MINT I
Multi-Institutional Neo-adjuvant Therapy
MammaPrint Project I

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1. INTRODUCTION AND RATIONALE FOR THE STUDY

Treatment of locally advanced breast cancer (LABC) with neoadjuvant chemotherapy measures the in vivo response to chemotherapy, assesses long-term clinical outcomes as associated with that response and affords the opportunity for some patients to undergo breast conservation therapy (BCT) as a result of therapeutic down staging of the tumor. The focus of surgical therapy is to accomplish the goals of accurate staging and local control of breast cancer.

Patients with LABC will traditionally receive surgical treatment following neoadjuvant therapy. This treatment usually consists of a modified radical mastectomy combined with radiation therapy while patients who are down staged by the treatment may be treated with breast conserving surgery. Recent studies have demonstrated that patients with LABC and positive axillae can also be treated with neoadjuvant chemotherapy prior to definitive surgery and can achieve a complete pathologic tumor and axillary response. Sentinel node staging before treatment can optimize post treatment prognostic stratification in clinically node negative patients.

Standardization of Neoadjuvant Chemotherapeutic Regimens

Neoadjuvant chemotherapy has become one of the standards of care for locally advanced breast cancer, as well as for patients who desire breast-conserving therapy but are not candidates based on the initial size of the tumor in relation to the breast. Treatment regimens that are considered “acceptable” include FAC, CAF, CEF and FEC according to Shenkier et al. when reviewing literature retrieved from MEDLINE for the British Columbia cancer agency in Vancouver. In the United States, these more traditional regimens have been adapted to be more consistent with standard adjuvant approaches and have included neoadjuvant anthracycline and taxane-based combinations for HER2-negative tumors and a combination of doxorubicin/cyclophosphamide and single agent taxane with Trastuzumab for HER2-positive tumors. In an effort to avoid anthracycline use, recent work using a combination of paclitaxel and carboplatinum has also demonstrated this combination to be “effective and well tolerated”. And additional such efforts are underway in the XeNA-trial, in which non-anthracycline preoperative regimens are a particularly interesting proposition in HER2-positive breast cancer, as they offer less cardiotoxicity and thus can be used concomitantly with preoperative trastuzumab therapy. Thus, it appears that several different combinations of chemotherapeutic agents are effective in the neoadjuvant setting.
Microarray Genomic Testing in the Treatment of Breast Cancer

In the broad overview of breast cancer treatment the prediction of which patients would be ideal candidates for adjuvant chemotherapy has been brought with controversy and difficulty in finding which patients would benefit most from chemotherapeutic strategies in the adjuvant setting. The development of the microarray technology has allowed genomic data to be used to classify breast tumors along with standard clinical prognostic factors to help define which patients would benefit least or most from adjuvant chemotherapeutic treatment. Gene signatures have been developed and validated against large retrospective databases and adopted by medical oncologists as a means to risk stratify patients to selectively recommend adjuvant treatments for breast cancer therapy. One approach using the intrinsic subtyping and p53 mutations as possible predictive test for response to therapy was used in the XeNA study.

MammaPrint is a 70 gene classifier and has been validated against multiple retrospective studies of patients with banked fresh frozen tissues. Development of a methodology to rapidly collect tissue and inhibit RNAsase activity has made the test widely available commercially. In 2007, MammaPrint became the first In Vitro Diagnostic Multivariate Index Assay (IVDMIA) to acquire clearance from the US Food and Drug Administration (FDA). The test classifies each breast cancer patient in one of two categories: "low risk" or "high risk" to develop metastases within the first 10 years after surgery.

The tumor sample collected for the MammaPrint test can also be used to determine additional gene profiles. These include TargetPrint, which determines the mRNA expression levels of Estrogen Receptor (ER), Progesterone Receptor (PR) and HER2. It offers the opportunity for objective and more quantitative measurements, as differences in immunohistochemistry (IHC) methods and interpretation can substantially affect the accuracy and reproducibility of results. Using TargetPrint in addition to standard IHC may improve molecular characterization of breast cancer tissue.

