Preliminary EDRN Benign Breast Disease Tissue Resource (6/27/2018)

Background and Rationale

The EDRN Breast and Ovarian Collaborative Group has established a tissue resource to discover and test potential biomarkers of progression from benign breast disease to invasive breast carcinoma. The major hypothesis is that biomarkers expressed in benign breast disease tissue of women who progressed to invasive breast cancer are quantitatively and/or qualitatively different from those expressed in tissue of women who do not progress to invasive breast cancer.

Overview of the Resource

The tissue in the resource consists of biopsy specimens obtained from women who received breast biopsies at Northwestern University (NU) or at the Geisinger Health System (GHS).

Specimens contributed by Northwestern University
Subjects were identified through the Enterprise Data Warehouse and Lynn Sage Database at NU. Subjects were designated “cases” if they were treated for invasive breast cancer and underwent a previous benign breast biopsy at least six months prior to the diagnosis of breast cancer. Controls are women who underwent a benign breast biopsy and do not have a breast cancer subsequently recorded in the data base. Slides for 30 cases and for 62 controls are available. Controls were matched 2:1 to cases on time of biopsy (within 1 year), age (within 2 years at the time of biopsy) and race. The tissue blocks of interest were retained in the Pathology Core Facility of the NU Cancer Center. Blocks were stored at room temperature and cut by the Pathology Core Facility over a single two week period of time. 25 slides per pathology block are available, each slide 5 microns thick and three slides (Slide 1, 13 & 25) are stained. Since subjects who received biopsies at NU were not uniformly followed for the outcome of breast cancer occurrence, the specimens do not satisfy the PRoBE criteria for biomarker study design.[1] For example, the specimen set may miss subjects whose breast cancers were diagnosed outside of the NU system and it may preferentially include controls who had other morbidities for which care was sought at NU.

Specimens contributed by the Geisinger Health System
A cohort of women was assembled that satisfied the following inclusion criteria:

1. Diagnosis of benign breast disease between January 1, 1994 and December 31, 2005
2. A recipient of health services at the Geisinger Health System at the time of the diagnosis and treatment of the BBD
3. A minimum of 6 months stay with the health care system post their initial diagnosis of BBD
4. Age 40 - 70 years at the time of BBD diagnosis.
5. No previous history of malignancy of the breast or other organ sites except for squamous or basal cell carcinoma of the skin
6. No history of exposure to medical radiation to chest area.
7. Breast cancer not diagnosed within 6 months of the diagnosis of BBD.

A set of 30 cases who developed invasive breast cancer > 6months after BBD contributed tissue specimens for the BBD pre-validation set. Controls were selected from a list of up to 20 possible matched controls for each case. Matching criteria included the time in months to cancer diagnosis after BBD diagnosis, and BBD tissue availability. Using the cohort database, the DMCC provided an ordered list of controls with pertinent data items. This list consisted of subjects in the cohort satisfying the following criteria as closely as possible:

i. Not already selected as a control for another case
ii. Date of last contact exceeding case's time in months to cancer diagnosis after BBD
iii. Without breast cancer at case's time in months to cancer diagnosis after BBD
iv. Matched with the case on presence of atypia
v. Matched on BBD related procedure: core biopsy; other biopsy; other procedure(eg implant removal, reduction mammoplasty, unknown)
vi. Matched on age: within 5 years
vii. Matched on date of biopsy: within 2.5 years

Starting at the top of the ordered list of potential controls, subjects were evaluated for eligibility until 2 satisfactory controls were identified.

a) Availability of BBD tissue block
b) BBD histology category matched with the case: non-proliferative or proliferative or atypia.
c) For controls diagnosed with BBD with atypia determine if the control is matched with the case on participation in the STAR trial and on same study arm if applicable

Tissue blocks were processed for each case and their 2 matched controls according to EDRN SOPs described in Appendix I. Slides for 30 cases and for 60 controls are available from the GHS site. 25 slides per pathology block are available, each slide 5 microns thick and three slides (Slide 1, 13 & 25) are stained. Since uniform extended follow up is the norm for subjects in the GHS, this set satisfies the PRoBE design criteria. Slides are stored at University of Kansas Medical Center on DryRite at 4^0C in sealed containers.

