1 were positive for PTEN.

Table 3 shows the results for the fraction of TMA spots that were scored in each category as negative, positive-heterogeneous, positive-homogeneous (for ERG, AR, PSA, NKX3.1, and PSMA), or, for PTEN as positive, loss-heterogeneous or loss-homogeneous. For Test TMA1 (Primary Matched LN), using the primary tumors and metastatic lymph nodes only (e.g., without the xenografts included), as expected most tumors were positive for AR, PSA, and NKX3.1 and PSMA (**Table 3**). Also, as seen in a number of prior studies, primary tumors or lymph node metastases that were ERG-positive more commonly had PTEN loss (65.1% of cases with any ERG-positive staining had any PTEN loss) than such tumors that were ERG-negative cases (42.1% of cases with any ERG negative staining had any PTEN loss; **Table 4**, $P = 0.037$).

Table 3 also shows the staining and scoring results for Test TMA2 (Pre vs. Post ADT), which consisted of tissue samples from RP, TURP, as well as the control tissues and xenografts. Excluding the xenografts, most cases of carcinomas stained adequately, and most were positive for the four prostate-enriched biomarkers (AR, PSA, NKX3.1, and PSMA). ERG was positive in at least a fraction of tumor cells in 42% of cases and PTEN was lost homogeneously in 47% of cases and lost heterogeneously in one additional case. PTEN loss was again more common in ERG-positive cases (55% of cases with any ERG-positive staining had any PTEN loss vs. 44.8% of ERG-negative cases had any PTEN loss), although this was not statistically significant ($P = 0.48$).

Test TMA3 (CRPC Met Heterogeneity) consists of metastatic tumor tissues from autopsies from three institutions as well as a small number of primary prostate carcinomas from one of the sites and the control xenograft tissues. Focusing on the metastatic autopsy tumor spots, the majority (84%) had at least some positive staining for PSMA (84%), AR (84%), and PSA (68%). NKX3.1 was positive in 48.3% of metastatic TMA cores. This relatively low fraction of cases staining positively using the NKX3.1 antibody may relate to either less frequent presence of NKX3.1 protein in these very late stage disease tissues, or, poor preservation of its protein target as a result of prolonged warm ischemic time. Deciphering which of these alternatives may be correct is difficult and represents an inherent limitation of using autopsy tissue for some biomarkers (see Discussion). ERG was positive in 28% and PTEN was lost in 90.5% of metastatic cores in test TMA3 (CRPC Met Heterogeneity). The majority of metastatic TMA cores with PTEN loss showed homogeneous loss (86%). This rate of PTEN loss is remarkably high and it will be interesting to determine if this rate of loss holds in the full GAP1 TMAs when PTEN is evaluated in those. Unlike in test TMAs 1 and 2, the fraction of ERG-positive cases with PTEN loss was lower than in ERG-negative cases in Test TMA3 (CRPC Met Heterogeneity; $P < 0.0001$).

Resource available for PC community: process

The process to access the UTMA resource is outlined in Supplementary Fig. S1. As part of a collaboration, the biomarker proposal form will be located on the Prostate Cancer Biorepository Network (PCBN; ref. 25) website (www.prostatebiorepository.org). Because the ultimate objective of the UTMA GAP1 program is to identify biomarkers and combinations thereof that would be helpful for clinicians to refine patient management, end users are required to agree to return their raw results (scoring results and protocols) back to the GAP1 coordinating center either after publication, or after 6 months from scoring completion, whichever is sooner.

Table 3. Staining results for Test TMAs 1, 2, and 3.

