Date: 10/18/2020 NCT04129216

THE EFFECTS OF SHORT-TERM PREOPERATIVE TREATMENT WITH HORMONAL THERAPY ON GENE PROFILES IN BREAST CANCER

JHM IRB - eForm A – Protocol

- **Use the section headings to write the JHM IRB eForm A, inserting the appropriate material in each. If a section is not applicable, leave heading in and insert N/A.**
- **When submitting JHM IRB eForm A (new or revised), enter the date submitted to the field at the top of JHM IRB eForm A.**

****

1. Abstract

- **Provide no more than a one page research abstract briefly stating the problem, the research hypothesis, and the importance of the research.**

Breast cancer is among the most common malignancies in women in the United States. Over the years breast cancer management have dramatically developed from the extensive surgical approach toward the breast conservative approach. This was mainly due to the introduction of chemotherapy and hormonal therapy. Hormonal therapy in particular has been shown to improve the oncological outcomes of the breast cancer. However, while this is well documented in the clinical outcomes. Little is known in regards what happens on the genetic level. As such in this study we would like to study the genetic, molecular and functional outcomes that results after a short term neoadjuvant hormonal therapy on patients with breast cancer.
The hypothesize of this study is that short-term, preoperative hormonal treatment will induce genetic changes associated with reduced proliferation, including lower Ki67 expression, and changes in ER and PR expression as well as reduced ER pathway activity. The data from such investigation will be very helpful in advancing the individualized care to women with breast cancer.

Hypothesis

- We hypothesize that short-term, preoperative hormonal treatment will induce genetic changes associated with reduced proliferation, including lower Ki67 expression, and changes in ER and PR expression. as well as changes in ER pathway activity measures

2. Objectives

The purpose of this study is to evaluate the effects of short-term preoperative treatment with hormonal therapy (tamoxifen, letrozole, and exemestane, administered according to standard of care) on gene expression profiles in patients with breast cancer. As such:

A. **Primary objective** is to determine whether there is a reduction in the expression of proliferation marker Ki67, measured by IHC as well as single-gene read out, following short-term preoperative treatment with hormonal therapy.

Secondary objectives are to determine whether short term treatment results in significant reductions in the Mammaprint risk score, or in ER or PR expression as measured by IHC and single-gene read out or a change in ER pathway activity.**C. Exploratory objectives:**

a. Identify differentially expressed transcripts after short-term pre-operative, hormone treatment

b. Identify transcripts for which gene expression is associate with measures of response, including changes in Ki67, ER and PR.

c. Perform full-transcriptome set analysis to identify pathway level changes associated with treatment.

d. Comparison of expression changes between two standard of care molecular diagnostics: 70-GS and 21-GA

e. Perform OncoSignal pathway activity analysis on pre and post treatment samples to related pathway activity to patient response

The participants of this study will be clinical breast cancer patients of the Principal Investigator (PI) or those referred by community physicians to the PI. The breast cancer patients will be evaluated by the PI or a study team member to assess if they meet inclusion/ exclusion criteria for this study. If the patient meets all the criteria, the consent process will take place.

Participants will include 30 breast cancer patients. These patients will be recruited from Johns Hopkins University cancer treatment centers.

This is a clinical trial with three treatment groups including tamoxifen, letrozole, and exemestane.Ten pre-menopausal patients and men will be in their own group and will recievetamoxifen. Twenty post-menopausal patients will in a separate groupand will recieve letrozole or exemestane. Treatment selection will be at the discretion of the medical oncologist. Ten patients will be allocated to each treatment group, for a total of 30 patients

Genomic data will be collected as a part of the standard of care 70 gene signature (70GS; trade name MammaPrint), which is read from the comprehensive-genome microarray at Agendia (Irvine, CA). The 70GS will be performed on the baseline biopsy specimen as SOC, and experimentally on the surgical specimen collected after short-course endocrine treatment. We will then compare the genomic profiling between the two samples to measure changes in gene expression before and after treatment. Special care will be taken to verify with pathology that the baseline biopsy is large enough to run the commercially available 21 gene assay (21GA; trade name Oncotype) on, as this assay is also currently considered standard of care. For the OncoSignal test about 0.25mm3 FFPE tissue is needed from the same biopsy or surgical material. The tumor content should be at least 50%. If on a slide of 5 µm thickness about 25-50 mm2 tissue is available, only 2 slides are required.

