Research Article

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The Prostate, Lung, Colorectal and Ovarian Cancer (PLCO) Screening Trial Pathology Tissue Resource

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Abstract

Background: Pathology tissue specimens with associated epidemiologic and clinical data are valuable for cancer research. The Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial undertook a large-scale effort to create a public resource of pathology tissues from PLCO participants who developed a cancer during the trial.

Methods: Formalin-fixed paraffin-embedded tissue blocks were obtained from pathology laboratories on a loan basis for central processing of tissue microarrays, with additional free-standing tissue cores collected for nucleic acid extraction.

Results: Pathology tissue specimens were obtained for prostate cancer ($n = 1{,}052$), lung cancer ($n = 434$), colorectal cancer ($n = 675$) and adenoma ($n = 658$), ovarian cancer and borderline tumors ($n = 212$), breast cancer ($n = 870$), and bladder cancer ($n = 204$). The process of creating this resource was complex, involving multidisciplinary teams with expertise in pathology, epidemiology, information technology, project management, and specialized laboratories.

Conclusions: Creating the PLCO tissue resource required a multistep process, including obtaining medical records and contacting pathology departments where pathology materials were stored after obtaining necessary patient consent and authorization. The potential to link tissue biomarkers to prospectively collected epidemiologic information, screening and clinical data, and matched blood or buccal samples offers valuable opportunities to study etiologic heterogeneity, mechanisms of carcinogenesis, and biomarkers for early detection and prognosis.

Impact: The methods and protocols developed for this effort, and the detailed description of this resource provided here, will be useful for those seeking to use PLCO pathology tissue specimens for their research and may also inform future tissue collection efforts in other settings. *Cancer Epidemiol Biomarkers Prev; 25(12); 1635–42. ©2016 AACR.*

Introduction

Pathology tissue specimens with accompanying epidemiologic and clinical data are increasingly important for cancer research. The Cancer Genome Atlas (TCGA) and other efforts have established that individual cancer types possess distinct molecularly defined subtypes with distinct etiologies, responses to therapy, and survival characteristics (1–4).

Fresh-frozen tissue samples are thought to be optimal for molecular analyses; however, obtaining such specimens poses multiple logistical challenges, requiring rapid access to tissues prior to histopathologic examination and specialized equipment for freezing, transport, and storage. In addition, tissue morphology is often compromised in fresh-frozen tissues. In contrast, routinely prepared formalin-fixed paraffin-embedded (FFPE) diagnostic tissue specimens archived in pathology laboratories are often available for research. Although variable preparation and preservation may pose challenges for using archived pathology samples in particular applications, technological advances have expanded the utility of FFPE tissue specimens (5).

Collecting archival FFPE tumor tissues within a large cohort or prevention trial, such as the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial, is cost effective due to the existing infrastructure established for the study. The value of such specimens is substantially increased by the detailed epidemiologic and clinical data available in PLCO, such as screening history, risk factors, and long-term survival data, as well as the serial prediagnostic blood collection. Furthermore, results of pathology studies can be linked to results of prior laboratory assays that have been performed by many investigators over the course of a decade.

Here, we describe the creation of the PLCO pathology tissue resource, including methods and protocols for patient and tissue

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selection, obtaining tissue materials from pathology laboratories, and processing the tissue blocks. Detailed descriptions of the tissue specimens collected and their epidemiologic and clinical characteristics are also provided. This resource was created by the NCI (Rockville, MD) as a public resource, available to all researchers whose proposals are approved through peer-review.

Materials and Methods

The PLCO Cancer Screening Trial

PLCO was a randomized cancer screening trial designed to assess the effectiveness of specified screening tests for the four cancers, as described elsewhere (6, 7). Briefly, over 154,000 healthy men and women, ages 55 to 74, were enrolled at 10 screening centers across the United States. Half of the participants were randomized into the screening arm and half into the control arm. Participants in the screening arm received annual screens for the four cancers for the first 6 years; participants in the control arm received usual care. Enrollment began in 1993 and concluded in 2001. Both arms were followed for cancer incidence and mortality for at least 13 years from baseline. Subjects were queried annually for the occurrence of any cancer (not just the trial-related cancers), and all reported cancer diagnoses were pathologically confirmed through medical record abstraction by trained staff. Incident cancers were also identified through death certificates for subjects with underlying cause of death from cancer. Epidemiologic data, including demographic and lifestyle information, were collected through multiple questionnaires administered at baseline and during follow-up, from all participants. PLCO also collected blood samples annually at each of the six screening rounds from the screened participants, and a one-time buccal cell sample from the control arm participants. These samples are stored in the PLCO biorepository, available for use by the entire scientific community through a peer-review application process (8, 9).