Biomarker	Test TMA1 (primary matched LN)			Test TMA2 (pre vs. post ADT)		Test TMA3 ^a (CRPC met heterogeneity)		
	Tissue type			Tissue type		Tissue type		
	Primary	LN Met	Xenograft	RP/TURP	Xenograft	Primary	Metastasis	Xenograft
PSMA staining, <i>n</i> (%)								
Negative	0 (0)	1 (3.1)	11 (50)	2 (4)	7 (50.0)	1 (12.5)	24 (16)	5 (41.7)
Positive heterogeneous	19 (27.9)	4 (12.5)	4 (18.1)	13 (26.0)	2 (14.3)	3 (37.5)	24 (16)	2 (16.7)
Positive homogeneous	49 (72.1)	27 (84.4)	7 (31.8)	35 (70)	5 (40.0)	4 (50)	102 (68)	5 (41.7)
Total	68	32	22	50	14	8	150	12
PTEN staining, <i>n</i> (%)								
Positive	35 (54.2)	13 (34.6)	8(40)	26 (48.2)	8 (57.1)	0 (0)	14 (9.3)	5 (35.7)
Loss heterogeneous	2 (2.8)	2 (7.7)	1 (5)	1 (1.9)	1 (7.1)	2 (25)	7 (4.7)	2 (14.4)
Loss homogeneous	31 (43)	15 (57.7)	11(55)	24 (44.4)	5 (35.7)	6 (75)	127 (84.6)	7 (50)
Cannot determine ^b	0 (0)	0 (0)	0 (0)	3 (5.6)	0 (0)	0 (0)	2 (1.3)	0 (0)
Total	68	30	20	54	14	8	150	14
ERG staining, <i>n</i> (%)								
Negative	39 (54.9)	22 (68.9)	21 (95.2)	29 (54.7)	13 (100.0)	7 (87.5)	105 (70.0)	12 (85.7)
Positive heterogeneous	7 (9.9)	5 (15.6)	0 (0)	9 (17.0)	0 (0)	1 (12.5)	21 (13.9)	2 (14.3)
Positive homogeneous	25 (35.2)	5 (0)	0 (0)	12 (22.6)	1 (7.1)	0 (0)	19 (12.6)	0 (0)
Cannot determine ^b	0 (0)	0 (0)	0 (0)	3 (5.7)	0 (0)	0 (0)	6 (4)	0 (0)
Total	71	32	21	53	14	8	151	14
AR staining, <i>n</i> (%)								
Negative	0 (0)	1 (3.1)	10 (47.6)	0 (0)	6 (42.9)	5 (62.5)	24 (16)	9 (64.3)
Positive heterogeneous	13 (18.3)	6 (18.8)	3 (14.3)	1 (1.9)	2 (14.3)	2 (25)	19 (12.7)	0 (0)
Positive homogeneous	58 (81.7)	25 (78.1)	8 (38.1)	51 (98.1)	6 (42.9)	1 (12.5)	107 (71.3)	5 (35.7)
Total	71	32	21	52	14	8	150	14
NKX3.1 staining, <i>n</i> (%)								
Negative	2 (2.8)	4 (12.5)	10 (47.6)	3 (5.6)	6 (46.2)	7 (87.5)	75 (51.7.0)	8 (61.5)
Positive heterogeneous	21 (29.6)	5 (15.6)	7 (33.3)	16 (29.6)	6 (46.2)	1 (12.5)	35 (24.1)	2 (15.3)
Positive homogeneous	48 (67.6)	23 (71.9)	4 (19.1)	35 (64.8)	1 (7.7)	0 (0)	35 (24.1)	3 (23.1)
Total	71	32	21	54	13	8	145	13
PSA staining, <i>n</i> (%)								
Negative	1 (1.3)	2 (6.3)		8 (15.1)		2 (25)	48 (32.4)	
Positive heterogeneous	30 (41.7)	5 (15.6)	NA ^c	20 (37.7)	NA ^c	2 (25)	42 (28.4)	NA ^c
Positive homogeneous	41 (56.9)	25 (78.1)		23 (43.4)		4 (50)	58 (39.1)	
Total	72	32		51		8	148	

^aThere are six metastatic tissues each from 20 patients from UW, four each from 5 patients from JHU, and two to four metastatic sites from each of 4 patients from OUH, and two samples of matched prostates from each of these patients from OUH.

^bIf there was a complete absence of staining in tumor cells and surrounding stromal cells, then that given TMA spot was considered inadequate for scoring and was not included.

^cThe anti-mouse antibody used for PSA is unable to be assessed on mouse xenografts.

Discussion

Tissue microarrays represent an efficient format to profile biomarkers from many different tumors at the same time. However, most studies use either a small number of samples or only include cases from a single institution. With the vision of building a unique resource for

prostate cancer researchers, Movember brought together 19 principal investigators from 13 institutions based in different continents. Through the development of specific guidelines, model documents for TMA design, and clinical data collection using an harmonized dictionary, we successfully created a unified resource representing different disease states of prostate cancer.