Another assay, BluePrint, is a molecular subtyping profile which determines the mRNA levels of 80 genes that best discriminate between three distinct subtypes; Basal-type, Luminal-type, and HER-2 type and may help medical oncologists in making treatment recommendations in the future. The Molecular Subtyping Profile BluePrint can classify MammaPrint High Risk breast tumors into biologically different molecular subgroups. We and others have shown that breast tumors of distinct molecular subtype have different benefit from (neo-)adjuvant chemotherapy, ranging from minimal response in Luminal A- to substantial response for HER2 type and Basal-type tumors. Further sub stratification of MammaPrint high risk
patients may therefore be useful for developing sub-type specific treatment regimens and potentially useful for future treatment decisions.

In addition to the commercially available tests as listed above, Agendia developed the TheratPrint Research Gene Panel (see Appendix I). The panel provides the mRNA expression of 56 genes (125 in 2012) that might have relevance in breast cancer therapy and prognosis, and could be used to study potential biomarkers and choices of effective cytotoxic agents in breast cancer.

**MINDACT**

The MINDACT trial (Microarray in Node-negative and 1-3 positive lymph node Disease may Avoid ChemoTherapy) will measure the clinical utility of MammaPrint in comparison with Adjuvant!Online.\textsuperscript{31} This prospective, randomized phase III clinical trial will compare risk assessment using MammaPrint with risk assessment using common clinico-pathologic criteria (Adjuvant!Online) in selecting patients for adjuvant chemotherapy in early stage breast cancer. The goal of including 6,000 women will be reached in 2011. If both MammaPrint and the clinical assessment are “High Risk” (n=3300), patients will be randomized to one of two chemotherapy regimens (docetaxal-capecitabine versus an anthracyclin regimen; anthracyclines followed by docetaxel for node positive disease). If both are “Low Risk” (n=780), then no chemotherapy will be administered. If the two forms of risk assessment are discordant (n=1920), then patients will be randomized to therapy based either on the clinical assessment or MammaPrint. Hormone receptor positive disease will be randomized to one of two hormonal regimens. The analysis of genome-wide expression data on 6,000 patients treated prospectively with several treatment regimens will likely yield clinically useful chemo-responsive profiles, potentially enabling cross-validation of such profiles in the current study.

**Development of new profiles for Neo-Adjuvant chemosensitivity**

One of the aims of the current study is to identify and/or cross validate a unique set of classifier genes that will accurately predict a complete Pathologic Response (pCR) to standard chemotherapeutic regimens in the neoadjuvant setting. Studies to date have demonstrated a 25-27% complete pathologic response in both breast and axilla which affords the patients a survival advantage of 80% in 5 years, which is double the expected survival of the remaining patients without complete pathologic response. Given that the subset of patients with complete pathologic response could be identified by a genomic signature then the remaining patients would best be suited to innovative new strategies for drug discovery. While in fact, the patients
with the genomic signature for chemotherapeutic response would be served well by current neoadjuvant chemotherapy protocols.

**Neo-Adjuvant Chemotherapeutic Trials With MammaPrint® and BluePrint®**

Two neoadjuvant studies on MammaPrint analysis have been performed. The first evaluated 167 patients from the Netherlands Cancer Institute NKI, and another studied 68 US patients from The City of Hope National Medical Center. MammaPrint was found to be a powerful predictor of chemotherapy response in patients treated with neoadjuvant chemotherapy using contemporary anthracycline-based regimens. Only patients with “high risk” profile achieved a pathologic complete response (pCR) and no pCR occurred in patients who were classified as “low risk” by MammaPrint. In a smaller study presented at the ASCO annual meeting 2010 in Chicago, the MammaPrint index was found to be significantly associated with pCR. A validation consisting of 133 in silico samples was performed to test the Molecular Subtyping Profile, BluePrint, as a predictor of pathological Complete Response (pCR) in patients treated with T/FAC neoadjuvant chemotherapy. Patients with a Basal-type profile achieved a 56% pCR, HER2-type patients achieved a 50% pCR, and patients with a Luminal-type 9% pCR.

In the current study, the chemosensitivity of MammaPrint and BluePrint will be assessed in the clinical diagnostic setting.
2. STUDY DESIGN

The main aim of this study is to determine the chemosensitivity predictiveness of MammaPrint and Blueprint in patients receiving neo-adjuvant chemotherapy.