3. Background

Breast cancer is the most common malignancy and the second leading cause of cancer deaths among women [1]. As a heterogeneous and phenotypically diverse disease, breast cancer may necessitate divergent treatment strategies, among which cytotoxic chemotherapy remains an integral part of therapy at any given stage. However, cytotoxic therapy is not considered a "targeted therapy", because it does not act on cellular pathways involved in cell growth and progression [1]. The real targeted therapy would be based on the identification of a molecular growth pathway and on the modification of its activity in order to block the growth and progression of cancer. With regard to breast cancer, the endocrine manipulation is considered as a targeted therapy [2]. The role of estrogen on tumor cell growth is well established in breast cancer. Identification of the estrogen receptors (ER) provided the first target for anti-estrogenic therapeutic agents [1-3]. Tamoxifen, the prototype SERM (Selective Estrogen Receptor Modulator), has been the gold standard treatment for early and advanced disease and its activity on ER is considered the first form of molecular targeted therapy [1, 4]. Aromatase inhibitors (e.g., anastrozole, letrozole, or exemestane) which directly inhibit the production of estrogen, have shown superiority over tamoxifen in adjuvant and metastatic setting [5-8], also they appear to fare better than tamoxifen in the neoadjuvant setting [8], where therapeutic agents are administered before the main treatment (e.g. in the preoperative phase). Following the success of adjuvant endocrine treatments [9-11], more studies have been emerging with the use of endocrine agents in the neoadjuvant setting (PO24 trial [12], IMPACT trial [13] and PROACT trial [14]). The objective of endocrine therapy in the neoadjuvant setting is primarily to produce sufficient response in the tumor to allow mastectomy in inoperable tumors and potentially breast conservation in patients initially proposed to undergo mastectomy [8]. Treatment response to these "targeted therapies" differs vastly across different populations; even patients with the same stages of disease can have markedly different responses and overall outcome [10]. Therefore, an ideal therapy should be tailored based on individual characteristics so that each patient receives a treatment with an appropriate response and the least adverse effects. Genetic breakthroughs in the last few decades have provided us with a unique opportunity to address this challenge. The emergence of genomic techniques (e.g. complementary DNA microarrays) and their ability to simultaneously measure the expression of thousands of genes have led to the identification of biology-based prognostic profiles [15-18], several of which have been validated and are in clinical use. The development of prognostic profiles has been particularly useful in guiding decision-making about adjuvant therapy along with well-established clinical prognostic factors (e.g. patient age, comorbidity, tumor size, grade, and nodal status) [19]. These molecular prognostic profiles can augment but do not replace classic clinical factors. They offer the potential for use in clinical practice for prognostic stratification and treatment selection for patients with breast cancer, particularly if they are hormone receptor-positive. Among the available prognostic profiles, the Amsterdam 70-gene prognostic profile (Mammaprint) was one of the first gene expression array-based marketed for prognostic purposes [20]. The test was developed using a supervised DNA microarray analysis of gene expression arrays on frozen tissue from primary breast tumors. A mathematical model is used to calculate a score that stratifies patients as having a breast cancer with an associated poor-prognosis (High risk: 29% risk of metastasis after 10 years, without adjuvant treatment) or good-prognosis (Low risk: 10% risk of metastasis after 10 years without adjuvant treatment) [21]. Validation of the Mammaprint gene expression profile demonstrated that the profile is a powerful prognostic factor to predict outcome in lymph node-negative and lymph node-positive breast cancer. Recent reports, that the most important of them is MINDACT trial [22, 23], confirm this predictive value for Mammaprint signature. Although it was originally approved for use with unfixed, frozen tissue, it has now been adapted for use with formalin-fixed, paraffin-embedded tissue. Neoadjuvant endocrine therapy has been increasingly employed in clinical practice to improve the disease outcome [6-8], but little is known of the molecular effects of these agents. Particularly, the genetic impact of endocrine therapy directly on breast cancer remains largely unexplored. Most studies that examined the anti-estrogen treatment of ER positive breast cancer, have measured changes in clinical, pathological, proliferative or surgical outcomes [7, 25, 26] and

