EDRN Breast Cancer Reference Set (9/26/2011)

Overview of the Resource

The EDRN has created a prospectively collected set of plasma and serum samples designed to test circulating biomarkers that may be useful for the detection and diagnosis of breast cancer. These samples are now available to any investigator who submits an approved proposal for analysis. Sample identity with respect to case/control status will remain blinded to the investigator and data analysis will be conducted by the Data Management and Coordinating Center (DMCC) of the EDRN.

The reference set is comprised of blood samples that were collected from subjects undergoing either diagnosis for breast cancer (i.e., at the time of a physician referred breast biopsy) or at the time of routine screening mammography. These subjects were consented and samples collected at four U.S. medical centers from 2008-2010: Dana Farber Cancer Institute, Duke University Medical Center, Fox Chase Cancer Center, and University of California, San Francisco. The four sites used the same standardized protocols for sample and data collection with variations noted below. The reference set contains samples from women with incident invasive cancer (n=207), carcinoma in situ (n=55), benign pathology with atypia (n=63), benign disease with no atypia (n=231), and women with no evidence of breast disease by screening mammography (BI-RADS 1 or 2, n=276). The specimens reside in liquid nitrogen at NCI-Frederick in 200 µl aliquots (the number of aliquots derived from an initial 4mls of serum and 4mls of plasma from most subjects). The protocol and forms used to abstract clinical elements are posted on the EDRN website. Variations in how the protocol was implemented are described below in Table 2 along with descriptive information for the included subjects.

Scenario for Use of Specimens

The preferred use of these samples is for validation studies on markers that have already been shown to have some ability to discriminate breast cancer. However, strong proposals for earlier phase research will also be considered. Typically, samples will be made available in 3 sets: (i) Set 1 will constitute a preliminary test of the marker in this cohort based on 30 subjects with invasive cancer and 30 subjects with benign disease without atypia collected at the time of breast biopsy. This preliminary set is powered to determine if a biomarker is associated with disease (80% power for an odds ratio of 5 for a biomarker with positive rate of 20% or more). If Set 1 indicates that the proposed biomarker(s) is associated with disease, a larger and more diverse set of samples (Set 2) will be delivered for biomarker evaluation; (ii) Set 2 is also comprised of samples collected at the time of referred breast biopsy. These samples are from 125 subjects with invasive cancer, 129 benign without atypia, 52 benign with atypia and 40 carcinomas in situ. In addition, samples collected at screening mammography from 125 subjects with normal screening mammograms are included in Set 2. If the biomarker(s) performs well in Set 2 the remaining samples collected at the time of screening mammography (Set 3) will be delivered; (iii) Set 3 is comprised of samples collected at screening mammography from 35 subjects with invasive cancer, 15 with carcinoma in situ, 83 with benign disease and 151 with normal screening mammograms. Samples from 17 subjects who developed invasive breast cancer subsequent to a normal screening mammogram (n=15) or a benign breast disease diagnosis (n=2) are also included in Set 3.

Application and Review Process

The application form can be found on the EDRN website. Investigators are encouraged to contact the Breast-Gynecologic Collaborative Group within the 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 applicants will be provided and revised applications may be submitted.

Descriptive Summary Data for the Breast Cancer Reference Set

Table 1: Total numbers of subjects contributing serum and plasma samples to the reference set. Normal controls=BIRADS score ≤ 2; BD=benign disease; DCIS=ductal carcinoma in situ; LCIS= lobular carcinoma in situ; Normal-late Ca=normal screening mammogram at blood draw but later found to have breast cancer; BD-late Ca= benign disease at blood draw but later found to have breast cancer.

Diagnosis	Duke	UCSF	FCCC	DFCI	Total
Normal Control	35	25	176	40	276
BD atypia	46	4	11	2	63
BD non-atypia	131	19	72	9	231
Case: DCIS	26	10	11	1	48
Case: LCIS	3	0	4	0	7
Case: Invasive	131	15	35	9	190
Normal-late Ca	0	0	15	0	15
BD-late Ca	0	0	2	0	2
Total	372	73	326	61	832

Study Protocol

The study protocol is available on the EDRN website. However, variations in how the protocol was implemented occurred across study sites (Table 2).

Table 2: Variations in the study protocol by study site

	Duke	UCSF	FCCC	DFCI
Main collection in the diagnostic radiology clinic	√	√		√
Main collection in mammography screening clinic			√	
Blood drawn prior to biopsy		√	√	
Blood drawn immediately post biopsy procedure	√			√
Normal controls enrolled at screening mammography	√	√	√	√
Possible previous inconclusive biopsy for current presentation of suspicious lesion		√		

Eligibility Criteria

Specimens included in the reference set were from women who satisfied the following eligibility criteria.