Table 4. Correlation between PTEN loss and ERG status.

Biomarker	ERG status			<i>P</i> value ^a
	Negative	Positive heterogeneous	Positive homogeneous	
PTEN status, <i>n</i> (%)				
Loss heterogeneous	4 (7.0)	0 (0.0)	0 (0)	0.037
Loss homogeneous	20 (35.0)	9 (75.0)	17 (59.0)	—
Positive homogeneous	33 (58.0)	3 (25.0)	12 (41.0)	—
Total	57	12	29	—

^aChi-squared test.

Demographics of the TMA series showed that the cohorts are representative of patients with prostate cancer in terms of age at diagnosis. The majority of the patients in the cohort are white. Therefore, the generalizability of these TMA resources are limited somewhat in terms of race. The serum PSA level at diagnosis was generally lower than expected for TMA1 (Primary Matched LN), given that only 10% of patients within the PSA range of TMA1 (Primary Matched LN) have LNI based on commonly used predictive tables (26). However, this may be partially explained by half of the patients having only one or two positive LN. The other clinico-pathologic variables such as Gleason score, staging, and pathologic findings are more in line with what would be expected for this cohort. TMA2 (Pre vs. Post ADT) and TMA3 (CRPC Met Heterogeneity) cohorts are composed of patients diagnosed with PC more than two decades ago and clinico-pathologic data are representative of the type of disease included in this series. In addition, treatment provided reflects the reality of limited therapeutic options for patients with advanced/CRPC during this period.

Although these results are preliminary because of small sample sizes in the test TMAs, biomarker expression patterns and frequencies were mostly as expected based on prior work. Although some of the findings were somewhat unexpected, such as the very high rate of PTEN loss in the autopsy samples (86%), current studies by our group are ongoing in which we are examining these markers in the full TMAs and details regarding them will be published separately. In concurrent preliminary analyses of PTEN and ERG, we found that any PTEN loss was more common in cases with ERG expression in Test TMAs 1 and 2 as has been seen previously (8), but present at an even rate between ERG-positive and ERG-negative specimens in the CRPC autopsy samples in Test TMA 3. Taken together, these results indicate that the staining for the biomarkers employed were robust in various types of tissues processed separately at multiple institutions and that the staining results are consistent with previous reports.

Some limitations of our study should be noted. Because of the relative paucity of well-annotated samples representing the clinical attributes chosen for these TMAs, the samples essentially represent convenience samples. We recognize this may introduce bias. For our planned follow-up study with the full TMAs (as opposed to the test TMAs reported here), we will compare clinical characteristics of patients at each institution for whom samples were used in the TMAs to those of patients for whom samples were unavailable. This will allow us to identify and potentially correct for any systematic differences in the TMA patients, or conduct sensitivity analyses to determine the impact of any differences. In addition, the resource is composed entirely of samples punched from older FFPE specimens, including various tissue types such as biopsies, TURP, prostatectomy, and metastatic lesions in which tissue handling varied across institutions in terms of time of fixation and protocols. We recognize that this could introduce variability into biomarker assessment. In addition, given the variation in block age this could be a limitation for RNA *in situ* hybridization (RNA-ISH) because it is known that RNA-ISH signals decrease over time (18) and hence this, as with many other TMAs, may not be highly suitable for ISH studies for RNA in general. Furthermore, a common concern of the use of TMAs is whether a small sampled area can accurately capture heterogeneity in the wider specimen. Although this issue is always a factor in all biomarker studies of tissues, including using biopsy specimens, this was accounted for to a certain extent in TMA design by selecting multiple TMA cores per specimen. Furthermore, a number of prior studies have addressed this issue and have generally reported that TMAs are often quite robust to tissue hetero-

geneity, especially when multiple replicates are included (27–29). An additional limitation is that complete pathology or follow-up clinical data were not available for all patients. Finally, rapid autopsy tissue has limitations. One limitation is that the time between death and tissue fixation is variable and can be from several hours up to 24 hours in this study. Although some biomarkers may be retained fully with such post-mortem intervals in which vascular tissues were studied (30), it cannot be known which others may not be robustly retained without experimental data on that given biomarker. In addition, bone lesions at autopsy underwent decalcification, generally in formic acid. Although prior studies by one of our groups have not identified negative effects of decalcification on expression of the analytes in prior studies using similar samples (31–35), analytes that we have not studied, such as phosphoproteins, could potentially be altered. Another limitation of these specimens is that they were collected from patients with CRPC no later than 2013, before late generation androgen deprivation therapy was widely used. Consequently, only two of the 50 patients received modern anti-androgens (abiraterone or enzalutamide) for a significant time during their treatment.