Overview of the PLCO pathology tissue collection project

Pilot. The PLCO pathology tissue collection project was conducted in three phases: pilot, main phase, and an add-on phase. The pilot phase was conducted in 2000 to 2001 to determine the availability of the pathology tissue materials, assess the feasibility of obtaining them either for permanent retention or for temporary loan, and investigate the overall process of requesting donor blocks with predetermined specifications (i.e., containing primary histology with adjacent normal tissue, etc.) from pathology laboratories. Additional information, such as costs and level of effort required, was also collected. The pilot phase was conducted at 3 of the 10 PLCO screening centers, with the aim of collecting up to 100 tumor samples from each screening center. Tumor tissues from 3 organ sites were collected: prostate, lung, and colorectal. For each case, one tumor block containing primary tumor and tumor-free margin was requested for permanent retention.

Main phase. The main phase was conducted in 2006 to 2011. Rather than requesting donor blocks for permanent retention, it was decided, based on the pilot results, that the requests would be to borrow donor blocks for the purpose of removing small cores (0.6–1.0 mm in diameter) containing targeted tissue types to create tissue microarrays (TMA) and to extract DNA/RNA. The original donor blocks would be returned to the pathology laboratories once the desired cores were extracted. Five tumor sites were selected on the basis of the trial and public health interest: colorectal, ovary, prostate, lung, and breast. The project began with colorectal cancer and colorectal adenoma and proceeded to ovary, prostate, lung, and breast. The PLCO pathology collection protocols were approved by the Institutional Review Boards (IRB) at NCI and each of the 10 screening centers.

Add-on phase. In 2012, the pathology tissue collection effort was resumed to add new tumor sites and newly ascertained cases to the previous collections. As of this writing, collection of tissue specimens for bladder cancer, breast ductal carcinoma *in situ* (DCIS), and the newly ascertained colorectal cancer cases has been completed.

Patient and tissue selection

Subjects in both the screened and the control arms who had a pathologically confirmed cancer diagnosis and who had signed the PLCO etiologic studies consent form (ESC) were eligible for selection. The ESC was implemented in 1998 to obtain patient consent for PLCO to collect biological specimens, including blood, buccal, and pathology tissue specimens for broad-based research purposes.

The project aimed to collect tissues from 50 to 300 patients from each of the 10 screening centers, per year, for 5 years. Table 1 details the sample selection criteria by cancer site, which generally included size of the tumor, pathologic features of the tumor (e.g., stage), and availability of matched blood or buccal cell samples. Cases with only biopsy tissues were not selected, except for lung cancer and colorectal adenoma. Some tumors with certain characteristics of particular research interest were oversampled. For examples, for prostate cancer, cases included in a prior genome-wide association study (10), cases with Gleason grade 7+, or African American ethnicity were oversampled.

Once the cases were selected, pathology reports were reviewed by qualified PLCO trial staff and a consulting pathologist to select the appropriate donor blocks from each case. In general, 1 to 3 representative donor blocks were selected per case: 2 blocks containing tumors of the dominant histologic features and 1 block containing adjacent normal tissue, with some variations depending on the tumor site (Table 2).

Importantly, a donor block could contain tumor only, normal only, or both normal and tumor, and a tumor could also contain one or more histologic features in a donor block. For tumors with complex histologic features, the donor block containing 2 or more histologic features were preferentially selected. Donor block containing adjacent normal tissues were selected whenever available. In some cases, metastatic tumors (from involved lymph nodes or distant sites) were also selected if available.

Requesting donor blocks from the pathology laboratories

Once the donor blocks were identified from pathology reports, source pathology laboratories were contacted to determine their loan policy (loan period, cost per sample, etc.), and any additional requirements, such as patient authorization.

