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The Prostate Cancer Pathology Resource Network (which has since been renamed the Prostate Cancer Biorepository Network or PCBN) is a collaboration between the Johns Hopkins School of Medicine (JHU) and the New York University School of Medicine (NYU). This Final Report covers the initial 3 year funding period plus a 4th “bridge year” that was awarded by DOD-PCRP to allow PCBN to submit a competitive renewal application in response to a RFA that was delayed. The PCBN has developed a biorepository with high quality, well annotated specimens that can be used by prostate cancer researchers. Specimens include prostatectomy tissues (frozen, paraffin embedded, and tissue microarrays (TMAs), serum, plasma, buffy coat, prostatic fluid, and derived specimens (DNA, RNA, and protein); these specimens are linked to clinical and outcome data and supported by an informatics infrastructure. In addition to providing specimens the PCBN provides technical advice to researchers and performs independent biospecimen science research. The PCBN is currently made accessible to outside researchers through a website. Funding for the PCBN began June 2010, and it has been open to researchers since July 1 2011. In addition to the large number of biospecimens made available to the research community, other accomplishments include creation of a website to facilitate access and specimen requests, high impact publications citing use of PCBN specimens, biospecimen science describing optimized methods for DNA extraction and comparison of RNA quality from open vs. robot assisted radical prostatectomy, hosting a symposium with researchers from around the country to discuss issues and advances in biomarker and biospecimen research, and assembling a consortium among JHU, NYU, Memorial Sloan Kettering and University of Washington that successfully collaborated on a PCBN competitive renewal application.
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INTRODUCTION

The Prostate Cancer Biorepository Network (PCBN) is a collaboration between the Johns Hopkins School of Medicine (JHU), the New York University School of Medicine (NYU), and the Department of Defense (DOD). The PCBN is organized with a Coordinating Center (JHU – led by Bruce Trock, Ph.D.), and Network Sites at NYU (led by Jonathan Melamed, M.D. and Peng Lee, M.D.) and JHU (led by Angelo De Marzo, M.D. and George Netto, M.D.). The goal of the PCBN is to develop a biorepository with high quality, well-annotated specimens obtained in a systematic, reproducible fashion using optimized and standardized protocols, and an infrastructure to facilitate the growth of the resource and its wide usage by the prostate cancer research community. The specimens in the PCBN include tissues from prostatectomies, serum, plasma, buffy coat, prostatic fluid, derived specimens such as DNA and RNA, linked to clinical and outcome data, and supported by an informatics infrastructure. A website has been established to make the PCBN accessible to the prostate cancer research community: http://prostatebiorepository.org.

BODY

Current status of the PCBN

By the end of the 3rd year of funding and operation of the PCBN, a fully developed infrastructure was in place, including a web site that describes the available specimen resources and policies of the PCBN, that provided a means for researchers to electronically submit requests for specimens or queries to help refine their applications, displays the standard operating procedures (SOPs) in place at the PCBN, and highlights PCBN biospecimen science. During this year it was uncertain whether the Department of Defense/PCRP would release a Program Announcement for competitive renewal of the Prostate Cancer Pathology Resource Network Award, or what form it would take. Because this delay meant the original PCBN award would expire before the time when a competitive renewal application could be submitted, the DOD/PCRP awarded the PCBN a 4th year of “bridge” funding to allow it to continue operations and ultimately, prepare a competitive renewal application. During the bridge year, planning began to expand the PCBN from 2 to 4 Network Sites by adding investigators from University of Washington (UW: PI Robert Vessella, PhD, Co-PI Colm Morrissey, PhD), and Memorial Sloan-Kettering Cancer Center (MSKCC: PI Anuradha Gopalan, MD, Co-PI Howard Scher, MD). These 2 Network Sites were chosen deliberately to add additional experience and tissue resources from men with advanced or metastatic disease, and from men in clinical trials or with specimens pre- and post-treatment for metastatic disease. With the expansion to 4 Network Sites we successfully competed and were awarded funding for the PCBN for 3 additional years.

