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# Prostate Cancer Pathology Resource Network

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). 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 and RNA); these specimens are linked to clinical and outcome data and supported by an informatics infrastructure. The PCBN is currently made accessible to outside researchers through a website. The PCBN has been open to researchers since July 2011. Because release of the Program Announcement for competitive renewal of the PCBN was delayed, the DOD awarded the PCBN a 4th year of "bridge funding" beyond the original 3 years to permit continued operation while applying for the competitive renewal. In this 4th year of operation the PCBN has continued to accrue new specimens, increase the number of TMAs available, increased usage from prostate cancer researchers around the world, and continued to conduct biospecimen science research. Accrual of specimens from men with advanced or metastatic disease is an increasing focus of the PCBN. A highlight of this year was the successful competitive renewal of the PCBN award for 3 more years, with expansion of the participating Network Sites to 4: Johns Hopkins, New York University, University of Washington, and Memorial Sloan Kettering Cancer Center.
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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, provides 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. This report covers activities during this 4th year, during which the PCBN was still comprised only of JHU and NYU.

During this 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.

The UW Prostate Cancer Program has a 25 year history of collecting and distributing biospecimens to investigators worldwide. Since 2002 they have provided biospecimens to more than 375 investigators. They also established one of the first rapid autopsy programs for prostate cancer and have accessioned 110 patients since 1991, collecting more than 2000 metastatic samples from bone and none-bone sites. Their current activity level is 8 rapid autopsies annually. In addition they have also collected over 2000 samples of peripheral blood circulating tumor cells (CTC) and bone marrow disseminated tumor cells (DTC). Serum has been collected from several thousand patients, including over 700 specimens.
from men with metastatic disease. Finally, a number of TMAs are available, including a TMA of bone and soft tissue metastases, and a TMA of post-neoadjuvant abiraterone tumor tissue.

At MSKCC tumor specimens are collected through a collaboration between the Pathology Core Facility and the Tissue Procurement Service and Tissue Bank, which are funded by the MSKCC NIH Cancer Center Support Grant and the Prostate Cancer SPORE. All patients seen for prostate cancer treatment are asked if they are willing to consent to donate biospecimens. Since 2008 the tissue bank has fresh frozen tissue from approximately 1700 primary prostate tumors, approximately half of which have matched benign tissue. Serum and plasma samples collected for routine PSA testing are also banked. Since 1999 over 6000 radical prostatectomy specimens have been whole-mounted and step-sectioned and archived with digital maps. For more than 800 of these cases there have matched needle biopsy tissue. In keeping with the enhanced focus of the PCBN shifting toward advanced and metastatic disease, MSKCC anticipates contributing patients in the following categories:

- post-neoadjuvant prostatectomy and matched lymph node metastasis specimens from patients with pT3N1 disease
- bone and visceral metastasis resection tissue with matched serum, plasma, and buffy coat
- serum, plasma and buffy coat samples from patients with progressive metastatic disease
- prostatectomy specimens from men with intermediate and high Gleason score

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 is coordinating 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.

With the expansion to 4 Network Sites we successfully competed and were awarded funding for the PCBN for 3 additional years.

**Biospecimen Accrual**

The table below shows specimens prospectively accrued to the PCBN since inception (June 2010) and during the 12 month period covered by this report. African American comprise 12% of patient with specimens accrued at JHU and 11% and NYU.
<table>
<thead>
<tr>
<th>Specimen Category</th>
<th>Total Since Start of Funding (JHU Total / NYU Total)</th>
<th>Last 12 months (JHU / NYU)</th>
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<tr>
<td>Total cases accrued</td>
<td>4362 / 857</td>
<td>919 / 141</td>
</tr>
<tr>
<td>Frozen tissue cases</td>
<td>1061 / 453</td>
<td>337 / 211</td>
</tr>
<tr>
<td>Seminal vesicle cases</td>
<td>3060 / 453</td>
<td>752 / 126</td>
</tr>
<tr>
<td>Prostatic fluid cases</td>
<td>2476 / 0</td>
<td>608 / 0</td>
</tr>
<tr>
<td>Seminal vesicle fluid cases</td>
<td>234 / 234</td>
<td>48 / 0</td>
</tr>
<tr>
<td>Metastatic** cases</td>
<td>209 / 139</td>
<td>23 / 17</td>
</tr>
<tr>
<td>Rapid Autopsy cases</td>
<td>6 / 2</td>
<td>2 / 0</td>
</tr>
</tbody>
</table>

** Lymph node metastases from patients undergoing prostatectomy

**Derived Specimens.** In the past 12 months the following specimens have been prepared from frozen tissue at JHU (specimens also have matched tumor and benign tissue available):

- 87 tubes cut for derivative extraction
- DNA (4 samples)
- RNA (10 samples)
- Protein (6 samples)

This accrual is much lower than in previous years because the Pathology Tech who performed the extractions and derivative preparations left at the beginning of the reporting period, and it was almost one year before another Pathology Tech could be hired and trained. Since the time of this report our derivative extraction effort is back at the level prior to when the previous Pathology Tech left. Derivatives were prepared using an optimized protocol that we developed.