A pathology tissue request package was mailed to affiliated pathology laboratories from the 10 PLCO screening centers. The package included a cover letter, a copy of the original signed consent form, a copy of the pathology report, the pathology request form (PRF), and if required by the laboratory, the signed HIPAA-compliant authorization form provided by the patient or if deceased, by next of kin. The PRF listed the sample ID, the

Table 1. Pathology tissue selection criteria

Tissue site	Year of selection	Subject selection criteria
Main phase		
Colorectal	2006	All eligible subjects were selected, including a small number of multiple-primary cases. The cases were split into pretreatment and posttreatment tissue.
Colorectal adenoma	2006	Subjects with adenomas $\geq$5 mm and who were in Aberrant Crypt Foci study, or African Americans, or those with recurrent adenomas, incident adenomas, or prevalent adenomas were selected from all eligible subjects; nonprevalent adenomas were preferentially selected. Number of subjects selected was limited to 300 from each study center, due to the large number of adenoma cases available.
Ovarian[a]	2006, 2010	Subjects with either pathologic staging or an oophorectomy were selected from all eligible subjects. Cancers included invasive ovarian, fallopian tube, and primary peritoneal.
Prostate	2007	Subjects with a prostatectomy were selected from all eligible subjects; those who were in CGEMS or had blood/buccal cell available, or African American, or had Gleason grade 7 or above, were preferentially selected.
Lung	2009	Subjects who had a biopsy or surgery were selected from all eligible subjects; those who were never smokers, or had an interval cancer or small aggressive lung cancer, or African American were preferentially selected.
Breast	2010	Subjects with tumor size 0.5 cm or greater were selected from all eligible subjects; those who were included in the CGEMS or had invasive cancers were preferentially selected.
Male breast	2010	All eligible subjects were selected, including cases where the cancer was reported but not yet confirmed. Unlike female breast cancer, tumor size was not taken into account in selection.
Add-on phase		
Bladder	2012	Subjects with tumor size 3 mm or greater were selected from all eligible subjects. Primary bladder (invasive or *in situ*) cancers were selected, including papillary and transitional bladder cancers; flat papilloma tumors of the bladder were excluded.
Breast DCIS	2012	Subjects with tumor size 3 mm or greater and who were not included in the main phase were selected from all eligible subjects. DCIS tumors with an *in situ* diagnosis only. Cases of mixed tumor behavior (with both *in situ* and invasive components) were not selected.
Colorectal	2013	Subjects with tumor size 3 mm or greater and who were not included in the main phase were selected from all eligible subjects, excluding those with carcinoids, extranodal lymphomas, sarcomas of the colorectal sites, and tissues resected after neoadjuvant treatment of CRC.

Abbreviations: CGEMS, Cancer Genetic Markers of Susceptibility Project; CRC, colorectal cancer; DCIS, ductal carcinoma *in situ*.
[a]Ovarian tumor collection was conducted in two phases; the first phase took place during 2006–2008, and the second during 2010–2011.

histologic findings in blocks of interest, and a preaddressed, postage-paid, bubble-wrap lined mailer for shipping the donor blocks. If the pathology laboratory was unable to send the requested donor blocks, it was asked to indicate the reasons on the PRF.

Donor block processing for TMAs and for nucleic acid extraction

Donor blocks were shipped in batches to a central pathology laboratory with expertise in TMA construction. One 4-micron H&E–stained section per block was prepared and reviewed by a pathologist to identify "region(s) of interest" (ROI) that contain (s) benign ("normal") or tumor tissue.

Tissue cores (0.6 mm in diameter for colorectal adenoma and lung tumors or 1.0 mm in diameter for all other tumor sites) removed from ROIs were placed in recipient blocks to create TMAs or placed into Eppendorf tubes (2 mL) for nucleic acid extraction. Coring was conducted to maximize sampling of histologically heterogeneous regions while preserving donor blocks. Typically, up to 15 cores, with some exceptions, were taken per patient: 6 tumor and 6 adjacent normal cores, to be split on duplicate TMA blocks (i.e., alpha and beta), and 3 tumor cores for DNA/RNA extraction. For most organ sites, the cores for DNA/RNA extraction were limited to tumors with the most representative invasive histology or with the highest grade. For a few organ sites, adjacent normal cores were also obtained (Table 2). Cores were taken in a representative fashion across all ROIs or blocks when there were multiple ROIs or blocks for the same histology.

When the size of tissue targets was limiting, construction of the alpha TMA was prioritized, followed by nucleic acid extraction, and finally, construction of the beta TMA (Table 2). For example, if tissue material was available for only 6 cores, 3 would be arrayed on the alpha TMA and 3 would be stored for DNA/RNA extraction; the case would be excluded from the beta TMA array.