Patients with suspected primary breast cancer on mammography and clinical examination will be assessed for eligibility by having a needle core biopsy to confirm invasive carcinoma. This will also be immunostained for ER, PR and HER2. Patients will also have routine histology to assess grade and histological type. They will also be assessed for tumor size and for the presence of distant metastases by appropriate imaging examinations. Axillary lymph nodes will be staged according to the following diagram:

Nodal Staging Schema:

A fresh unfixed tumor specimen, incisional or core biopsy (see section 5) will be sent to Agendia to determine the MammaPrint risk profile, the BluePrint molecular subtyping profile, the TargetPrint ER, PR and HER2 single gene readout, the 56-geneTheraPrint Research Gene Panel and the additional genes as measured on the whole genome (44k) array.
Extra breast specimen handling and tumor assessment are described in detail in the attached pathology protocol (attachment II).

Eligible patients will receive the recommended neo-adjuvant chemotherapy treatment. At the end of the neo-adjuvant chemotherapy, all patients will have definitive surgery and complete axillary dissection if the initial node biopsy or SLN biopsy was positive. If the SLN biopsy prior to neoadjuvant chemotherapy was negative then no additional axillary surgery would be required. Response will be measured by pathological CR and by centrally assessed RCB. 

A total of 226 eligible patients will be enrolled from multiple institutions.

3. STUDY OBJECTIVES

1. To determine the predictive power of chemosensitivity of the combination of MammaPrint and BluePrint as measured by pCR.

2. To compare TargetPrint single gene read out of ER, PR and HER2 with local and centralized IHC and/or CISH/FISH assessment of ER, PR and HER2.

3. To identify possible correlations between the TheraPrint Research Gene Panel outcomes and chemoresponsiveness.

4. To identify and/or validate predictive gene expression profiles of clinical response/resistance to chemotherapy.

5. To compare the three BluePrint molecular subtype categories with IHC-based subtype classification.
4. **STUDY POPULATION**

**Inclusion Criteria:**
- Women with histologically proven invasive breast cancer and no distant metastases and:
  - Lymph node negative and a clinical tumor classification of T2 (≥3.5cm)-T4
  - or with 1-3 positive lymph nodes and a clinical tumor classification of T2-T4
  - DCIS or LCIS are allowed in addition to invasive cancer at T2 or T3 level.
- Age ≥ 18 years.
- At least one lesion that can be accurately measured in two dimensions utilizing mammogram, ultrasound, or MRI images to define specific size and validate complete pathologic response.
- Adequate bone marrow reserves (neutrophil count >1.5 x10^9/l and platelet count >100 x10^9/l), adequate renal function (serum creatinine ≤ 1.5 x upper limit of normal) and hepatic function (ALAT, ASAT ≤ 2.5 x upper limit of normal, alkaline phosphatase ≤ 2.5 x upper limit of normal and total bilirubin ≤ 2.0 x upper limit of normal).
- Signed informed consent of the patient

**Exclusion Criteria:**
- Any patient with confirmed metastatic disease.
- Patients with inflammatory breast cancer.
- Tumor sample shipped to Agendia with ≤ 30% tumor cells or that fails QA or QC criteria.
- Patients who have had any prior chemotherapy, radiotherapy, or endocrine therapy for the treatment of breast cancer.
- Any serious uncontrolled intercurrent infections, or other serious uncontrolled concomitant disease.

5. **TISSUE COLLECTION**

Tissue should be collected by incisional biopsy (when placing port) or via core needle biopsy. Sufficient tissue should be submitted to Agendia, to ensure the tissue collection for both gene expression analysis on 44k array as well as DNA isolation for p53 mutation detection.

The preferred method to obtain the tissue is by incisional biopsy. The tissue sample should be 3 to 4 mm in thickness (maximum of 4 mm) and between 8 and 10 mm in diameter. This size allows timely and thorough perfusion of the RNARetain® preservative.
Core needle biopsies should be obtained with a 14 gauge or larger needle. To increase the probability of tumor-positive biopsies the following number of cores are obtained:

If a 14 gauge needle is used please **provide 5 cores**.
If a 11 gauge needle is used please **provide 4 cores**.
If a 9 gauge needle is used please **provide 3 cores**.

In order to minimize sampling failures, one of the cores selected for the Agendia test should be the first or second core obtained.

### 6. NEO-ADJUVANT CHEMOTHERAPY

In order to provide some consistency in management and have a treatment policy in place only recommended therapy with several well accepted and presumed equivalent chemotherapy regimens will be used. The recommended length of therapy is felt to be 6 – 8 cycles to achieve a maximum tumor response prior to proceeding with definitive surgery.