one study investigated the effect of neoadjuvant treatment on ER pathway activity (34; Inda et al; Mol Can Ther 2020 19 680-9) To our knowledge, there are few studies that have shown molecular effects as the early (10-14 days) or late (3-4 months) changes in gene profile after preoperative anti-estrogen treatment of ER-positive breast cancer [26, 27]. Most reports of the transcriptional profiling of estrogen responses in breast cancer cell lines are results of in vitro [28-31] or animal models [32]. Thus, identification of in vivo responses needs to be more elaborated. On the other hand, these studies generally examined one type of neoadjuvent therapy, therefore a comparative study on this subject can be very useful. Preoperative endocrine therapy in neoadjuvant settings revealed to be ideal for this purpose because it allows the direct assessment of response to treatment, and tumor is readily available for multiple time point biopsies [7].

The FLEX Registry (NCT03053193) is a large-scale, population based, prospective registry. All patients with stage I to III breast cancer who receive MammaPrint testing on a primary breast tumor are eligible for entry into the FLEX Registry, which is intended to enable additional substudies at low incremental cost. This study will utilize FLEX's adaptive study infrastructure to capture clinical data.

4. Study Procedures

a. Study design, including the sequence and timing of study procedures (distinguish research procedures from those that are part of routine care).

The breast cancer patients will be evaluated by the PI or a study team member to assess if they meet inclusion/ exclusion criteria for this study. If the patient meets all the criteria, the consent process will take place. This protocol is designed to be a companion substudy of FLEX Registry, which prospectively observes patients receiving MammaPrint as a part of their care, and data collection will occur within FLEX's eCRF infrastructure. All patients consenting to screening for this study will be co-enrolled in FLEX, with appropriate dual informed consents obtained to participate in both studies.

Participants will include 30 breast cancer patients. These patients will be recruited from Johns Hopkins University cancer treatment centers.

This is a clinical trial with three treatment groups including tamoxifen, letrozole, and exemestane. Ten pre-menopausal patients and men will be in their own group and will receive tamoxifen. Twenty post-menopausal patients will in a separate group and will receive either letrozole or exemestane. Treatment selection will be at the discretion of the medical oncologist. Ten patients will be allocated to each treatment group, for a total of 30 patients.Full genome Mammaprint will be run on the biopsy specimen and the final surgical specimen. We will then compare the gene profiling between the two results. OncoSignal pathway analysis will make use of the same biopsy specimen for evaluating the ER pathway activity scores.

The Oncosignal results will not be shared with patients(subjects), treating physicians; nor will be used for patients' management; It only will be used for research purposes.

In addition, some clinical data will be collected from the patient's medical records, operative/pathology reports, and clinical notes. This information will include information such as details of the cancer diagnosis, details of the surgery and further treatment of the disease.

Study design is illustrated in Figure 1.