Likewise, when a tumor contained mixed histologic patterns, and sampling all morphologically distinct areas would exhaust the tissue in the donor blocks, certain histologic regions were prioritized, particularly for ovarian and lung cancers, which often demonstrate mixed histologic patterns. The order of prioritization generally applied was as follows: the primary invasive histology, the secondary invasive histology, carcinoma *in situ*, adjacent normal, metastasis involving the lymph node, and metastasis involving distant organ site(s).

TMA blocks were constructed following standard methods (11). They were prepared as four quadrants, each containing about 50 to 125 cores depending on the core size, with empty rows and columns between quadrants to facilitate orientation. Cores belonging to the same patient, from both tumor and adjacent normal regions, were grouped contiguously, but patients were randomly disbursed across the TMA blocks, arrayed in the order they were received. Each TMA block also contains a set of 10 standard control tissue samples not from PLCO, arrayed on the outer border of the quadrants.

Protocol changes during add-on phase

In 2011, after 13 years of active follow-up of clinical outcomes, PLCO began to transition into a new phase of continued follow-up using a more cost-effective centralized approach. As a result, pathology reports were available for only those who reconsented to the centralized active follow-up or were already deceased, which is about 80% of all otherwise eligible cases. The tissue collection protocol was modified to prioritize collection of

Table 2. Tumor histologies targeted and coring protocol by cancer site

	Donor blocks requested/tumor histology targeted	Coring protocol	Sampling priority	
			Cores	Histology
Main phase				
Colorectal cancer	3 blocks of primary tumor as well as any colorectal adenomas and hyperplastic polyps removed with the primary tumor (if available), and up to 3 blocks of normal tissue, where tumor and normal may be on the same blocks if tissues are distinct.	3 cores of tumor and 3 cores of adjacent normal per alpha and beta TMA block; 3 cores of tumor for DNA/RNA. Maximum 15 cores per case.	Alpha TMA; DNA/RNA; beta TMA	NA
Colorectal adenoma	3 blocks of colorectal adenomas and hyperplastic polys. Adjacent normal not targeted.	3 cores of tumor per alpha and beta TMA block; 3 cores of tumor for DNA/RNA. Maximum 9 cores per case.	Alpha TMA; DNA/RNA; beta TMA	NA
Ovarian cancer	2 blocks of tumors from each ovary, fallopian tube, and omentum or peritoneum. If endometrioid or clear cell, 2 blocks of tumor and 2 blocks of normal endometrium. If primary fallopian tumor, 2 blocks of tumor and 2 blocks adjacent normal.	3 cores of tumor per histology per alpha and beta block; 3 cores of tumor per histology per for DNA/RNA. Adjacent normal tissue was collected for both TMA and DNA/RNA when present on the donor blocks. Maximum 24 cores per case.	Alpha TMA; DNA/RNA; beta TMA	2 dominant patterns
Prostate cancer	3 blocks per case: 1 containing Gleason major grade, 1 containing Gleason minor grade, and 1 containing benign tissue.	2 cores of Gleason major, Gleason minor, and benign prostate tissue per alpha and beta TMA block; 3 cores of highest grade tumor for DNA/RNA. Maximum 15 cores per case.	Alpha TMA; beta TMA; DNA/RNA;	Major Gleason, minor Gleason, benign
Lung cancer	3 blocks of primary and secondary invasive histology, carcinoma *in situ*, adjacent normal, metastases from lymph node, proximal or distal bronchi, at least 1 of which containing adjacent normal, and 1 block of involved lymph node or distant met when available.	3 cores of tumor per histology and 3 cores of adjacent normal per alpha and beta block; 3 cores per histology and 3 cores of adjacent normal for DNA/RNA. Maximum 18 cores per block, 39 cores per case.	Alpha TMA; DNA/RNA; beta TMA	Primary, secondary, *in situ*, adjacent normal, metastases (lymph node or distant), benign lymph nodes, proximal, and/or bronchi
Breast cancer	3 blocks of primary main histology, secondary histology, carcinoma *in situ*, adjacent normal, lymph node or distant metastases, at least 1 of which containing adjacent normal, and 1 block of involved lymph node or distant metastases when available.	3 cores of tumor per histology and 3 cores of adjacent normal per alpha and beta block; 3 cores per histology and 3 cores of adjacent normal for DNA/RNA. Maximum 18 cores per block, 39 cores per case.	Alpha TMA; DNA/RNA; beta TMA	Primary, secondary, normal breast, metastases (lymph node or distant)
Add-on phase				
Bladder cancer	3 blocks per case: 2 primary invasive tumors and *in situ* carcinoma, and 1 of adjacent normal.	2 cores per tumor per alpha and beta block. Up to 24 cores of tumor for DNA/RNA. Maximum 28 cores per case.	DNA/RNA; alpha TMA; beta TMA	NA
Breast DCIS	3 blocks per case: 2 DCIS tumors and 1 containing adjacent normal.	Twelve 5-μm sections per case, for immediate staining of PGR (PR), ESR-1 (ERα), EGFR, KRT5 (CK5/6), MKI67 (Ki67), and ERBB2 (HER2); three 10-μm sections per case.	NA	NA
Colorectal cancer	3 blocks per case: 2 primary invasive and *in situ*, and 1 adjacent normal.	2 cores of tumor and 2 cores of adjacent normal per case per alpha and beta block. Up to 24 cores of tumor and up to 24 cores of adjacent normal per case for DNA/RNA (4 cores/tube).	DNA/RNA; alpha TMA; beta TMA	NA