In summary, we have developed a set of TMAs from a multi-institutional initiative supported by the Movember Foundation. This effort focused on rare but clinically important samples made possible through an international collaboration. It required leveraging existing institutional resources and multidisciplinary prostate cancer expertise across multiple continents to provide a unique resource that will serve the wider prostate cancer research community to support discovery based research and enhance the overall impact of biomarker validation studies.

Authors' Disclosures

V. Berge reports grants from Movember during the conduct of the study. X. Zhou reports grants from Movember Foundation during the conduct of the study. B.J. Trock reports grants from Movember during the conduct of the study as well as grants from Myriad Genetics, Inc. and MDxHealth, Inc. outside the submitted work. P. Taimen reports grants from Movember Foundation during the conduct of the study as well as grants from The Academy of Finland, Sigrid Juselius Foundation, The Finnish Foundation for Cardiovascular Research, The Cancer Foundation Finland, and The Hospital District of Southwestern Finland outside the submitted work. F. Saad reports grants from Movember Foundation during the conduct of the study. B.S. Knudsen reports grants from Movember during the conduct of the study. A.M. De Marzo reports grants from Movember Foundation, US NIH, and US Department of Defense during the conduct of the study as well as grants from Janssen R&D and personal fees from Cepheid Inc. and Merck outside the submitted work. No disclosures were reported by the other authors.

Authors' Contributions

V. Ouellet: Resources, writing—original draft, project administration, writing—review and editing, project coordination, participation in project coordinators committee, ongoing project design and quality control, coordinated supply of 13.9% of samples, principal role in writing. **A. Erickson:** Resources, data curation, writing—original draft, writing—review and editing, principle role in writing, co-supplied 19.9% of samples. **K. Wiley:** Resources, project administration, writing—review and editing, project coordination, data management, coordinated supply of 13.1% of samples, quality control, review of draft. **C. Morrissey:** Resources, writing—review and editing, supplied 9.6% of samples, review of draft. **V. Berge:** Resources, writing—review and editing, supplied 3.5% of samples, review of draft. **C.S. Moreno:** Resources, writing—review and editing, co-supplied 3.9% of samples, review of draft. **K.A. Tasken:** Conceptualization, formal analysis, writing—review and editing, project design, assisted in scoring the immunohistochemical stains, review of draft. **D. Trudel:** Resources, writing—review and editing, quality control and histology review of supplied specimens, review of draft. **L.D. True:** Resources, data curation, formal analysis, writing—review and editing, rapid autopsy pathology, pathology annotation of slides, quality control of autopsy samples, review of draft. **M.S. Lewis:**

