Cooperative Human Tissue Network (CHTN)
Breast Carcinoma Progression Tissue Microarray: Version CHTN BrCaProg1

Purpose:
To provide researchers with a tissue microarray of formalin fixed paraffin embedded samples of human breast epithelium representing the stages of tumor progression in breast adenocarcinoma. This TMA represents a limited number of cases that may detect strong trends in differential gene expression, and is intended for pilot surveys and generation of hypotheses. This TMA does not contain case numbers of sufficient quantity to prove the clinical utility of a marker.

Note: **Pre-invasive breast neoplasia (ductal carcinoma in situ) has proven difficult to reproducibly capture in tissue microarray format.** Thus a specific number of DCIS cases is not guaranteed to be present in the TMA sections.

Tissue samples:

The following tissue types are represented in the original TMA design:

- 7 cases of non-neoplastic breast epithelium from subjects with no history of breast cancer (reduction mammoplasties).
- 7 cases of non-neoplastic breast epithelium from subjects with breast cancer.
- 7 cases of DCIS, low grade.
- 7 cases of DCIS, high grade.
- 7 cases of low to moderate grade invasive ductal adenocarcinoma (Grades 1 & 2, modified Scarff-Bloom-Richardson criteria).
- 7 cases of high grade invasive ductal adenocarcinoma (Grade 3, modified Scarff-Bloom-Richardson criteria).
- 7 cases of invasive lobular carcinoma.
- 7 cases of breast carcinoma metastatic to regional lymph nodes.

TMA design:

Each target tissue sample was sampled one time with a 2 mm needle core in the original array design. There are three replicate CHTN BrCaProg1 TMA blocks slated for dispersal, designated B, C & D. Each array block is serially sectioned at 4 micron thickness. The histologic sections are placed on charged glass slides (Superfrost Plus, Fisher Scientific). At intervals, sections are stained and examined by a pathologist for quality assurance (QA) purposes.

Quality assurance procedures:

Due to the variability inherent to tissue samples and histologic methodology, *most of the tissue microarray sections will not contain all of the tissue spots in the original design.* The number of tissue spots in which the desired tissue types resides will vary from section to section.

The following guidelines are used during histologic quality assessment:
A tissue spot is defined as being present if tissue occupied at least 25% of the possible surface area OR if at least 10 cells of target tissue are present.
Target tissue is defined as being present if at least 10 cells were present in a tissue spot.

TMA slides are being released up to the histologic level in which the number of cases with target tissue present does not drop below the cutoff limit for each of the following 3 categories:

Tissue type	Tissue Codes	# original cases	QA cutoff
Non-neoplastic breast	NB-NC, NB-C	14	7
Primary carcinomas	IDC-L, IDC-H, ILC	21	10
Metastatic carcinoma	LNM	7	4

Thus at a minimum, there will be 21 tissue samples on the TMA section, representing benign and malignant breast epithelium. As noted above, DCIS has proven to be too difficult to effectively capture in TMA format. Researchers requiring DCIS samples to study are recommended to get whole histologic sections from the Cooperative Breast Cancer Tissue Resource (http://www-cbctr.ims.nci.nih.gov/) or from the Cooperative Human Tissue Network (http://www.chtn.ims.nci.nih.gov/index.html). If despite our efforts you are missing more than the minimum number of target tissue samples on your array sections, please contact us at (434) 924-9879 or UVA-CHTN@virginia.edu. Copies of the QA scoring sheets are also available upon request.

THE TISSUE SAMPLES HAVE BEEN ANONYMIZED, NO FURTHER DATA ON THE DONORS IS AVAILABLE OTHER THAN THAT FOUND IN THE ACCOMPANYING GUIDE SHEETS.

Guide sheets that include representative histologic figures can be downloaded in Microsoft Excel format from the CHTN TMA website: http://faculty.virginia.edu/chtn-tma.

Frequently asked questions:

Why aren't all there as many tissue spots on my section of the array as are listed on the TMA key?

> The key represents the original TMA design. Tissue cores are of various lengths, hence at deeper sections, some cores have been exhausted while others remain. In addition, some tissue spots may be lost during the process of transferring the TMA section to the glass slide.

Why isn't the target tissue type present in the tissue spot?

> Although TMA manufacture is guided by a histologic section that represents the surface of the donor tissue, this target tissue may not be uniformly represented in the deeper sections of the tissue. This problem is greatest with small structures (e.g. breast ducts and lobules).

Why doesn't the representative microscopic image of the target tissue exactly match the tissue spot on my TMA?

> The representative images have been taken from a single spot from a single QA section from a single array. Four different replicate TMA blocks were made for this series, each of which

has different tissue cores. Even the same tissue core at a deeper section would not exactly match a more superficial section due to the variability inherent in tissue architecture.

Can I use antigen retrieval methods (boiling, microwave, pressure cooker, etc) on these sections?

Yes.

Can I perform in situ hybridization on these sections?

Yes.

Definitions and abbreviations:

Donor block: a tissue paraffin block (see below) that contains tissue of the desired type to be placed into the tissue microarray.

DCIS: Ductal carcinoma in situ

Histologic section: a flat sheet of paraffin and embedded tissue cut from a paraffin block on a microtome. The thickness of the section can vary, but a typical thickness is 4 microns (micrometers).

QA: quality assurance

Recipient block: The blank paraffin block into which tissue cores are inserted to form the tissue microarray.

Tissue core: the cylindrical tissue sample removed from the donor block, which is placed in the recipient block.

TMA: tissue microarray. A recipient paraffin block into which tissue cores have been inserted in a gridded array.

Tissue paraffin block: a sample of tissue that has been fixed in formalin, processed to remove water, then infused with molten paraffin, which is allowed to harden within and around the tissue in a square mold. This is the standard method of preparing tissue for clinical histologic analysis. The paraffin block is subsequently cut on a microtome to produce thin histologic sections which are placed on glass slides. In the manufacture of TMAs, these become the donor blocks.

Tissue spot: the tissue sample present on a histologic section of a tissue microarray that corresponds to a tissue core.