**Autopsy and Advanced Disease Specimens.** Two rapid autopsies have been completed at JHU during this year, with successful harvest of soft tissue and bone metastatic sites. As described above, the rapid autopsy program has been revised with input from Dr. Pienta. 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). We have also constructed a new TMA using specimens from 15 rapid autopsies; these autopsies were part of a collection of 33 rapid autopsies performed prior to the original PCBN award, but the specimens were annotated and the TMAs prepared during the current reporting period using PCBN resources.
In a previous report we described the possible acquisition of an existing collection of serum samples at JHU from men with biochemical recurrence or metastatic disease. Unfortunately, one of the 2 freezers housing this collection failed and all specimens were lost. Furthermore, difficulties with the annotation made it problematic to ascertain the clinical status of the samples. Therefore, we have not pursued this collection.

**New TMAs.** 4 new TMAs have been constructed. The TMAs have the following designs/purposes:

- evaluate the effect of obesity on outcomes in men undergoing prostatectomy
- biospecimen science: 20 patients with replicate samples to evaluate the effect of temperature on TMA storage and slide storage. Conditions are room temperature, 4°C and 20°C.
- metastatic tissue from 15 rapid autopsies
- test TMA for optimizing immunohistochemistry assays or testing new antibodies

This brings the total number of TMAs available to 30, with 6 more TMAs actively in development.

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.

**Usage:**

Usage continues to increase, and we continue to receive queries and requests from researchers who have not previously contacted the PCBN. Usage for 7/13 – 7/14 is summarized below:

- 45 queries received and responded to
- 19 applications for tissue received from 17 institutions. Of these 19 requests:
  - 14 requests completed – samples shipped to investigators
  - 4 requests – awaiting additional information from the investigator
  - 1 requests MTA in process
  - requests represented 5 countries (USA, Hong Kong, Sweden, Switzerland, Spain)
  - of the 17 institutions, 16 were academic and 1 was from industry
Number of samples distributed:
- Number of unstained sections of TMAs: 18
- Number of other sample types (DNA/RNA/serum/FFPE tissues/frozen tissues, etc): 250
- Total number of samples distributed 7/2013-7/2014: 268

In addition to the above biospecimens, during this period the PCBN provided letters of support for 13 grant applications, of which 7 were to the DOD, 5 were to NIH, and one was to the Veteran’s Administration. Of these, we have heard that 7 were not funded, one is under review, and the remainder have not yet responded to our request for an update. Access to tissues or the quality of tissues does not appear to have been a criticism of any of the applications that were not funded.

**Biospecimen Science**

In the previous report we described submission of an abstract describing our SOP for derivative extraction from frozen tissue, and our demonstration that DNA and RNA quality were similar for tissues obtained from standard open prostatectomy compared to robotic assisted laparoscopic prostatectomy. This manuscript has now been published (Darshan M 2014).

**Publications**

Eight publications that cited tissue provided by the PCBN were published during the reporting period or submitted during the period but published subsequently. These include the high impact paper on clonal origin of metastatic prostate cancer cited above, and a potentially practice-changing investigation of AR-V7 and resistance to abiraterone and enzalutamide in metastatic prostate cancer (Antonarakis 2014). All 8 publications are listed below. Papers were published in high impact journals including Nature, New England Journal of Medicine, Cancer Research, Journal of Clinical Investigation, and Oncogene.

**KEY RESEARCH ACCOMPLISHMENTS**

a. Continued prospective collection of high quality biospecimens, including tissues now totaling nearly 4300 prostate cancer cases and over 10000 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.

c. PCBN support cited in 8 manuscripts, including 2 very high impact papers in J Clin Invest and New Engl J Med.

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

This report detailed activities and achievements during a 4th “bridge” year of funding, generously awarded by the DOD/PCRP to allow continued operations while preparing a competitive renewal application. This 4th year was notable for the successful creation of a consortium of 4 institutions comprising the 4 Network Sites, successful competitive renewal award, increased focus on advanced/metastatic disease patients including rapid autopsy, continued increase in biospecimen accrual and usage from the international prostate cancer research community, and citation of PCBN support in 8 publication, including 2 very high impact publications. In addition, the PCBN through JHU and UW is participating in the Movember Global Action Plan Unique TMA project to develop 3 high quality high demand TMAs from multiple institutions around the world.

Activities for 2014-2015 will focus on integrating MSKCC and UW fully into the PCBN, revising the website to reflect the expanded composition and capabilities of the PCBN, continuing to increase access to specimens from advanced disease patients, adding specimens from patients enrolled in active surveillance protocols, completing TMAs currently under construction, and planning a biomarker/biospecimen science workshop as a follow-up to our successful 2013 workshop.
PUBLICATIONS