Figure 1. Study Scheme

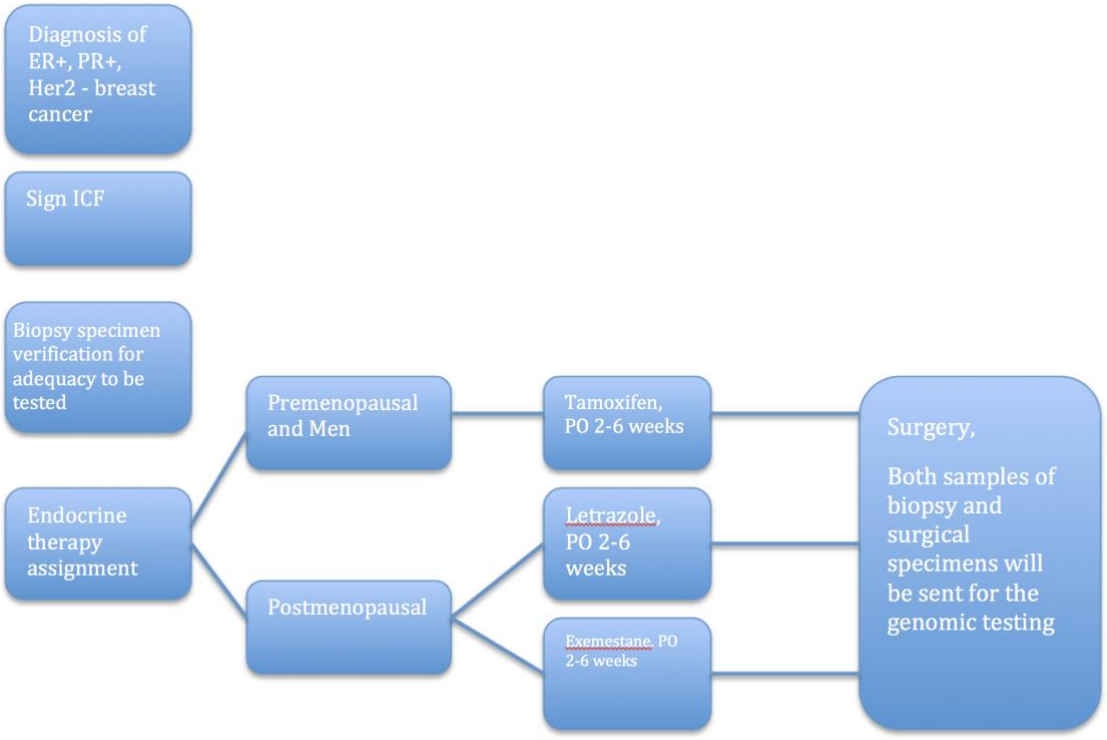

Figure 1. Study Scheme

Assays and Tests used in the study:

Mammaprint: This a strong diagnostic tool that will be used in the study as Breast Cancer Assay and will provide invaluable information regarding the genomics of the patient and their tissue sample. We will be running the test twice to ascertain the differences between the samples due to a short term hormonal therapy course.

- OncoSignal: This is a test that measures biologically active pathway activities of 4 different pathways (ER, AR, PI3K and MAPK). In this study especially the ER-pathway activity is

relevant since ER staining positive tumors are selected with an intend for hormonal treatment. From previous studies it is known that, although tumors can be ER positive in staining, the pathway is not always equally active (34). High ER pathway activity on the primary biopsy leads to a better response to hormonal therapy as compared to samples with a low ER activity on the primary biopsy, although all are ER staining positive. Also the change in pathway activity is higher in patients that respond to hormonal therapy as compared to patients that do not, or suboptimally respond, to hormonal treatment. The test requires 0.25mm3 FFPE tissue with a tumor percentage of at least 50%. In principle the same biopsy material can be used as collected for mammaprint but the processing of the FFPE samples is different

Study Drug Information(Dosage, Administration Frequency, Duration):

Tamoxifen: 20mg is administered daily. Patients take this drug for 2-6 weeks.

Letrozole: 2.5mg is administered daily. Patients take this drug for 2-6 weeks

Exemestane: 25mg is administered daily. Patients take this drug for 2-6 weeks.

b. Study duration and number of study visits required of research participants.

We anticipate this study to be done in 2 years. Following consent, and prescription of hormonal therapy, patients will have only one study visit prior to surgery, to take a baseline core research biopsy. The surgical sample will be used for post-treatment analysis

c. Blinding, including justification for blinding or not blinding the trial, if applicable.

d.