Abbreviations: NA, not applicable; DCIS, ductal carcinoma *in situ*.

free-standing cores for nucleic acid extraction, and TMAs were constructed according to a slightly different design.

The collection of breast DCIS tissue posed unique challenges, as the tumor size was unusually small and calcifications in these tumors often rendered coring impossible. Thus, no attempt was made to obtain tissue cores; instead, 4-micron slides were cut for immediate immunohistochemical staining of the following common breast cancer protein antigens: PGR (PR), ESR-1 (ERα), EGFR, KRT5 (CK5/6), MKI67 (Ki67), and ERBB2 (HER2). In addition, 3 to 5 tissue sections (10-micron) were made from each of the donor blocks and stored on membrane slides; these membrane slides are readily amenable to laser capture microdissection to isolate tissue areas of interest for molecular analyses.

Results

Pilot study

In the pilot phase (2000–2001), tissues for 250 cancer cases (152 prostate, 29 lung, 43 colorectal, and 26 colorectal adenoma)

Table 3. Overall success rates by cancer site

Cancer site	Phase	Tissue requested (patients)	Tissue obtained (patients)	Overall success rate
Bladder	Add-on	859	204	24%
Breast (F)		1,841	853	46%
Invasive	Main	1,238	692	56%
In situ	Main	293	109	37%
DCIS	Add-on	310	52	17%
Breast (M)	Main	27	17	63%
Colorectal	Main	1,755	658	37%
Adenoma				
Colorectal		1,390	675	49%
	Main	873	500	57%
	Add-on	517	175	34%
Lung	Main	1,017	434	43%
Resected		736	417	57%
Biopsy		281	17	6%
Ovarian	Main	320	212	66%
Prostate	Main	1,541	1,052	68%
Total		8,750	4,105	47%

Abbreviations: F, female; M, male; DCIS, ductal carcinoma *in situ*.

were requested, of which PLCO received donor blocks from 85 (34%) cases for permanent retention and 30 (12%) cases on loan basis. The most frequently cited reasons for not providing specimens were: lack of reimbursement to the pathology department (s) for the effort involved (no compensation was provided to the pathology laboratories during the pilot), hospital policy, and specimens not available. The average cost for donor block retrieval was $6 per block according to the survey.

Success rate for main and add-on phases

The overall success rate, defined by the percentage of the cases with any tissue materials collected (TMA or DNA/RNA cores) out of all cases requested, was about 47% for all cancer sites combined. Table 3 shows the success rate for each cancer site. The success rate was highest for prostate cancer (68%), likely because of the abundant tissue materials available from prostatectomy. Not surprisingly, lung biopsy and breast DCIS had the lowest success rate (6% and 17% respectively), due to the small size of the specimens.

More than 400 pathology laboratories were contacted during the add-on phase. Of these, a majority (77%) required patient authorization for medical record release (in addition to informed consent for sample collection). A small portion (7%) of the pathology laboratories did not require patient authorization. The remaining 17% had a no-loan policy.