Furthermore, during this time Kenneth Pienta, MD came to Johns Hopkins as Director of Research for the Brady Urological Institute. Dr. Pienta had established a highly successful rapid autopsy program while at University of Michigan, and he became a PCBN Co-Investigator , where he helps to coordinate rapid autopsy efforts to meet the needs of the PCBN and the broader needs of the Urology and Oncology departments. In addition to rapid autopsy, Dr. Pienta is also establishing bone marrow biopsy from
selected patients with metastatic disease, which will provide additional valuable biospecimens for the PCBN.

**PROGRESS**

Progress will be described in 7 areas: governance, policies for specimen access, informatics, biospecimens, standard operating procedures (SOPs), marketing and usage by the research community, and biospecimen science.

**Governance**

Two committees have been formed:

**Steering Committee:** The SC includes 2 voting members from each institution; also to include consumer advocates and an outside expert. Members from NYU are Jonathan Melamed (Site PI), Peng Lee, and Ira Smolin (Consumer Advocate). Members from JHU are Bruce Trock, Angelo De Marzo, and Karen Sfanos. SC membership may be fluid, with members changing from time to time as specific expertise is needed. The functions of the SC include the following:

1. Define policies and criteria for prioritization of, and access to samples.
2. Assure that SOPs conform to NCI Best Practices.
3. Review progress
4. Resolve conflicts
5. Define policies for collaborations and publications
6. Regulatory and IP issues, with institutional consultants brought in as needed.

**Bioinformatics Committee:** The BC included James Morgan from JHU and Stuart Brown and James Robinson from NYU. They defined the requirements for the informatics infrastructure, oversaw the harmonization of data elements, developed SOPs for data quality control, and operationalized caTISSUE at both institutions (demonstrating interoperability with caTISSUE was a required element of the original RFA).

Because of the limited number of participants with only 2 funded PCBN sites, other governance activities beyond those defined for the SC or Bioinformatics Committee are taken up as they present themselves, either by those 2 committees, or by ad hoc committees. Although JHU, as the Coordinating Center has formal oversight of the PCBN, all decisions have been made jointly with input from NYU and JHU investigators.

**Scientific Advisory Board:** At the recommendation of the External Advisory Board (EAB; convened by DOD- PCRP) the Scientific Advisory Board was convened to provide independent scientific guidance. Members are:

Daniel Lin, MD: Dr. Lin is Professor of Urology and Chief of Urologic Oncology at University of Washington and Associate Member at Fred Hutchinson Cancer Research Center.
M. Scott Lucia, MD: Dr. Lucia is Associate Professor of Pathology at the University of Colorado. He is Vice Chair of the Dept. of Pathology and Co-Director of the PCa Research Laboratory.

William Grizzle, MD, PhD: Dr. Grizzle is Professor of Pathology at the University of Alabama at Birmingham. He is a well-known expert on uropathology and PCa biomarkers.

Material Transfer Agreement (MTA): A process has been established between the Technology Transfer offices of both institutions to handle requests for materials/data. For transfers to academic researchers and not-for-profit entities a Uniform Biological Material Transfer Agreement (UBMTA) is used. This process also covers sharing of materials between JHU and NYU. Requests from for-profit entities are determined on a case-by-case basis, with intellectual property considerations formulated by the Technology Transfer office.

**Policies for Specimen Access**
The SC developed **3 categories of specimens** to reflect a prioritization according to their rarity/research value:

**Priority 1 specimens:** Specimens that are readily available and have little or no linked clinical data. These specimens are made available for early stage research, e.g. to demonstrate that a particular biomarker is differentially expressed in normal vs. tumor. **Ex.** 40 case TMA with matched tumor and normal tissue; no clinical data. **Little or no preliminary data are required to justify the request; however, the applicant must still provide evidence that the proposed assay performs well in human prostate samples (including analytical validation of antibodies for IHC assays in their hands).** An expedited review procedure will be used to review applications for Priority 1 specimens.