The proposed chemotherapy regimens recommended include:

**For HER2 negative:**

1. **TAC chemotherapy**
   
   - Docetaxel 75 mg/m² IV day 1
   - Doxorubicin 50 mg/m² IV day 1
   - Cyclophosphamide 500 mg/m² IV day 1
   
   Cycled every 21 days for 6 cycles

2. **TC chemotherapy**
   
   - Docetaxel 75 mg/m² IV day 1
   - Cyclophosphamide 600 mg/m² IV day 1
   
   Cycled every 21 days for 6 cycles
3. Dose Dense AC or FEC100 followed by paclitaxel or docetaxel chemotherapy
   
   Doxorubicin 60 mg/m² IV day 1
   Cyclophosphamide 600 mg/m² IV day 1
   Cycled every 14 days for 4 cycles
   Or
   5-Fluorouracil 500 mg/m² IV day 1
   Epirubicin 100 mg/m² IV day 1
   Cyclophosphamide 500 mg/m² IV day 1
   Cycled every 21 days for 3 cycles
   Followed by
   Paclitaxel  80 mg/m² by 1 h IV infusion weekly for 12 weeks
   or
   Docetaxel 100mg/m² IV day 1 cycled every 21 days for 3 or 4 cycles

For HER2 positive patients include:

1. TCH chemotherapy
   
   Docetaxel  75 mg/m² IV day 1
   Followed by
   Carboplatin AUC 6 IV day 1
   Cycled every 21 days for 6 cycles
   Trastuzumab
   Initial dose of 4 mg/kg over 90 minute IV infusion, then 2 mg/kg over
   30 minute IV infusion weekly for 52 weeks,
   Or
   Initial dose of 8 mg/kg over 90 minutes IV infusion, then 6 mg/kg over 30-90 minutes IV infusion
   every three weeks for 52 weeks.

2. T + trastuzumab followed by CEF + trastuzumab
   
   Trastuzumab 4 mg/kg IV for one dose beginning just prior to first dose of paclitaxel.
   Followed by
Trastuzumab 2 mg/kg IV weekly for 23 weeks
Paclitaxel 80 mg/m² by 1 h IV infusion weekly for 12 wks
Followed by
5-Fluorouracil 500 mg/m² IV on days 1 and 4
Epirubicin 75 mg/m² IV on day 1
Cyclophosphamide 500 mg/m² IV on day 1 cycled every 21 days for 4 cycles
Trastuzumab 6 mg/kg IV every 21 days for 9 cycles to complete 1 yr

Dose adjustments
Hematological and non-hematological toxicities should be managed by treating oncologist as per routine clinical practice.
Adverse events will be graded using the NCI Common Terminology Criteria for Adverse Events Version 3.0 (CTCAE). Only grade 4 or 5 adverse events will be recorded in the clinical report form.

Treatment withdrawal criteria
The treatment should be withdrawn if:
- The patient, at any time, withdraws consent to participate.
- The Investigator judges that the decision is in the best interest of the patient
- The treatment must be interrupted for more than 3 weeks
- There is evidence of disease progression
- The patient becomes pregnant

7. CLINICAL DATA COLLECTION

Clinical data will be collected at baseline and post surgery as outlined in Appendix V. Source data must be available to document the existence of the study patients and should substantiate integrity of study data collected. Source data must include the original documents relating to the study, the medical treatment and medical history of the patient.

Data will be entered directly by each participating center using an on-line electronic Case Report Form (eCRF).
Each participating centre will be assigned a unique centre number and will receive a center-specific password. In addition, each CRF will be encoded with a unique serial number. Patient names, initials and date of birth are not collected as part of the study data. Agendia will receive only encoded data. All data will be treated as confidential at all times under all circumstances.

Participating centers will only have access to the data of their own study patients. A site can only get access to their entered data after successfully logging in with their center-specific password. Sites have the ability to view, add or revise data in the database. Agendia also has controlled password-protected access to review and modify data. When any change to a data record is made, the date and the name of the person initiating the change are electronically captured.