The EDRN will distribute blinded tissue slides to investigators that successfully apply for use of the resource to discover or test biomarkers. Data will be returned to the EDRN for data analysis. Clinical data elements that are available include: age at biopsy, race, laterality of biopsy, presence of atypia, date of previous mammogram and date of last contact. In addition for the cases, the following data items are available: date and laterality of cancer, stage, grade, hormone receptor status(ER, PR, HER2) and KI67 and P53. Table 1 summarizes characteristics of cases and controls. Additional data pertaining to the cases are listed in Tables 2 and 3.

Table 1. Characteristics of cases that developed invasive cancer and of controls that did not develop cancer in the preliminary benign breast disease tissue specimen set.

	Northwestern University		Geisinger Health System	
	Cases (n=30)	Controls (n=62)	Cases (n=30)	Controls (n=60)
Biopsy Year				
average	2002.6	2003.6	2000.6	2000.8
sd	3.2	3.0	3.7	3.6
range	1997–2007	1998–2009	1994–2005	1994–2005
<2000	6 (20%)	4 (6%)	10 (33%)	19 (32%)
2000–2005	17 (57%)	36 (58%)	20 (67%)	41 (68%)
≥2006	7 (23%)	22 (35%)	0 (0%)	0 (0%)
Age at Biopsy				
average	52.8	53.4	56.9	56.4
sd	8.4	8.2	8.0	8.2
range	40–68	38–68	43–68	41–69
Race				
white	21 (70%)	48 (77%)	30 (100%)	57*(95%)
black	5 (17%)	7 (11%)	—	—
other	4 (13%)	7 (11%)	—	—
Atypia				
no	24 (80%)	59 (95%)	27 (90%)	59 (98%)
yes	6 (20%)	3 (5%)	3 (10%)	1 (2%)
Side of BBD				
right	16 (53%)	35 (56%)	10(33%)	25 (42%)
left	10 (33%)	24 (39%)	17 (57%)	30 (50%)
both	4 (13%)	3 (5%)	3 (10%)	1 (2%)
Previous Mammogram				
no	15 (50%)	27 (44%)	7 (33%)	17 (28%)
yes	15 (50%)	35 (56%)	23 (77%)	43 (72%)
average years before biopsy (sd)	1.1 (0.6)	1.6 (1.7)	0.3 (.37)	0.2 (.25)

*3 unknown

Table 2. Characteristics specific to the 30 cases who developed invasive cancer after benign breast disease.

Years from biopsy to cancer	Northwestern University	Geisinger Health System
average	4.6	4.8
sd	2.4	2.0
range	1.3–10.1	1.1–7.7
ER receptor		
positive >10%	30 (100%)	26 (87%)
unknown		4(13%)
PR receptor		
positive		25(83%)
positive >10%	25 (83%)	
positive 1–10%	1 (3%)	
negative	4 (13%)	2(7%)
unknown		3(10%)
Her2 receptor		
positive		3(10%)
positive >10%	1 (3%)	
positive 1–10%	2 (6%)	
negative/null	27 (90%)	19(63%)
equivocal		1(3%)
unknown		7(23%)
kI67		
null	10 (33%)	—
not done	20 (67%)	—
p53		
negative	24 (80%)	—
pos >10%	3 (10%)	—
not done	3 (10%)	—
Laterality of cancer		
same as BBD	10 (33%)	11 (37%)
opposite of BBD	15 (51%)	14 (47%)
both sides involved for BBD or cancer	5 (16%)	5 (17%)

Table 3. Additional characteristics specific to the cases who developed invasive cancer after benign breast disease.