**Priority 2 specimens:** Specimens that have greater research value, either due to their relative abundance or the richness of linked data or other linked specimen types. **Ex.** 80 case TMA with a range of Gleason scores and pathology stages; limited clinical data. **Access to these specimens requires preliminary data showing that the biomarker assay performed well and that the biomarker is differentially expressed in tumor vs. normal.**

**Priority 3 specimens:** Rare and/or data-rich specimens. **Ex.** 52 case TMA with matched primary tumor and lymph node metastases from hormone naive patients; detailed clinical data. Requests for these specimens **require more mature preliminary data, e.g. demonstration that the biomarker is correlated with established prognostic factors such as Gleason grade or stage.**

Review criteria for specimens are linked to these 3 priority categories. Requests for specimens of **any type** must also meet the following requirements:
1. Scientifically valid objective
2. PI and institution have suitable experience and resources to conduct the study.
3. Methods and sample amount/number requested are reasonable.
We have operationalized these definitions and criteria and posted them on the website. Requests for samples are first reviewed by Dr. Sfanos or Dr. Trock to determine that the necessary information is provided, the requested samples are available, and to flag unreasonable requests for revision with the investigator (e.g. a request for 600 frozen tissues for a discovery project). The requests are then forwarded for review by the Scientific Advisory Board members with Jonathan Melamed included to represent the PCBN.

Specimens requiring collaboration. It is important to note that some of the previously existing specimens/data available through the PCBN were developed by non-PCBN investigators at JHU and NYU prior to the funding of the PCBN, or with funds from other sources. For such resources that are particularly valuable or were very labor intensive to develop, the specimens may be made available to users in the form of a collaboration with the originator, rather than providing the samples without restriction. The collaboration is worked out between the originator and the requesting investigator and can be as little as an acknowledgement in a publication or as much as the originator taking an active role in the research. Specimens requiring collaboration are so designated on the website.

**Informatics**

Common data elements (CDEs): Through a series of face to face meeting as well as teleconferences and many email exchanges, JHU and NYU developed a set of CDEs used for general demographics, RP specimens, and clinical outcomes. We were mindful of College of American Pathology standards as well as CDEs developed by the NCI and used for caTISSUE. Going forward we will continue to monitor and implement as appropriate CDEs from these national efforts into our network site and across sites.

**Informatics platform:** caTISSUE Suite was initially chosen as a common export format for both sites. Rather than deploying caTISSUE as the sole system it was decided to use it as a secondary system to which both JHU and NYU could send data to meet the requirements of the Award (and also to satisfy IRB requirements for de-identification). Both institutions have mapped data elements to caTISSUE and can automatically export their data to a caTISSUE-accessible format. Currently neither institution is using caTISSUE as their primary informatics platform. NYU is moving to an enterprise-wide instance of LabVantage, and is using a RedCap system in the interim. JHU currently has a private instance of caTISSUE for PCBN, and the university is setting up an enterprise-wide caTISSUE instance. However, for PCBN functions during the last 3 years, we have used TMAJ, a database previously developed by Dr. De Marzo and Mr. Morgan which stores pathology and specimen data and images, and our Master Radical Prostatectomy database, which stores clinical and outcome data on over 21,000 prostatectomies.

Numerous projects over many years have shown that these 2 databases can be easily linked to query or output all data types necessary for PCBN, and these databases are compatible with the CDEs used by both JHU and NYU. Because the PCBN is a virtual biorepository, with most specimens remaining at JHU or NYU rather than stored centrally, the current informatics approach is adequate.

**Annotation of pre-analytical variation:** Currently we are collecting the following annotation variables from >90% of cases: (1) time of incision start / incision close (as surrogate for time of devascularization), (2) time when surgical pathology is paged to get the specimen, (3) the time the specimen is frozen or placed in fixative, and (4) time in formalin fixative.
Website. The website www.prostatebiorepository.org was made available to the public in June 2011, and has been continuously updated. It is the main portal through which investigators can contact the PCBN, have questions answered, and submit applications for biospecimens. It includes the following:

- a description of the PCBN and listing of the people involved
- information regarding PCBN governance
- listings and descriptions of available specimens and those in development
- policies, requirements, confidentiality, prioritization scheme and review criteria for specimen requests
- updated application forms with instructions and automatic “submit” feature
- SOPs used in PCBN
- FAQs
- useful links to other websites
- a query feature that automatically directs questions to the entire PCBN team
- biospecimen science activities including links to posters and abstracts