Table 4 shows the reasons for failure of obtaining tissue specimens. Tissue not available at the pathology laboratories accounted for 57% of the all failed cases. Hospital no-loan policies accounted for 19%, and failure to obtain patient authorization accounted for 10% of all failed cases. The remaining 14% of failed cases had insufficient amount of tissue to be processed.

For all cancer sites, the success rate for obtaining tissue specimens was significantly lower for subjects diagnosed greater than 10 years before the selection date compared with those diagnosed within 10 years. For prostate, resected lung, colorectal, ovarian, and invasive breast cancers, success rates ranged from 37% to 73% (median, 55%) for specimens requested less than 10 years from diagnosis, as compared with 20% to 51% (median, 40%) for specimens requested $\geq$10 years from diagnosis. For bladder and *in situ* breast cancer, success rates were 30% for less than 10-year specimen requests versus 15% to 24% for $\geq$10-year specimen requests.

Epidemiologic and clinical characteristics of tissues collected

Epidemiologic and clinical characteristics, including age, gender, vital status, cancer stage, and mode of detection (screen detected, interval, postscreening, or never screened cancer for trial-related cancers in the intervention arm), were examined to assess the potential for selection bias among the subset of cases with tissue material obtained as compared with the overall group of cases selected as eligible for specimen collection. Overall, there were few statistically significant differences in any of the above factors between those with material obtained and those who were selected but had no material obtained; the magnitude of any such differences was generally small and may reflect multiple comparisons.

For resected lung cancer, those with material were significantly older than those without material (median age 70 vs. 68, $P = 0.0005$); furthermore, cases with material obtained were less likely to be screen detected (35% vs. 48% among intervention arm cases, $P = 0.006$). For colorectal cancer, cases with material were more likely to be alive (at time of selection) than those without material (81% vs. 74%, $P = 0.001$), less likely to be stage I/II (60% vs. 67%, $P = 0.006$), and less likely to be screen detected (26% vs. 36% among intervention arm cases, $P = 0.008$). For bladder cancer, cases with material were more likely to be alive at the time of selection (86% vs. 74%, $P = 0.0004$) and more likely to be female (24% vs. 16%, $P = 0.008$).

Summary of tissue materials collected from main and add-on phases

Table 5 summarizes the tissue specimens collected by cancer site. In total, 4,048 cases (8,971 donor blocks) were arrayed on 193 TMA blocks (98 alpha + 95 beta). The beta blocks are near-perfect replicates of the alpha blocks, as intended, containing an average of 97% of the cores on the alpha blocks, except for those of bladder cancer, which contained only 35% of cores on the alpha blocks. Except for bladder cancer and colorectal adenoma, the TMAs contained an average of 38% of cores from adjacent normal tissues; these were usually arrayed next to the corresponding tumor tissues. Most of tissue specimens arrayed on the TMAs had one or more corresponding DNA/RNA cores. Breast DCIS tissue specimens were collected as thick tissue sections (10-micron) of the donor blocks and stored in membrane films for laser microdissection.

Tissue specimen storage and dissemination

TMA blocks and Eppendorf tubes containing tissue cores were initially stored at ambient temperature, but later moved to vacuum pouches filled with nitrogen (for humidity control) and stored at 4°C. Unstained TMA slides, once cut, were promptly placed in vacuum pouches with nitrogen at 4°C. Stained slides are stored at ambient temperature. Researchers may request TMA slides or an aliquot of the extracted DNA/RNA via the PLCO Etiologic and Early Markers Studies program by submitting an application (more information available on the website https://biometry.nci.nih.gov/cdas/plco/; ref. 8).

TMA slides will be cut close to the time of use, in batches of 20 to 30 slides. An H&E slide will be cut periodically to reassess the core coverage. DNA/RNA will be extracted centrally, using