Formal analysis, writing–review and editing, pathology annotation of slides, review of draft. **A. Svindland:** Formal analysis, writing–review and editing, pathology annotation of slides, review of draft. **O. Ertunc:** Formal analysis, writing–review and editing, pathology annotation of slides, TMA construction, review of draft. **I.D. Vidal:** Formal analysis, writing–review and editing, pathology annotation of slides, review of draft. **A.O. Osunkoya:** Formal analysis, writing–review and editing, pathology annotation of slides, review of draft. **T. Jones:** Data curation, writing–review and editing, data management; review of draft. **G.S. Bova:** Resources, writing–review and editing. **T. Lamminen:** Data curation, patient identification and follow-up; review of draft. **A.H. Achtmann:** Writing–original draft, project administration, writing–review and editing, project management, review of draft, coordination of publication process. **M. Buzza:** Project administration, writing–review and editing, project management, review of draft. **M.M. Kouspou:** Writing–original draft, project administration, writing–review and editing, project management, revising section on project timelines, review of draft. **S.A. Bigler:** Resources, writing–review and editing, co-supplied 12.5% of samples, review of draft. **X. Zhou:** Resources, writing–review and editing, co-supplied 5.4% of samples, review of draft. **S.J. Freedland:** Resources, writing–review and editing, co-supplied 12.5% of samples, review of draft. **A.-M. Mes-Masson:** Resources, writing–original draft, writing–review and editing, co-supplied 13.9% of samples, review of draft, writing methods. **I.P. Garraway:** Conceptualization, resources, data curation, formal analysis, writing–review and editing, co-supplied 12.5% of samples, project design, data collection, data analysis, review of draft. **B.J. Trock:** Conceptualization, resources, data curation, formal analysis, supervision, writing–original draft, project administration, writing–review and editing, participation in project coordinators committee, ongoing project design and quality control, data harmonization and management, data analysis, co-supplied 13.1% of samples; writing of statistics sections. **P. Taimen:** Resources, writing–original draft, writing–review and editing. **F. Saad:** Conceptualization, resources, formal analysis, supervision, project administration, writing–review and editing, co-supplied 13.9% of samples, high-level project coordination, high-level project design, high-level quality control, review of draft. **T. Mirtti:** Resources, writing–original draft, project administration, writing–review and editing, supplied 18.9% of samples, writing lessons learned and collaboration history, review of draft. **B.S. Knudsen:** Conceptualization, resources, formal analysis, project administration, writing–review and editing, co-supplied 12.5% of samples, participation in project coordinators committee, ongoing project design and quality control, staining data analysis, data analysis, review of draft. **A.M. De Marzo:** Conceptualization, resources, formal analysis, supervision, writing–original draft, project administration, writing–review and editing, participation in project coordinators committee, ongoing project design and quality control, writing

background on TMAs, table describing samples, purpose statement, co-supplied 13.1% of samples, principal role in writing.

Acknowledgments

C. Morrissey, V. Berge, C.S. Moreno, K.A. Tasken, D. Trudel, X. Zhou, S.J. Freedland, A.-M. Mes-Masson, I.P. Garraway, B.J. Trock, P. Taimen, F. Saad, T. Mirtti, B.S. Knudsen, and A.M. De Marzo were funded by Movember. B.J. Trock and A.M. De Marzo from JHU were supported by U.S. NIH/NCI SPORE in Prostate Cancer: P50CA58236, the U.S. Department of Defense Prostate Cancer Research Program (PCRP): W81XWH-18-2-0015 to A.M. De Marzo. Johns Hopkins Sidney Kimmel Comprehensive Cancer Center Oncology Tissue Services Laboratory supported by U.S. NIH/NCI P30 CA006973 (to B.J. Trock and A.M. De Marzo). CRCHUM was supported by the FRQS (to A.-M. Mes-Masson, F. Saad, and D. Trudel) and their Biobanking at the CRCHUM was done in collaboration with the Réseau de recherche sur le cancer supported by the Fonds de Recherche Québec - Santé (FRQ-S) affiliated to the Canadian Tissue Repository Network (CTRNet). F. Saad holds the Raymond Garneau Chair in Prostate Cancer. D. Trudel was supported by the Chercheure boursière clinicienne niveau junior 2 of the FRQ-S awards. C. Morrissey and L.D. True had their tissue acquisition activity supported by U.S. NIH/NCI SPORE in Prostate Cancer: P50CA97186 and the UW Institute for Prostate Cancer Research. We thank all patients for providing specimens and data to the UTMA-affiliated biobanks. We thank Éloïse Adam-Granger, Kathy Doan, Andrée-Anne Grosset, Belinda Nghiem, Olov Ögren, Tiina Vesterinen, RRcancer-CRCHUM prostate cancer biobank staff, and the CRCHUM molecular pathology platform for TMA preparation, sample collection, and clinical data integration and Yasser Amhdak, Lauri Elo, and Lori Kollath for sample and clinical data collection. We thank the rapid Autopsy Team, Celestia Higano, Pete Nelson, Bruce Montgomery, Evan Yu, Elahe Mostaghel, Paul Lange, Robert Vessella, Xiaotun Zhang, and Martine Roudier for contributing to the rapid autopsy programs and Gayle Walters for legal support.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received June 7, 2021; revised October 26, 2021; accepted January 31, 2022; published first February 7, 2022.