Data is stored on the web-server in a secure database, which is replicated for backup purposes. Data sent to, and retrieved, from the web-servers is encrypted using SSL (Secure Sockets Layer) if so required. Only the ICT Director and Operations Director at ActiveReaction (the company responsible for creating and maintain the study database) will have access to the data entered. ActiveReaction's Directors have signed a Confidentiality Agreement to ensure that data is kept private.

8. STATISTICS

It is anticipated that a total of 226 patients will be enrolled over a period of 24 months. This sample size calculation was based on a power calculation, assuming a ratio of 20% Low Risk MammaPrint samples and 80% High Risk samples. To achieve a statistical significant difference of 20% in chemotherapy sensitivity for patients stratified by MammaPrint, a total of 205 samples is needed (significance level 0.05 and power of 0.90). A treatment withdrawal of 10% can be expected in this study, leading to a total sample size of 226 samples.

Baseline characteristics include age, menopausal status, ER/PgR status, HER-2 status, nodal involvement, tumor size, differentiation, method of axillary evaluation (sentinel only, dissection).
Baseline characteristics will be summarized by incidence table.

Pathological complete response (pCR) is defined as the absence of invasive carcinoma in both the breast and axilla at microscopic examination of the resection specimen, regardless of the presence of carcinoma in situ.
Response rate and corresponding confidence intervals will be presented as a proportion of all patients enrolled. The confidence intervals will be calculated using the normal approximation to the binomial distribution.

The differences in patients and tumor characteristics between MammaPrint High and Low risk will be tested using Pearson Chi-square test (Fisher’s Exact test when a cell total does not exceed 5) for categorical variables and Students t-test for continuous variables. To assess the association between the response of the tumor and the outcome of MammaPrint, Pearson Chi-square test will be used. The association of the MammaPrint index with treatment response will be tested using Wilcoxon signed rank test.

Correlation of TargetPrint ER, PR, and HER2 microarray readout with IHC assessment will be determined using Pearson correlation and linear fit models. Agreement measurements between binary microarray and IHC classifications will be based on 2-way contingency table analysis and includes overall concordance, positive agreement defined as the number of samples classified positive by both IHC and TargetPrint divided by the number of positive samples using IHC, negative agreement and Cohen's Kappa coefficient score.

Chemoresponsiveness is measured as a binary response: complete response or no complete response.

Comparison of response rates between BluePrint molecular subtype will be conducted using Pearson Chi-square test.

The correlation between chemoresponsiveness and the TheraPrint Research Gene Panel outcomes will be measured by means of Logistic Regression with chemoresponsiveness as the dependent variable. Possible relationships will be further explored after a positive significant correlation (p>0.05).

“Whole genome” complex arrays will be performed for all patients providing a unique opportunity to investigate the relationship between gene expression patterns and response to treatment. In addition to MammaPrint, TargetPrint, BluePrint and TheraPrint, the complex microarrays used will yield information on gene expression of 44,000 genes (i.e., oligonucleotides that represent thousands of genes on a microarray).
End of study occurs when all of the following criteria have been satisfied:
1. Thirty days after all patients have completed surgery
2. The database has been fully cleaned and frozen for the analysis

9. ETHICAL CONSIDERATIONS

All patients must be appropriately informed about the participation in this study and sign an IRB approved consent form. A template informed consent document is attached to the protocol (Appendix VI). It is the responsibility of the participating investigators to collect human material (meaning tissue and/or data) for this research project in accordance with applicable local laws and guidelines. The responsible investigator will ensure that this study is conducted in agreement with the Declaration of Helsinki (Tokyo, Venice, Hong Kong, Somerset West and Edinburgh amendments). The protocol has been written, and the study will be conducted according to the ICH Harmonized Tripartite Guideline for Good Clinical Practice.
10. REFERENCES


The following genes are assessed:

<table>
<thead>
<tr>
<th>AKT1</th>
<th>CCNE1</th>
<th>ECGF1</th>
<th>FLT4</th>
<th>KRT17</th>
<th>PDGFRB</th>
<th>RAD51L3</th>
</tr>
</thead>
<tbody>
<tr>
<td>AURKA</td>
<td>CDH1</td>
<td>EGFR</td>
<td>FRAP1</td>
<td>KRT5</td>
<td>PIK3CA</td>
<td>RAF1</td>
</tr>
<tr>
<td>BCL2</td>
<td>CDH3</td>
<td>ERBB3</td>
<td>GSDML</td>
<td>KRT8</td>
<td>PIK3R1</td>
<td>TRIM29</td>
</tr>
<tr>
<td>BRAF</td>
<td>CRYAB</td>
<td>ERBB4</td>
<td>IGF1R</td>
<td>MAP2K1</td>
<td>PITX2</td>
<td>TYMS</td>
</tr>
<tr>
<td>BRCA1</td>
<td>CSK</td>
<td>ESR2</td>
<td>IGF2R</td>
<td>MAP2K2</td>
<td>PRKCB1</td>
<td>VEGFA</td>
</tr>
<tr>
<td>BRCA2</td>
<td>CXCL12</td>
<td>FANCF</td>
<td>KDR</td>
<td>NFkB1</td>
<td>PTHLH</td>
<td>VEGFB</td>
</tr>
<tr>
<td>C11orf30</td>
<td>CXCL14</td>
<td>FLT1</td>
<td>KIT</td>
<td>NFkB2</td>
<td>RAD51C</td>
<td>XRCC2</td>
</tr>
<tr>
<td>CCND1</td>
<td>DHFR</td>
<td>FLT3</td>
<td>KRAS</td>
<td>PDGFRA</td>
<td>RAD51L1</td>
<td>XRCC3</td>
</tr>
</tbody>
</table>

- Readout is the log2 intensity of each gene as measured on the array ranging from 0 to 16.
- For each Research Panel Gene, the expression is compared to expression in the reference distribution.
- The relative expression of the patient’s gene is given as a percentile score. This percentile score indicates the percentage of reference samples with a lower intensity.
- There are no cutoffs known to determine whether a given readout is high / low or active / inactive.
- Reference distribution was established using 373 samples from newly diagnosed untreated breast cancer patients.
- To indicate the expression distribution of the individual gene, the reference distribution is given as the 5%-95% expression range on the 0 to 16 log2 intensity scale.
- More genes will be added in 2012

Appendix II Pathology protocol

The primary aim of the study is to assess the predictive power of chemosensitivity of the combination of MammaPrint and BluePrint as measured by pCR. It is thus imperative that institutions participating in the project process specimens and evaluate tumor characteristics uniformly. As such, the following outlines breast specimen handling and tumor assessment protocol.

Initial core/incisional biopsy specimen

1. Upon collection of the initial biopsy specimen, fresh tissue will be appropriately divided for: 1) formalin-fixation for histopathological analysis, and 2) Gene Expression Profiling (GEP) analysis for MammaPrint, BluePrint, TargetPrint and TheraPrint.
   a. Cold ischemic time (time from collection of specimen to fixation solution) should be restricted to < 1 hour
   b. Tissue submitted for histopathological analysis will be fixed in 10% buffered formalin for 6 to 48 hours
   c. Tissue submitted for GEP analysis should be placed within RNARetain according to the sampling instructions provided by Agendia
   d. The pre-neoadjuvant estimation of tumor size will be recorded, with data collected from the following modalities in preferential order: MRI > ultrasound > mammogram

2. All initial core/incisional biopsy specimens will be reviewed centrally
   a. Participating institutions must send 1 H&E, original ER, PR, and HER2 immunohistochemical stains, and 5-10 unstained sections of representative tumor on positive-charged glass slides for central review (Appendix II).
   b. If receptor studies are not available, an additional 10 unstained sections on positive-charged glass slides of representative tumor will be required.

Gross specimen processing

3. Lumpectomy/partial mastectomy
   a. The specimen is oriented, inked in 6 colors, and sectioned at 0.3-0.4 cm intervals.
   b. If gross residual tumor is identified:
      i. Dimensions (width, length, heighth) are recorded
      ii. Distance from all margins if < 0.2 cm is noted, otherwise the closest margin is recorded
      iii. The gross residual tumor is entirely submitted in sequential sections (Appendix IV)
      iv. Sampling of margins is performed
   c. If no-grossly identifiable residual tumor is present:
      i. The dimensions of the biopsy site and surrounding fibrosis/induration is recorded
      ii. The specimen is entirely submitted sequentially (Appendix IV)
      iii. Description of margin status is recorded as noted above
4. Total mastectomy/modified radical mastectomy
   a. The specimen is oriented and inked accordingly
   b. If gross residual tumor is identified:
      i. Dimensions (width, length, heighth) are recorded
      ii. Distance from all margins if < 0.2 cm is noted, otherwise the closest margin is recorded
      iii. The gross residual tumor is entirely submitted in sequential sections (Appendix IV)
      iv. Sampling of margins is performed
   a. If no-grossly identifiable residual tumor is present:
      i. The dimensions of the biopsy site and surrounding fibrosis/induration is recorded
      ii. The area of fibrosis (“tumor bed”), to include biopsy site, is entirely submitted sequentially (Appendix IV)
      iii. Description of margin status is recorded as noted above