Table 4. Reasons for failure of obtaining tissue specimens

Cancer site	Phase	No-loan policy Patients (%)	Patient refusal/ not contactable Patients (%)	No tissue available[a] Patients (%)	Insufficient tissue[b] Patients (%)	Total not collectable Patients
Bladder	Add-on	146 (22%)	109 (17%)	311 (47%)	89 (14%)	655
Breast (F)		200 (20%)	47 (5%)	569 (58%)	172 (17%)	988
Invasive	Main	117 (21%)	24 (4%)	321 (59%)	84 (15%)	546
In situ	Main	34 (18%)	3 (2%)	102 (55%)	45 (24%)	184
DCIS	Add-on	49 (19%)	20 (8%)	146 (57%)	43 (17%)	258
Breast (M)	Main	3 (30%)	1 (10%)	5 (50%)	1 (10%)	10
Colorectal adenoma	Main	102 (9%)	22 (2%)	760 (69%)	213 (19%)	1,097
Colorectal		123 (17%)	178 (25%)	314 (44%)	100 (14%)	715
	Main	32 (9%)	50 (13%)	238 (64%)	53 (14%)	373
	Add-on	91 (27%)	128 (37%)	76 (22%)	47 (14%)	342
Lung	Main	116 (20%)	9 (2%)	390 (67%)	68 (12%)	583
Resected		85 (27%)	9 (3%)	172 (54%)	53 (17%)	319
Biopsy		31 (12%)	0 (0%)	218 (83%)	15 (6%)	264
Ovarian	Main	47 (44%)	3 (3%)	42 (39%)	16 (15%)	108
Prostate	Main	124 (25%)	105 (21%)	251 (51%)	9 (2%)	489
Total		861 (19%)	474 (10%)	2,642 (57%)	671 (14%)	4,648

Abbreviations: F, female; M, male; DCIS, ductal carcinoma *in situ*.

[a]No tissue specimens meeting the requirements were available at the pathology lab.

[b]Tissue specimens were received but found to contain insufficient amount of tissue to be processed.

state-of-the-art methods. To date, only cores obtained from colorectal cancer and adenomas have been extracted. These were extracted using a method optimized for simultaneous extraction of DNA and RNA; the RNA portions are stored at −80°C in lysate form for further purification.

Discussion

We have described here an effort to create a tissue resource suitable for molecular pathologic epidemiology studies. To date, TMAs have been created for prostate, lung, colorectal, ovarian, breast, and bladder cancers, with additional free-standing tissue cores available for molecular extraction. A significant advantage of the PLCO tissue resource is the availability of detailed, high-quality, prospectively collected patient data, including various lifestyle and other risk factors for cancers, as well as prediagnostic blood and buccal cell samples.

The process of creating this resource was complex, involving multidisciplinary teams with expertise in pathology, epidemiology, information technology, project management, as well as specialized laboratory technologies. The protocols and procedures described here may be specific to the PLCO trial, as the effort was largely built upon the existing trial infrastructure. Nonetheless, readers may find certain aspects of the process applicable for their future endeavors. The ability to obtain patient medical records, including the pathology report, is critical to be able to confirm diagnosis, to determine whether suitable donor blocks exist, and to identify and contact the pathology laboratories to request the donor blocks. Obtaining patient consent for research use of their tissue materials and authorization to release medical records can be a time-consuming and labor-intensive process. In PLCO, this was relatively streamlined due to the existing trial infrastructure, prior patient consent, and medical records already collected during trial follow-up periods.

The overall success rate, defined as the percentage of the cases with any tissue materials obtained out of all cases selected for collection, was about 47%. This rate compares favorably with the rate reported for the Cancer Prevention Study II Nutrition Cohort Colorectal Tissue Repository (43%; ref. 12). The CPS II effort also reported a higher success rate (87%) for cases diagnosed within 10 years of the collection date than those diagnosed more than 10 years (32%). We observed a similar trend across tumor sites, albeit to a lesser extent; the combined success rate for all cases diagnosed within 10 years was 54%, compared with 34% for those diagnosed more than 10 years ago. This may reflect differences in pathology laboratory practices and policies in retaining tissue specimens.

Success rates varied widely with tumor type. The success rate for bladder cancer tumors was especially low, due to these tumors frequently being small and vascular with limited tissue. Similarly, the success rate was very low for breast DCIS tumors, due to the small tumor size and widespread calcification.

From the resource management perspective, there are several logistic challenges facing the distribution of the tissue source. Ideally, TMA slides should be cut close to the time of use to preserve antigenicity. On the other hand, each time new slides are cut from a TMA block, the first few cuts are inevitably discarded due to oxidization at the surface of the block. Therefore, it is more resource efficient if multiple requests for the same TMA slides are made at the same time; in reality, however, this can be difficult to achieve. To date, PLCO has adopted a middle ground; each time a request is approved, 20 to 30 slides will be cut from the block, even if the request is for fewer slides. The remaining slides are stored in vacuum pouches filled with nitrogen at 4°C, ready for the next request. Long-term storage and distribution of RNA samples is another challenge, as RNA is extremely unstable in storage. Our current strategy is to store RNA in lysate form at −80°C to be extracted at the time of use.