This is a single arm trial and so will not be blinded.

e. Justification of why participants will not receive routine care or will have current therapy stopped.

Participants will receive routine care. Normally, patients are scheduled for surgery after radiologic and other workups are completed. After surgery, they will receive hormonal therapy. In our study, hormonal therapy will commence after patients are recruited to the study and informed consent is signed, prior to surgery. Hormone therapy will continue after surgery.

f. Justification for inclusion of a placebo or non-treatment group.

There is no placebo or non-treatment group.

g. Definition of treatment failure or participant removal criteria.

Treatment failure happens if the patient doesn't comply with the hormonal therapy treatment plant. At such cases, the patient will be removed from the study. If any patient miss more than two doses of the medication they will be removed from the study.

Because the duration of the study is short, and the medications are well tolerated, we expect very few non-compliant patients. We will add additional patients to the study if we lose any due to this clause.

h. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.

This is expected to be a rare event as patients are only followed for 2-6 weeks, until surgery, and the hormonal therapy administered in this study is well studied and carries minimal risk of long term effects or consequences. In such cases, patients will continue their standard of care plan as advices by their care providers.

5. Inclusion/Exclusion Criteria

A. Inclusion criteria:

- Treatment-naïve, histologically confirmed invasive ductal breast cancer between stages 1 to 3.
- Co-enrollment in the FLEX Registry
- ER+ PR+ confirmed hormone receptor status measured by immunohistochemistry(IHC)
- Patients should understand their condition and be able to give informed consent to participate

B. Exclusion Criteria:

- History of hormonal therapy, chemotherapy, radiation therapy, or novel therapy to treat the current breast cancer.
- Allergic reactions/hypersensitivity to tamoxifen, letrozole, or exemestane or any of their ingredients.
- Any contraindication to hormonal therapy, such as history of thromboembolic disease or uterine cancer.
- Patients without invasive disease (stage 0)
- Patients with metastatic breast cancer(stageIV)
- Patients that are HER2+ by IHC/FISH.

Drugs/ Substances/ Devices

a. The rationale for choosing the drug and dose or for choosing the device to be used.

The drugs in this study are FDA approved mediations and drugs of choice for hormone therapy according to NCCN (National Comprehensive Cancer Network) guidelines. We will use the standard recommended daily doses of the drugs.

MammaPrint has 510k FDA clearance as a device and is a cost-effective vehicle for evaluating the genomic objectives of this study. The patient population considered for enrollment in this trial are candidates for receiving MammaPrint as a part of their standard of care.

The rationale for using the Oncosignal in addition to Mammaprint is that Oncosignal shows differences in pre treatment ER pathway activity scores between patients, although all patients are ER staining positive this does not always relate to an active pathway. Mammaprint test can

show the gene level changes and oncosignal can show the whole pathway activity before and after treatment

b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.

The drugs that will be used are FDA approved to be administered for an FDA approved indication.

c. Justification and safety information if non-FDA approved drugs without an IND will be administered.

No non-FDA approved drugs without an IND will be administered

Study Statistics

a. **Primary outcome variable**: The primary outcome variable is Ki67 expression, a measure of tumor proliferation
a. **Secondary outcome variables:** Secondary outcomes include ER and PR expression and pathway activity patterns pre and post treatment
b.
c. **Statistical plan including sample size justification and interim data analysis**.

Primary Objective: Change in Ki67 expression after treatment will be calculated by subtracting %Ki67 expression at time of surgery from the baseline level. We will then fit a linear model to determine whether short-term treatment significantly reduces expression, while adjusting for choice of treatment, ER and PR level, and other possible cofounders. In a secondary analysis, we will evaluate individual treatment groups separately.

Sample size justification: Power calculations are based on the use of a one-sided, one-sample t-test to evaluate a decrease in Ki67. The proposed sample size of 30 subjects offers at least 90% power at an alpha of 0.05, to detect a decrease greater than 0.55s.d. For the analysis of individual treatment, groups of 10 offer at least 90% power to detect decreases greater than 1s.d.