Size of Tumor	Nodes Removed	Nodes Positive	Grade[1]	Histology
10 cm	23	22	IIIC	inv lobular
0.5 cm	3	0	IA	inv ductal|
9.4 cm	5	1	IIIA	inv lobular
8 mm	13	3	IIB	inv ductal|
2.5 cm	5	0	IIB	inv ductal
2.0 cm	1	0	II	mixed/mamm
1.0 cm	4	0	IA	inv ductal
1.1 cm	22	1	IIA	inv lobular
1.1 cm	3	0	I	inv lobular
10 mm	2	0	IA	tubular
1.0 cm	2	0	0	inv ductal
2.1 cm	18	1	IIB	inv ductal
9 mm	3	0	IA	inv ductal
1.8 cm	15	1	IB	inv ductal
4 cm	16	1	II	inv ductal
1.2 cm	2	0	IA	inv ductal
0.8 cm	1	0	IA	inv ductal
4.9 cm	10	1	IIB	mixed/mamm
0.7 cm	0	0	0	inv ductal
2.5 cm	1	1	IIB	mixed/mamm
1.5 cm	1	1	0	mixed/mamm
0.6 cm	1	0	IA	inv ductal
1.2 cm	17	0	IA	mixed/mamm
1.5 cm	22	8	IIIA	inv ductal
3.1 cm	17	3	IIB	inv ductal
0.6 cm	2	0	I	inv ductal
1.2 cm	1	1	0	tubular
0.8 cm	2	0	IA	inv ductal
4 cm	16	2	IV	inv ductal
0.5 cm	0	0	IA	inv ductal
0.2 cm	0	0	G1	lobular
0.3 cm	3	0	G1	ductal
0.5 cm	1	0	G2	ductal
0.5 cm	19	0	G2	ductal
0.5 cm	3	0	G2	ductal
0.6 cm	0	0	G1	ductal
0.8 cm	0	0	G2	ductal
1.0 cm	1	0	unknown	ductal
1.0 cm	1	1	G2	ductal
1.0 cm	11	1	G1	ductal
1.0 cm	1	0	G2	lobular
1.2 cm	2	0	G1	ductal
1.2 cm	2	0	G3	ductal

1.2 cm	2	1	unknown	ductal
1.2 cm	1	0	G1	ductal and tubular
1.4 cm	1	0	G2	ductal
1.5 cm	3	1	G2	ductal
1.5 cm	0	0	G3	malignant phyllodes tumor
1.5 cm	29	0	G3	ductal and lobular
1.5 cm	14	7	G3	ductal
1.6 cm	0	0	G2	ductal and lobular
1.7 cm	unknown	unknown	G1	ductal
2.1 cm	9	4	G2	ductal
2.5 cm	3	0	G1	cribriform
2.5 cm	unknown	12	G3	comedo
3.0 cm	14	2	G1	lobular
3.2 cm	1	0	G1	cribriform
5.0 cm	26	1	G1	tubular
5.0 cm	3	0	G2	lobular
unknown	unknown	unknown	G1	comedo

[1]G1 low grade (well differentiated); G2 intermediate grade (moderately differentiated); G3 high grade (poorly differentiated)

Application and Review Process

The application form can be found on the EDRN website and it is also included as an appendix to this document. Investigators are encouraged to contact Christos Patriotis (patriotisc@mail.nih.gov) of the Breast Gynecologic Collaborative Group within EDRN prior to submitting an application. Proposals will be reviewed as they are received by a standing committee within the collaborative group. This committee will report its recommendations for each application back to the collaborative group which in turn reports to the EDRN Steering and Executive Committees. Feedback to the applicant will be provided and revised applications may be submitted.

References

1. **Pepe MS**, Feng Z, Janes H, Bossuyt P and Potter J. Pivotal evaluation of the accuracy of a biomarker used for classification or prediction: Standards for study design *Journal of the National Cancer Institute* 100(20):1432-1438; 2008 PCMID: PMC2567415

2. APPENDIX I

Standard Operating Procedure (SOP) For Processing of FFPE Blocks from GHS to Support the Benign Breast Disease (BBD) Study

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PURPOSE
To provide a standard operating procedure for tissue sectioning and mounting of samples for biomarkers studies of BBD.

SCOPE
This procedure applies to sectioning, mounting, and storing of archival FFPE tissue sample section to support the EDRN biomarker studies.