From the user perspective, this tissue resource can be used for molecular characterizations of the tumors, such as genetic mutations, epigenetic changes, and abnormally expressed gene products. Such research is likely to be critical for early detection and diagnosis of cancer, as well as for etiologic research (13). Key applications of tumor markers in molecular epidemiology include studies to relate tumor alterations to suspected

Table 5. Summary of pathology tissue materials collected

	TMA				DNA/RNA cores			Tissue sections	
Tumor site	Subjects (donor blocks)	TMA blocks per set (alpha/beta)	Total # of TMA cores (alpha/beta)	# TMA tumor cores (alpha/beta)	Subjects (donor blocks)	# of DNA/RNA cores	# of tubes	Subjects (donor blocks)	# of sections
Bladder	201 (209)	5/2	406/144	406/144	159 (231)	931	336		
Breast	801 (2028)	24/24	5,302/5,302	3,210/3,210	769 (1416)	4,562	2,688		
Breast: DCIS only								52 (59)	207
Breast: male	17 (44)	1/1	107/107	86/86	17 (30)	90	54		
Colorectal adenoma	658 (811)	4/4	2,028/1,750	2,028/1,750	656 (815)	2,021	816		
Colorectal	661 (1,746)	20/20	3,424/3,400	1,690/1,678	643 (1,369)	2,548	1,500		
Colorectal: posttreatment	12 (37)	1/1	72/72	45/45	12 (25)	34	25		
Lung	434 (1,175)	8/8	3,687/3,687	1,806/1,806	433 (793)	3,164	1,062		
Ovarian	212 (666)	8/8	1,426/1,426	1,198/1,198	211 (597)	1,314	672		
Prostate	1,052 (2,255)	27/27	5,609/5,609	3,505/3,505	702 (704)	2,085	704		

Abbreviations: TMA, tissue microarray; DCIS, ductal carcinoma *in situ*.

carcinogenic exposures or hereditary factors, to examine whether associations vary by tumor molecular phenotype, to reduce disease misclassification and strengthen causal inference, to identify molecular characteristics associated with tumor progression, and to validate putative early-disease markers. In PLCO, several study designs can be applied including case–case, nested case–control, and case–cohort design as reviewed previously (14).

Molecular pathology is a rapidly evolving area within epidemiology with great promise (15, 16). Within this context, it is important to recognize that PLCO has strengths and weaknesses, as do all settings for molecular pathologic epidemiology. Set within a large screening trial, PLCO has prospectively collected exposure and risk factor data, which reduces differential recall and selection bias relative to cross-sectional case–control studies. Nevertheless, we observed varying success in tissue retrieval by PLCO screening center and cancer type. Therefore, it is important to compare clinical and epidemiologic characteristics of cancer cases with and without tissue specimens. We provide general comparisons for each collected cancer type here, but users should carefully consider possible biases in their particular study of interest. Also, for those cancers, such as prostate and lung, where certain subgroups were oversampled for the tissue collection, it may be important to consider appropriate sampling weights such that resulting data are not biased. It is also important to consider sample size. Pooling or meta-analysis approaches with other studies can overcome such limitations and also provide opportunities for replication.

Other practical issues should be considered when using FFPE samples in epidemiologic studies (15, 17). First, potential variations in tissue fixation protocol and storage time among FFPE samples could influence assay results. The specifics of the protocol, such as type of fixatives, length of the fixation, and time elapsed from surgery to fixation can all have adverse effects on tissue integrity. Unfortunately, these data were not available from the pathology laboratories. Users should note this caveat when interpreting study results. To ensure the productive use of these unique samples, investigators must have conducted prior pilot work to optimize and validate their proposed assays before applying for the PLCO tissue specimens. Finally, investigators should address multiple hypothesis testing using appropriate statistical methods when analyzing multiple tumor subtypes to reduce false-positive findings.

In summary, the PLCO tissue resource offers a large number of well-annotated pathology tissue specimens suitable for the application of state-of-the-art molecular methods for cancer etiology and early-detection research.

Disclosure of Potential Conflicts of Interest

C.D. Berg is a consultant/advisory board member for Medial Early Sign, LLC. No potential conflicts of interest were disclosed by the other authors.

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