- Age>=18 years
- Not pregnant or breast feeding
- No history of invasive breast or other cancers (except basal or squamous cell carcinoma of the skin)
- Undergoing diagnosis to determine if breast cancer is present or undergoing routine mammographic screening (FCCC)
- Blood drawn 0-14 days prior to biopsy procedure

Sample Collection and Preparation

1) Blood collection was performed before or on the day of biopsy
2) A minimum of 28 mls of whole blood was collected.
3) Four x 7 ml tubes were obtained, 2 red top tubes for serum and 2 EDTA (lavender top) tubes for plasma.
4) Blood was spun within 5 hours of collection. Blood tubes were spun at 3000 x g for 10 min at 4 °C and the serum or plasma removed by pipetting. Time to processing was noted.
5) For the specimens going to NCI Frederick, serum was stored in four 1 ml aliquots and the plasma in four 1 ml aliquots. All samples were stored at -80 °C.

Characteristics of Study Subjects

Table 3 Some characteristics of study subjects by major diagnostic categories.

	Invasive Cancer	**Benign Disease Without Atypia**	**Benign Disease With Atypia**	**DCIS**	**Normal**
Study Site					
Duke	131	131	46	26	35
UCSF	15	19	4	10	25
FCCC	35	72	11	11	176
DFCI	9	9	2	1	40
Age (years)					
mean(sd)	58.5(12.2)	53.1 (12.3)	52.3(10.3)	57.5(9.2)	59.9(11.8)
Menopausal Status(%)					
pre-menopausal	31%	42%	44%	25%	25%
post-meopausal	68%	58%	56%	75%	74%
Sample Timing(%)					
day of biopsy	89%	80%	87%	79%	--
1-7 days prior	6%	7%	6%	8%	--
8-14 days prior	4%	7%	5%	4%	--
>14 days	2%	5%	2%	8%	--
Ever on HRT (%)					
no	62%	69%	68%	63%	62%
yes	37%	31%	32%	35%	38%
Family History of Breast Cancer					
yes (% of known)	41(24%)	49(24%)	13(22%)	12(32%)	81(32%)
no	132	152	46	26	169
unknown	17	30	4	10	26
Race (%)					
White	76%	78%	75%	75%	90%
White-Latino	3%	3%	0%	0%	0%
Black/African Am	16%	15%	16%	17%	6%
Other/mixed	5%	4%	10%	8%	4%
Body Mass Index (kg/m^2)					
mean (sd)	28.8(7.7)	28.4(7.2)	27.1(7.4)	28.6(8.7)	27.1(5.9)

Table 4: Some characteristics of subjects with invasive cancer whose samples were collected at biopsy (Sets 1 and 2 combined) and whose samples were collected at screening mammography (Set 3).

	Samples drawn at time of biopsy	Samples drawn at screening mammography
Receptor Status		
ER positive yes (% of known) no unknown	 115 (76%) 37 3	 29(83%) 6 0
PR positive yes (% of known) no unknown/indeterm	 96 (64%) 51 8	 23(66%) 12 0
Her2/neu positive yes (% of known) no equivocal unknown	 20 (13%) 120 10 5	 8 (25%) 20 4 3
Tumor Stage		
Stage Grouping I IIA IIB IIIA IIIC Unknown	 48 (51%) 36 (38%) 11 (12%) 0 0 60	 15 (45%) 9 (27%) 6 (18%) 1 2 2

Supplementary Tables and stuff

Table 2: Total numbers of eligible subjects enrolled in the prospective study with serum and plasma samples collected. These are the denominators for Table 1. All samples were included in the reference set from most diagnostic categories with two exceptions. Random subsets of 'normal controls' and 'benign disease non-atypia controls' were selected to match the invasive cancer subjects according to study site, age and race/ethnicity.

Diagnosis	Duke	UCSF	FCCC	DFCI	Total
Normal Control	35	28	935	64	1062
BD atypia	46	4	11	2	63
BD non-atypia	235	29	72	61	397
Case: DCIS	26	10	11	1	48
Case: LCIS	3	0	4	0	7
Case: Invasive	131	15	35	9	190
Normal-late Ca	0	0	15	0	15
BD-late Ca	0	0	2	0	2
Total	476	86	1085	137	1784