Secondary objectives
Determine whether short term treatment results in significant reductions in Mammaprint risk score, ER or PR expression as measured by IHC.

Change in risk score ER and PR expression will be evaluated as described above for Ki67, using linear regression to model the change in each variable while adjusting for ki67 in addition to the covariates described above. Expected power is the same as for the primary analysis.

Exploratory analyses
Identify differentially expressed transcripts after short-term pre-operative, hormone treatment

Identify transcripts for which gene expression is associate with measures of response, including changes in Ki67, ER and PR expression.

We will fit empirical Bayes linear models [32] to identify genes for which expression is associated with response to treatment, controlling type I error at an FDR of <10%. Wilcoxon Rank Sum tests will be used to perform Gene set enrichment analysis on the results to identify pathways that are significantly altered after short-term treatment. [33]

Show differences in pre treatment ER pathway activity scores between patients, although all patients are ER staining positive this does not always relate to an active pathway. Show differences in ER pathway activity scores between pre and post treatment samples, indicating a measurable biological effect of the treatment.

d. Early stopping rules.

There are no early stopping rules.

6. Risks

a. Medical risks, listing all procedures, their major and minor risks and expected frequency.

Important drug adverse effects like increased risk of thromboembolism would be considered a major risk. The other non-specific side effects may be considered as minor risk. Since the drugs are administered over a short period, it is highly unlikely to develop an adverse event that requires chronic use of the drug.

The Oncosignal test has minimal risks:

It is noninvasive, does not require an invasive sampling that presents significant risk, does not introduce energy into a subject and is not used as a diagnostic procedure without confirmation of the diagnosis by another medically established diagnostic product or test

The Oncosignal results will not be shared with patients(subjects), treating physicians; nor will be used for patients' management; It only will be used for research purposes.

b. Steps taken to minimize the risks.

Known adverse effects of tamoxifen and anastrozole will be recorded using a checklist every week by the research assistant. Medical monitoring will be made by both the principle investigator and the Institutional Data and Safety Monitoring Board. Patients are informed about all common and important adverse effects and they are advised to report as soon as they notice them. They are also advised to take some preventive measures for example for the prevention of

deep vein thrombosis. In any circumstances, all adverse effects are taken care of by the research team immediately and effectively.

Participants are provided with the investigators contact information and a list of frequently asked questions for any concerns they might have.

c. Plan for reporting unanticipated problems or study deviations.

Any unanticipated problem or study deviation will be recorded and reported per IRB guidelines.

d. Legal risks such as the risks that would be associated with breach of confidentiality.

There is a minimal risk of losing confidential data. However, all measures advised by IRB to minimize this risk will be done. Best practices will be followed, including the assignment of a study ID and the de-identification of PHI from study data.

e. Financial risks to the participants.

N/A

7. Benefits

a. Description of the probable benefits for the participant and for society.

You will not directly benefit from being in this study. If you take part in this study, you may help others in the future.

8. Payment and Remuneration

a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.

Participants will not receive compensation.

9. Costs

a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them.

Costs of the study will be paid for using the grant funding this study.

References:

1. Orlando L, Schiavone P, Fedele P, Calvani N, Nacci A, Rizzo P, et al. Molecularly targeted endocrine therapies for breast cancer. Cancer treatment reviews. 2010;36 Suppl 3:S67-71. Epub 2010/12/07.
2. Miller WR, Larionov A. Molecular effects of oestrogen deprivation in breast cancer. Molecular and cellular endocrinology. 2011;340(2):127-36. Epub 2011/05/25.
3. Osborne CK, Schiff R. Estrogen-receptor biology: continuing progress and therapeutic implications. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(8):1616-22. Epub 2005/03/10.
4. Coates AS, Keshaviah A, Thurlimann B, Mouridsen H, Mauriac L, Forbes JF, et al. Five years of letrozole compared with tamoxifen as initial adjuvant therapy for postmenopausal women with