**Biospecimens**

One of the strengths of both the NYU and JHU sites is the large number and variety of biospecimens, both in existing archives and those newly available due to large patient volumes. In particular, both teams have extensive experience building and sharing biospecimens in the form of TMAs. Other specimens include fixed tissue (radical RP, TURP, suprapubic RP), snap frozen tissue (radical RP, seminal vesicles), body fluids (serum, plasma, buffy coat, prostatic fluid; most can be matched to tumor and benign tissue), and derived specimens (DNA, RNA, protein). It is notable that the NYU site contributed a substantial number of biospecimens during the last 12 months despite hospital closures resulting from Superstorm Sandy; the 3 hospitals that constitute the NYU site were closed from 2-9 months following the hurricane. The table below shows total specimens prospectively accrued to PCBN since June 2010. African Americans comprise 12% of patients accrued at JHU and 11% at NYU:

<table>
<thead>
<tr>
<th>Specimen Category</th>
<th>Total Since Start of Funding (JHU Total / NYU Total)</th>
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<tbody>
<tr>
<td>Total cases accrued</td>
<td>4362 (3505 / 857)</td>
</tr>
<tr>
<td>Frozen tissue cases</td>
<td>1061 (608 / 453)</td>
</tr>
<tr>
<td>Seminal vesicle cases</td>
<td>3060 (2607 / 453)</td>
</tr>
<tr>
<td>Prostatic fluid cases</td>
<td>2476 (2476 / 0)</td>
</tr>
<tr>
<td>Seminal vesicle fluid cases</td>
<td>234 (0 / 234)</td>
</tr>
<tr>
<td>Metastatic** cases</td>
<td>209 (70 / 139)</td>
</tr>
<tr>
<td>Rapid Autopsy cases</td>
<td>6 (4 / 2)</td>
</tr>
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** Metastatic cases primarily reflect pelvic lymph node metastases excised during prostatectomy
TMAs. 18 sets of TMAs are currently available to the PCBN with 6 more in development (this does not include TMAs that will be made available as part of the renewal with the addition of UW and MSKCC). Some of the more notable TMAs include (a full listing and description is on the website, under the “Specimens” tab):

- Metastatic tissue from 15 rapid autopsies (see below)
- Natural history of PCa progression: 237 cases with recurrence, followed for metastasis
- PSA progression: cases with recurrence matched to non-recurrence, total 726 cases
- Lymph node metastases: matched primary tumor and lymph node from 79 cases
- Hormone sensitivity: hormone naïve matched to hormone refractory, 56 cases
- Race disparity: 2 TMA sets of African American matched to Caucasian patients, 264 cases
- Fixation time: variation in time in fixative, 5 time points per case, 27 cases
- Warm ischemia/fixation delay: variation in delay prior to fixation, 4 time points per case, 15 cases

In addition, the PCBN at JHU has been selected as the Coordinating Center for the Movember Global Action Plan (GAP 1) Unique TMA project, to construct high quality TMAs from high demand specimens collected from multiple institutions in multiple countries (UW is also a participant). This project is constructing 3 TMAs with contributions from multiple institutions:

TMA 1: Matched primary tumor and lymph node metastasis tissue from prostatectomy patients.

TMA 2: Matched pre-treatment and post-treatment tissue from men treated with androgen deprivation therapy.

TMA 3: Metastatic tissue from men with multiple metastatic lesions.

Autopsy and Advanced Disease Specimens. NYU has been approved by the IRB to start a rapid autopsy program at both NYU and VA hospitals. Dr. Melamed has arranged for the necessary permissions and Personnel to perform autopsies on an on-call basis. Currently, 2 rapid autopsies have been performed, and 7 men with advanced metastatic disease have signed consent agreeing to rapid autopsy in the event of their death.

A similar program at JHU has now been re-activated under the aegis of the PCBN. Five rapid autopsies have been completed at JHU since the reactivation, with successful harvest of soft tissue and bone metastatic sites. One of the rapid autopsies led to a high impact paper describing the first prostate cancer case for which it was possible to carry out detailed longitudinal characterization of the lethal cell clone from the primary to multiple distant metastases (tissue was available from the prostatectomy performed at JHU 17 years before death) (Haffner 2013). The rapid autopsy program is being revised with input from Dr. Kenneth Pienta, who recently joined JHU as Director of Research for the Brady Urological Institute, and who formerly directed a highly successful rapid autopsy program at University of Michigan. The previous rapid autopsy program at JHU (prior to the funding of the PCBN) had collected
tissue from 33 autopsies. These specimens are being catalogued, and a TMA has been developed using tissues from 15 of the autopsies. In addition to the 5 rapid autopsies, metastatic lymph node tissue has been obtained from 50 RP cases.