References

- Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin* 2018;68:7–30.
- Culp MB, Soerjomataram I, Efstathiou JA, Bray F, Jemal A. Recent global patterns in prostate cancer incidence and mortality rates. *Eur Urol* 2020;77:38–52.
- Loeb S, Bjurlin MA, Nicholson J, Tammela TL, Penson DF, Carter HB, et al. Overdiagnosis and overtreatment of prostate cancer. *Eur Urol* 2014;65:1046–55.
- Schröder FH, Hugosson J, Roobol MJ, Tammela TLJ, Zappa M, Nelen V, et al. Screening and prostate cancer mortality: results of the European Randomised Study of Screening for Prostate Cancer (ERSPC) at 13 years of follow-up. *Lancet* 2014;384:2027–35.
- Klotz L. Cancer overdiagnosis and overtreatment. *active surveillance for localized prostate cancer*. Humana Press, Cham; 2018 p. 1–8. [cited 2017 Dec 22]. https://link.springer.com/chapter/10.1007/978-3-319-62710-6_1.
- Silberstein JL, Pal SK, Lewis B, Sartor O. Current clinical challenges in prostate cancer. *Transl Androl Urol* 2013;2:122–36.
- Cucchiara V, Cooperberg MR, Dall'Era M, Lin DW, Montorsi F, Schalken JA, et al. Genomic markers in prostate cancer decision making. *Eur Urol* 2018;73:572–82.
- Jamaspishvili T, Berman DM, Ross AE, Scher HI, De Marzo AM, Squire JA, et al. Clinical implications of PTEN loss in prostate cancer. *Nat Rev Urol* 2018;15:222–34.
- Lotan TL, Tomlins SA, Bismar TA, Van der Kwast TH, Grignon D, Egevad L, et al. Report from the international society of urological pathology (ISUP) consultation conference on molecular pathology of urogenital cancers. I. molecular biomarkers in prostate cancer. *Am J Surg Pathol* 2020;44:e15–29.
- Touijer KA, Mazzola CR, Sjöberg DD, Scardino PT, Eastham JA. Long-term outcomes of patients with lymph node metastasis treated with radical prostatectomy without adjuvant androgen-deprivation therapy. *Eur Urol* 2014;65:20–5.
- Chipollini JJ, Pow-Sang JM. Lymph node positive prostate cancer: the evolving role of adjuvant therapy. *Transl Cancer Res* 2018;7:S94–5.
- Lee RJ. Initial systemic therapy for castration-sensitive prostate cancer. In: Vogelzang N, Savarese DMF, editors. *UpToDate*. Waltham, MA: UpToDate; 2020 [cited 2021 Aug 1]. Available from: <https://www.uptodate.com/contents/initial-systemic-therapy-for-castration-sensitive-prostate-cancer>.
- Pound CR, Partin AW, Eisenberger MA, Chan DW, Pearson JD, Walsh PC, et al. Natural history of progression after PSA elevation following radical prostatectomy. *J Urol* 1999;154:8.
- Roudier MP, True LD, Higano CS, Vessella H, Ellis W, Lange P, et al. Phenotypic heterogeneity of end-stage prostate carcinoma metastatic to bone. *Hum Pathol* 2003;34:646–53.
- Shah RB, Mehra R, Chinnaiyan AM, Shen R, Ghosh D, Zhou M, et al. Androgen-independent prostate cancer is a heterogeneous group of diseases: lessons from a rapid autopsy program. *Cancer Res* 2004;64:9209–16.
- Beltran H, Prandi D, Mosquera JM, Benelli M, Puca L, Cyrta J, et al. Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. *Nat Med* 2016;22:298–305.
- Faith DA, Isaacs WB, Morgan JD, Fedor HL, Hicks JL, Mangold LA, et al. Trefoil factor 3 overexpression in prostatic carcinoma: prognostic importance using tissue microarrays. *Prostate* 2004;61:215–27.
- Baena-Del Valle JA, Zheng Q, Hicks JL, Fedor H, Trock BJ, Morrissey C, et al. Rapid Loss of RNA detection by in situ hybridization in stored tissue blocks