ROLES AND RESPONSIBILITIES
The SOP applies to all personnel from the EDRN breast working group who are responsible for sectioning tissue preserved in paraffin blocks.

MATERIALS, EQUIPMENT AND FORMS
The materials, equipment and forms listed below are recommendations only and may be substituted by alternative/equivalent products more suitable for the site-specific task or procedure.

Materials and Equipment Materials and Equipment (Site Specific)

- Solvent resistant markers, ink, pencils, and pens
- Microscope
- Microtome
- Hot water bath
- Microtome blades
- Fine tipped paint brush
- Fine tipped tissue separator
- Appropriate labels for slides (provided by DMCC*: one label for FFPE block and 25 serial labels for slides along with corresponding worksheets)
- Labeled glass slides
- Tray to hold slides
- Ice tray
- Oven
- Labeled electrostatically charged slides (such as SurgiPath Plus slides)
- Film for sealing slide boxes such as Parafilm
- Slide storage boxes (labeled with DMCC Box#)
- Harris Haematoxylin (filtered)
- Eosin

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Health/Safety Warning
When working with potentially hazardous materials, follow EPA, OSHA, and other specific health and safety procedures. Be prepared in case of emergency (e.g., telephone numbers, first aid kit). Personnel should wear an apron or lab coat, gloves and safety glasses when handling hazardous chemicals.

PROCEDURES

This procedure is intended to ensure that tissue samples preserved for research studies are sectioned in a safe and consistent manner while eliminating the risks of contamination and loss of molecular and structural integrity. It also ensures rationing of the tissue blocks associated for each case for multiple assays and projects and maintenance of the block orientation. Consistency in procedure is important for obtaining comparable and reliable test results.

These procedures also outline minimum steps that should be followed to ensure that tissue samples collected, stored and distributed are of sufficient morphological and molecular caliber to meet the research needs of the investigators.

Selection of blocks

- For each case, identify the "most significant" category of BBD (atypical lesions > proliferative lesions without atypia > nonproliferative lesions). Try to sample all lesions in that category (e.g., adenosis, apocrine change and simple fibroadenoma). Ideally the different lesions should be captured in one paraffin block, but multiple blocks per case may be selected to capture all relevant lesions.
- For each block one BBD Pathology Form should be filled out.

Sectioning Formalin Fixed Paraffin Embedded Tissue

- Treat all tissue as potentially infectious.
- Since some sections will be used for nucleic acid studies, whenever possible all instruments and equipment should be pre-cleaned and wiped down with RNAse-away before and between each specimen. Gloves must be worn. Molecular grade water is recommend to be used for floating sections for RNA extraction.
- Sectioning is performed by the laboratory or histology technician/technologist or personnel trained to use a microtome and cut histological sections.
- Have materials and equipment ready. Have as many slides as needed labeled and ready.
- Pre-cool paraffin blocks, tissue side down, on a tray of ice. In some cases this may facilitate sectioning. Using a steel microtome knife or disposable blade cut 25 sections/block at 5 microns/section for histological and nucleic acid extraction purposes.
- If there is insufficient tissue for complete sectioning, only cut as many sections as possible without depleting the tissue. Note, tissue sections without the actual lesion may still be important to study "field effects" of the biomarkers, so whenever possible prepare 25 slides.
- Label slides serially from 1 (top) to 25 (bottom) using the labels provided by the DMCC, indicate on worksheet which slides were created and whether or not the slide is designated H&E.
- Dry paraffin sections at room temperature overnight.
- Sections 1, 13 and 25 are to be stained with H&E using standard procedures.
- Since benign breast lesions may be very small, it is possible that they may not be present on deeper levels. In that case their absence should be noted by the reviewing pathologist.
- The unstained sections are stored at 4˚ C prior to shipping to the centralized EDRN pathology core (University of Kansas Medical Center*) in slide holder boxes.
- The FFPE block needs to be associated to the Participant ID in VSIMS (Validation Study Information Management System - a web-based data entry and specimen tracking system provided by the EDRN:DMCC). The individual slides need to be entered in VSIMS and then the slide box needs to be shipped to KUMC (to address provided below). Some sites may choose to store the slide sections locally until requested to provide for biomarker analysis. Prior to any samples being shipped for analysis either the collection site or the KUMC Central Pathology Lab will need to re-label the slides with a unique ID provided by the DMCC and associate that ID to the serial ID in VSIMS prior to shipment.
    - Example: Site ID XXX has entered Clinical Information for Participant ID XXX in VSIMS. Collection site will associate Participant ID XXX to FFPE ID.