Derived Specimens. In the past 12 months and since February 2011 the following have been extracted from frozen tissue at JHU:

**Total Since February 2011**

<table>
<thead>
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<th>DNA</th>
<th>177 samples from 76 cases</th>
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<tr>
<td>RNA</td>
<td>166 samples from 80 cases</td>
</tr>
<tr>
<td>Protein</td>
<td>110 samples from 46 cases</td>
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Almost all samples also have matched tumor and benign tissue available. *All were obtained since funding began, thus “prospective.”* These samples were extracted following a comprehensive quality control process to determine the optimal protocol.

**Standard Operating Procedures**
The SOPs at both sites for the major tissue-oriented processes (such as harvesting RP fresh frozen tissue, fixation and processing, extracting DNA, RNA and protein, QC for DNA and RNA, and extracting serum and plasma) have all been posted on the PCBN website. SOPs for harvesting fresh frozen tissue at both sites are identical, however protocols for fixation and processing are those that have been established on the basis of clinical considerations at each institution and do have some variations. In an effort to harmonize these protocols NYU has begun injecting formalin into RP specimens, and will begin microwave fixation. The protocol for extracting serum, plasma and buffy coat from blood samples was provided by Dr. Hans Lilja (MSKCC), one of the world’s foremost authorities on blood-based biomarkers for PCa. Prostatic fluid is harvested by manual compression of *ex vivo* surgical specimens in the pathology suite; the SOP is posted on the website, using a protocol developed at JHU prior to PCBN and since then, used on over 6000 RPs.

**Marketing and Usage by Research Community**
Usage continues to increase (usage through the first 9 months of 2013 was equal to that for all of 2012), and we continue to receive queries and requests from researchers who have not previously contacted the PCBN. Usage is summarized below:

- 123 queries were received and responded to
- 48 applications for specimens approved by the Review Committee, from 46 individual investigators
- 7 additional applications from 6 investigators are pending (bringing the total received to 55 from 52 investigators) – awaiting additional information from the investigators
- 50 applications came from academic institutions, 3 from commercial entities, 1 from a government agency, and 1 from a non-profit institution (these include the pending applications)
- the applications came from investigators in 6 countries
In addition to these requests, we provided 23 letters of support to investigators applying for DOD (13 letters), NIH (9 letters), and Veterans Administration (1 letter) grants.

Importantly, 19 manuscripts have been published or are in press citing the PCBN as the source of TMAs or other samples, including high impact journals: one each in Nature, PNAS, New England J Medicine, Cancer Research, Clinical Cancer Research, Oncogene, PLoS One, and J Clinical Investigation. Citations for these manuscripts can be found on the PCBN website at http://prostatebiorepository.org/publications.

Marketing has occurred largely through presentations and exhibit booths at national meetings. In 2011 we had a presentation and exhibit at the PCRP-sponsored Innovative Minds in PCa Today (IMPaCT) Conference (Orlando). In 2012, PCBN presentations and/or exhibit booths were at the annual meetings of ISBER, AACR, the NCI Office of Biorepository and Biospecimen Research, and the Society for Basic Urological Research. Also in 2012 the PCa Foundation graciously agreed to send a PCBN survey regarding biospecimen needs to researchers on their mailing list under Dr. Soule’s signature. The survey was sent to 296 researchers representing a majority of the most active PCa scientists and 84 responded; website traffic recorded 265 new visits. Some of the interesting survey results are as follows:

- 91% said their current needs for tissue were not met by available resources
- 89% were interested in primary tumor tissue
- >80% were interested in metastatic tissues
- nearly 80% were interested in TMAs with matched recurrent and non-recurrent cases
- 70-80% were interested in DNA and RNA
- >60% were interested in serum

In 2013 PCBN exhibit booths were included at the AACR, American Urological Association, the ASCO Genitourinary Cancer Symposium. Finally, in recent program announcements from the DOD PCRP about opportunities for PCa research awards, the PCBN was specifically highlighted and applicants were encouraged to request biospecimens from the PCBN. In 2014 PCBN flyers were distributed to investigators at the AUA and the Prostate Cancer Foundation Annual meeting.