Sentinel lymph node processing

5. Sentinel lymph nodes will be serially sectioned along the long axis at 2-mm intervals. If the lymph node measures 0.5 cm in greatest dimension, it may be bivalve. Specimens are then entirely submitted (Appendix IV).

Central assessment of pathologic tumor response

6. The Residual Cancer Burden (RCB) system will be used to assess for pathologic response, as previously described (see references)
7. In brief, a pathologic complete response (pCR) is defined as no residual microscopic tumor in the breast and axillary lymph nodes
8. Non pCRs will be evaluated according to percent tumor volume reduction based on a 3-tiered-system, as outlined by the RCB grading scale
9. Data will be entered on the on-line calculator (see appendix) at http://www3.mdanderson.org/app/medcalc/index.cfm?pathname=jsconvert3
10. The RCB class will be recorded on the Microscopic Specimen Worksheet (see below)
Initial core/incisional biopsy Microscopic Worksheet (central review)

1. Specimen type:
   a. Core biopsy
   b. Incisional biopsy
   c. Excisional biopsy

2. Type of carcinoma
   a. Invasive ductal carcinoma, NOS
   b. Invasive lobular carcinoma
      i. Classical
      ii. Pleomorphic
      iii. Other
   c. Other

3. Nottingham grade (modified Bloom-Richardson)
   a. Tubule formation score
   b. Nuclear pleomorphism score
   c. Mitotic score

4. In-situ component
   a. Present
      i. Grade
   b. Absent

5. Inflammatory infiltrate
   a. Present
      i. Mild
      ii. Moderate
      iii. Intense
   b. Absent

6. Receptors
   a. Estrogen receptor
      i. Positive
         1. H-score
      ii. Negative
   b. Progesterone receptor
      i. Positive
         1. H-score
      ii. Negative
   c. HER2
      i. IHC
         1. Negative
         2. Equivocal
         3. Positive
      ii. FISH
         1. Negative
         2. Equivocal
3. Positive

7. Molecular subtype based on histopathological features
   a. Luminal A
   b. Luminal B
   c. HER2
   d. Basal-like
   e. LumA/HER2 hybrid
   f. LumB/HER2 hybrid

**Gross Specimen Worksheet (central review)**

1. Specimen type
   a. Partial mastectomy/lumpectomy
   b. Total mastectomy
   c. Modified radical mastectomy
   d. Sentinel lymph node

2. Specimen size
   a. _____ x _____ x _____

3. Nipple
   a. Present
      i. Normal
      ii. Abnormal
   b. Absent

4. Skin
   a. Present
      i. Normal
      ii. Abnormal
   b. Absent

5. If grossly identifiable tumor present
   a. Tumor dimensions: _____ x _____ x _____
   b. % gross necrosis:

6. If no grossly identifiable tumor present
   a. Tumor bed/area of induration dimensions: _____ x _____ x _____

**Microscopic Specimen Worksheet (central review)**

1. Residual invasive tumor cells
   a. Present
      i. In breast: _____ x _____ x _____
      ii. In sentinel lymph nodes
         1. # positive
         2. # negative
         3. Size of largest met: _____
   b. Absent (pCR)
2. Pre-neoadjuvant tumor size (as determined by the following modalities in preferential order: MRI > CT > ultrasound > mammogram > clinical exam)
   a. ____ x ____ x ____

3. RCB-class (as determined by on-line calculator at
   a. RCB-0
   b. RCB-1
   c. RCB-2
   d. RCB-3

4. Therapy-related changes (TRC)
   a. Present
      i. Breast
      ii. Axillary lymph nodes
         1. # of axillary lymph nodes with TRC:
   b. Absent

5. Type of TRC, if present
   a. Fibroelastosis
   b. Lipid-laden macrophages
   c. Necrosis
   d. Metaplasia
   e. Other: _______

RCB will be determined by entering specimen data into the on-line calculator at
http://www3.mdanderson.org/app/medcalc/index.cfm?pagename=jsconvert3, pictured below
Figure 1. Schematic diagram of calculating primary tumor bed dimension, cellularity, and size of largest nodal metastasis.