- BBD FFPE IDs will start with Protocol ID 351 followed by 5 digit number. Each Slide ID will include the Protocol ID 351 followed by the same 5 digit number as the FFPE ID and appended with 1, 2, 3, 4, etc… serially.

- Note to biomarker validation sites. Prior to any immunohistochemical staining, the slides should be heated at 60 degrees C for 40 min using either an oven or warming tray.

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Quality Assessment – General Considerations for Section Review

- At a minimum, assessment must consist of morphologic review of tissue sections.
- Use researcher feedback about section quality to refine practices and guide evolution of Quality Control procedures.

Quality Assessment – Issues Concerning Quality of Sections

- Make sure that representative tissue remains in the block after sections are cut for an assay. Do not deplete paraffin blocks.
- Since some sections are intended for Polymerase Chain Reaction (PCR)-based molecular studies make sure that all attempts are made to eliminate or minimize nucleic acid contamination from equipment or other samples.
- Ensure that section thickness is consistent and appropriate for intended use.
- Ensure that sections are not scored or torn by the microtome knife as this will obscure microscopic observation and may cause uneven staining or bias assay results.
- Ensure that thin sections are placed on electrostatically charged slides to avoid loss of the section during the immunohistochemical assay.
- Ensure that paraffin sections are stored and shipped under appropriate conditions and temperatures.

Quality Assessment – General Sectioning Regimen for QA Safeguards

The use of this schema is recommended to ensure that representative sections from a sectioned block are kept for quality assessment purposes. Perform these steps at the time the block is being sectioned for a research application.

- Ensure that a representative Hematoxylin and Eosin (H&E) section is retained from the block within the biobank.
- If no H&E is available from the last sectioning of the block retain a “top” section for H&E review.
- If many sections are taken from a block, it may be useful to retain “intermediate” sections from the tissue block for H&E review.
- Label sections serially. Also record the date the section is cut on the worksheet.

APPLICABLE REFERENCES, REGULATIONS AND GUIDELINES

1. Declaration of Helsinki
http://www.wma.net/en/30publications/10policies/b3/index.html
2. Tri-Council Policy Statement 2; Ethical Conduct for Research Involving Humans; Medical Research

Council of Canada; Natural Sciences and Engineering Council of Canada; Social Sciences and Humanities Research Council of Canada, December 2010.
http://www.pre.ethics.gc.ca/eng/policy-politique/initiatives/tcps2-eptc2/Default/
3. Human Tissue and Biological Samples for use in Research. Operational and Ethical Guidelines. Medical Research Council Ethics
http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC002420
4. Best Practices for Repositories I. Collection, Storage and Retrieval of Human Biological Materials for Research. International Society for Biological and Environmental Repositories (ISBER).
http://www.isber.org/Search/search.asp?zoom_query=best+practices+for+repositories
5. US National Biospecimen Network Blueprint
http://biospecimens.cancer.gov/resources/publications/reports/nbn.asp
6. Jewell, S. et al. 2002, Analysis of the Molecular Quality of Human Tissues, an experience from the Cooperative Human Tissue Network. Am. J. Clin. Pathol. 118:733-741.
7. Guideline – Fresh Tissue Working Group of BIG and NCI breast cancer Cooperative Groups
8. SOP No.3 (Draft 1). November 15, 2005. Standard Tissue Sectioning. NCIC CTG. Ontario.
9. Snell L. and P. H. Watson. 2006, Breast Tissue Banking: Collection, Handling, Storage and Release of Tissue for Breast Cancer Research. Methods Mol Med. 120:3-24.
10. Recommendations of FFPE Working Group of BIG and North American breast Cancer Groups.