The PCBN sponsored a one day Workshop in conjunction with the ASCO Genitourinary Cancer Symposium in Orlando FL in February 2013. The title was “Validating Tissue Biomarkers in Primary and Metastatic PCa. “ The workshop featured 9 internationally regarded speakers from academia and industry and was attended by 50 PCa scientists (by invitation) from around the country. There was a great deal of productive discussion, including problems of sampling metastatic tissue, pros and cons of rapid autopsy programs, addressing tumor heterogeneity, and neuroendocrine tumors arising after targeted therapy. The Workshop was a deliverable required in the original Program Announcement.

**Biospecimen Science**

We have been actively conducting studies to test standard operating procedures developed for derivative extraction from frozen tissue. These studies have demonstrated that DNA and RNA quality, and 7 RNA and protein biomarkers were comparable for derivatives from standard open RP compared to robotic assisted laparoscopic RP. A manuscript describing the comparison of derivatives from open vs. robotic assisted laparoscopic RP has been published (Darshan, Prostate 2014). We have also used TMAs that
we developed with varying warm ischemia times and varying fixation times (described above) to evaluate the impact of pre-analytical variation on commonly used IHC markers that localize to a number of different cell types and cellular compartments (p63, basal cell specific keratins AR, NKX3.1, ERG, PTEN, MYC, p27) and have begun to quantify expression using TMAJ and FrIDA for image analysis. Similarly we performed in situ hybridization for a number of mRNAs (e.g. p63, ERG, PTEN, PCA3, MYC) on these same arrays.

KEY RESEARCH ACCOMPLISHMENTS

a. Continued prospective collection of high quality biospecimens, including tissues now totaling nearly 4300 prostate cancer cases and over 1,000 frozen tissue cases since inception.

b. Publication of biospecimen science paper describing PCBN protocol for derivatives and testing DNA and RNA quality in robot assisted vs. open radical prostatectomy.


d. Establishment of rapid autopsy program and construction of TMA with metastatic specimens from rapid autopsies.

e. Holding a workshop with prostate cancer leaders from around the country discussing biospecimen and biomarker issues critical to clinical translation including tumor heterogeneity and neuroendocrine phenotype arising after exposure to targeted therapy.

REPORTABLE OUTCOMES

Successful competitive renewal of the PCBN award with expansion to 4 Network Sites, adding highly experienced sites from MSKCC and UW, and expanding emphasis on advanced disease and metastatic disease biospecimens.

CONCLUSIONS

The concept of the PCBN demonstrates recognition of the critical need for improved quality, consistency, representativeness, and information content of biospecimens used for translational cancer research, and the shift in focus toward prevention and treatment of metastatic disease and away from low risk disease. The Network combines considerable expertise in multi-disciplinary tissue-based PCa research, excellence in PCa histopathology and molecular pathology and biospecimen quality control, significant experience in collecting and providing high quality research biospecimens, a proven infrastructure, and support from urology and pathology departments with strong histories of PCa
biospecimen research. All of the key positions in the Coordinating Center and the Network Sites are held by individuals with significant related experience. Of particular importance is the proposed expansion to include University of Washington and MSKCC under the competitive renewal, and the participation of Dr. Robert Vessella at UW and Dr. Ken Pienta at Johns Hopkins, two of the world’s leaders in programs to collect and evaluate metastatic tissues. Leadership and management of the PCBN is enhanced by a committee structure that triages and streamlines the process for planning and problem solving, and incorporates planning for scientific, regulatory, and intellectual property requirements. We believe the combination of expertise, experience in leadership of biobanking efforts, and substantial availability of biospecimens representing critical disease categories will contribute to the continued success of the PCBN in its mission to advance understanding of tumor biology and speed the clinical translation of biomarker research.
PUBLICATIONS