- and preservation by cold storage of unstained slides. *Am J Clin Pathol* 2017; 148:398–415.
19. Bethel CR, Faith D, Li X, Guan B, Hicks JL, Lan F, et al. Decreased NKX3.1 protein expression in focal prostatic atrophy, prostatic intraepithelial neoplasia, and adenocarcinoma: association with gleason score and chromosome 8p deletion. *Cancer Res* 2006;66:10683–90.
 20. Lotan TL, Gurel B, Sutcliffe S, Esopi D, Liu W, Xu J, et al. PTEN protein loss by immunostaining: analytic validation and prognostic indicator for a high risk surgical cohort of prostate cancer patients. *Clin Cancer Res* 2011;17: 6563–73.
 21. Guedes LB, Morais CL, Fedor H, Hicks J, Gurel B, Melamed J, et al. Effect of Preanalytic Variables on an Automated PTEN Immunohistochemistry Assay for Prostate Cancer. *Arch Pathol Lab Med* 2019;143:338–48.
 22. Lahdensuo K, Erickson A, Saarinen I, Seikkula H, Lundin J, Lundin M, et al. Loss of PTEN expression in ERG-negative prostate cancer predicts secondary therapies and leads to shorter disease-specific survival time after radical prostatectomy. *Mod Pathol* 2016;29:1565–74.
 23. Ahearn TU, Pettersson A, Ebot EM, Gerke T, Graff RE, Morais CL, et al. A prospective investigation of PTEN loss and ERG expression in lethal prostate cancer. *J Natl Cancer Inst* 2016;108:djv346.
 24. Lotan TL, Wei W, Morais CL, Hawley ST, Fazli L, Hurtado-Coll A, et al. PTEN loss as determined by clinical-grade immunohistochemistry assay is associated with worse recurrence-free survival in prostate cancer. *Eur Urol Focus* 2016;2: 180–8.
 25. Darshan M, Zheng Q, Fedor HL, Wyhs N, Yegnasubramanian S, Lee P, et al. Biobanking of derivatives from radical retropubic and robot-assisted laparoscopic prostatectomy tissues as part of the prostate cancer biorepository network. *Prostate* 2014;74:61–9.
 26. Swanson GP, Thompson IM, Basler J. Current status of lymph node-positive prostate cancer: Incidence and predictors of outcome. *Cancer* 2006;107:439–50.
 27. Zhang D, Salto-Tellez M, Putti TC, Do E, Koay ES-C. Reliability of tissue microarrays in detecting protein expression and gene amplification in breast cancer. *Mod Pathol* 2003;16:79–84.
 28. Leversha MA, Fielding P, Watson S, Gosney JR, Field JK. Expression of p53, pRB, and p16 in lung tumours: a validation study on tissue microarrays. *J Pathol* 2003; 200:610–9.
 29. Rosen DG, Huang X, Deavers MT, Malpica A, Silva EG, Liu J. Validation of tissue microarray technology in ovarian carcinoma. *Mod Pathol* 2004;17:790–7.
 30. Halushka MK, Cornish TC, Lu J, Selvin S, Selvin E. Creation, validation, and quantitative analysis of protein expression in vascular tissue microarrays. *Cardiovasc Pathol* 2010;19:136–46.
 31. Zhang X, Morrissey C, Sun S, Ketchandji M, Nelson PS, True LD, et al. Androgen receptor variants occur frequently in castration resistant prostate cancer metastases. *PLoS One* 2011;6:e27970.
 32. Akfirat C, Zhang X, Ventura A, Berel D, Colangelo ME, Miranti CK, et al. Tumour cell survival mechanisms in lethal metastatic prostate cancer differ between bone and soft tissue metastases. *J Pathol* 2013;230: 291–7.
 33. Zhang X, Coleman IM, Brown LG, True LD, Kollath L, Lucas JM, et al. SRRM4 expression and the loss of REST activity may promote the emergence of the neuroendocrine phenotype in castration-resistant prostate cancer. *Clin Cancer Res* 2015;21:4698–708.
 34. Haider M, Zhang X, Coleman I, Ericson N, True LD, Lam H-M, et al. Epithelial mesenchymal-like transition occurs in a subset of cells in castration resistant prostate cancer bone metastases. *Clin Exp Metastasis* 2016;33:239–48.
 35. Labrecque MP, Coleman IM, Brown LG, True LD, Kollath L, Lakely B, et al. Molecular profiling stratifies diverse phenotypes of treatment-refractory metastatic castration-resistant prostate cancer. *J Clin Invest* 2019;129: 4492–505.