**Residual Cancer Burden System**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard Ratio (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary tumor bed dimension (d₁, d₂)</td>
<td>1.24 (1.04 to 1.48)</td>
<td>.02</td>
</tr>
<tr>
<td>Cellularity fraction of invasive cancer (f₀)</td>
<td>7.37 (2.16 to 25.11)</td>
<td>.001</td>
</tr>
<tr>
<td>Size of largest metastasis (dₘ)</td>
<td>1.17 (0.99 to 1.38)</td>
<td>.06</td>
</tr>
<tr>
<td>No. of positive lymph nodes</td>
<td>1.11 (1.04 to 1.19)</td>
<td>.002</td>
</tr>
</tbody>
</table>

\[
RCB = 1.4(f₀)^{0.17} + \left[4(1 - 0.75^{LN})dₘ\right]^{0.17}
\]

Factors are combined mathematically to produce a continuous variable, which is used to define 4 categories of residual cancer burden (RCB):

<table>
<thead>
<tr>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCB 0: No carcinoma in breast or lymph nodes (pCR)</td>
</tr>
<tr>
<td>RCB I: Partial response, minimal residual disease</td>
</tr>
<tr>
<td>RCB II: Partial response, moderate residual disease</td>
</tr>
<tr>
<td>RCB III: Chemoresistant, extensive residual disease</td>
</tr>
</tbody>
</table>

Figure 2. RCB Classification System.
Appendix III Pathology Worksheet for Participating Institutions (Core/incisional biopsy worksheet)

MINT Project
Pathology Worksheet for Participating Institutions
Core/incisional biopsy worksheet

Please send the following, and complete the form below:

- One H&E section of tumor
- ER immunohistochemical stain
- PR immunohistochemical stain
- HER2 immunohistochemical stain or FISH/CISH/SISH report
- 5-10 unstained sections on positively-charged glass slides
- If ER, PR, and/or HER2 studies not available, please send an additional 10 unstained sections on positively-charged glass slides

1. Laterality
   a. Left breast
   b. Right breast
2. Location
   a. UOQ
   b. LOQ
   c. UIQ
   d. LIQ
3. Time in formalin
   a. ≥ 6 to ≤ 48 hours
   b. Other
APPENDIX IV Pathology Worksheet for Participating Institutions (Grossing worksheet for post-neoadjuvant specimens)

MINT Project
Pathology Worksheet for Participating Institutions
Grossing worksheet for post-neoadjuvant specimens

Please send the following, and complete the form below:

☐ All original or recut H&E slides
☐ Copy of final pathology report (including gross description)

1. Specimen
   a. Lumpectomy/partial mastectomy
   b. Total mastectomy
   c. Skin-sparing, nipple-sparing total mastectomy
   d. Nipple-sparing total mastectomy
   e. Modified radical mastectomy
   f. Other

2. Residual tumor
   a. Grossly identified
      i. Dimensions: ____ x ____ x ____ cm
      ii. Percent gross necrosis:
   b. Not grossly identified
      i. Fibrotic tumor bed dimensions: ____ x ____ x ____ cm

   NOTE: If no definitive tumor grossly identified, please submit entire tumor bed sequentially
   c. Distance from closest margin: ____
Appendix V Data collection

CRF1 will be completed 4 weeks after receiving the MammaPrint, BluePrint and TargetPrint results. CRF2 will be completed 4 weeks after surgery.

The following clinical data will be collected, by means of an online Clinical Report Form:

- Breast Cancer Requisition number
- Site specific patient ID, age at diagnosis, ethnicity
- Biopsy date
- Menopausal status (pre/post)
- Pre treatment clinical staging
- Vascular invasion
- Histology, differentiation grade
- Tumor size (mm)
- Size largest metastatic LN
- ER (%), PR (%), HER-2 status (IHC and/or FISH)
- Comments
- Neoadjuvant chemotherapy regimen
- Any grade 4 or 5 toxicities
- Carcinoma surgery date
- Type of surgery
- ypTNM
- Response: lesion size and LN