endocrine-responsive early breast cancer: update of study BIG 1-98. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2007;25(5):486-92. Epub 2007/01/04.
5. Coombes RC, Kilburn LS, Snowdon CF, Paridaens R, Coleman RE, Jones SE, et al. Survival and safety of exemestane versus tamoxifen after 2-3 years' tamoxifen treatment (Intergroup Exemestane Study): a randomised controlled trial. Lancet. 2007;369(9561):559-70. Epub 2007/02/20.
6. Dixon M, Macaskill J. Neoadjuvant endocrine therapy: ASCO educational book; 2009.
7. Forbes JF, Cuzick J, Buzdar A, Howell A, Tobias JS, Baum M. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 100-month analysis of the ATAC trial. The lancet oncology. 2008;9(1):45-53. Epub 2007/12/18.
8. Geisler J, Smith I, Miller W. Presurgical (neoadjuvant) endocrine therapy is a useful model to predict response and outcome to endocrine treatment in breast cancer patients. The Journal of steroid biochemistry and molecular biology. 2012;131(3-5):93-100. Epub 2011/12/31.
9. Mathew J, Asgeirsson KS, Jackson LR, Cheung KL, Robertson JF. Neoadjuvant endocrine treatment in primary breast cancer - review of literature. Breast (Edinburgh, Scotland). 2009;18(6):339-44. Epub 2009/10/20.
10. Dowsett M, Smith I, Robertson J, Robison L, Pinhel I, Johnson L, et al. Endocrine therapy, new biologicals, and new study designs for presurgical studies in breast cancer. Journal of the National Cancer Institute Monographs. 2011;2011(43):120-3. Epub 2011/11/02.
11. Miller WR. Clinical, pathological, proliferative and molecular responses associated with neoadjuvant aromatase inhibitor treatment in breast cancer. The Journal of steroid biochemistry and molecular biology. 2010;118(4-5):273-6. Epub 2009/10/20.
12. Mackay A, Urruticoechea A, Dixon JM, Dexter T, Fenwick K, Ashworth A, et al. Molecular response to aromatase inhibitor treatment in primary breast cancer. Breast cancer research : BCR. 2007;9(3):R37. Epub 2007/06/09.
13. Cicatiello L, Scafoglio C, Altucci L, Cancemi M, Natoli G, Facchiano A, et al. A genomic view of estrogen actions in human breast cancer cells by expression profiling of the hormone-responsive transcriptome. Journal of molecular endocrinology. 2004;32(3):719-75. Epub 2004/06/03.
14. Dowsett M, Smith IE, Ebbs SR, Dixon JM, Skene A, A'Hern R, et al. Prognostic value of Ki67 expression after short-term presurgical endocrine therapy for primary breast cancer. Journal of the National Cancer Institute. 2007;99(2):167-70. Epub 2007/01/18.
15. Frasor J, Danes JM, Komm B, Chang KC, Lyttle CR, Katzenellenbogen BS. Profiling of estrogen up- and down-regulated gene expression in human breast cancer cells: insights into gene networks and pathways underlying estrogenic control of proliferation and cell phenotype. Endocrinology. 2003;144(10):4562-74. Epub 2003/09/10.
16. Mrusek S, Classen-Linke I, Vloet A, Beier HM, Krusche CA. Estradiol and medroxyprogesterone acetate regulated genes in T47D breast cancer cells. Molecular and cellular endocrinology. 2005;235(1-2):39-50. Epub 2005/05/04.
17. Itoh T, Karlsberg K, Kijima I, Yuan YC, Smith D, Ye J, et al. Letrozole-, anastrozole-, and tamoxifen-responsive genes in MCF-7aro cells: a microarray approach. Molecular cancer research : MCR. 2005;3(4):203-18. Epub 2005/04/16.
18. Early Breast Cancer Trialists' Collaborative Group. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet. 2005;365(9472):1687-717. Epub 2005/05/17.
19. Smith IE, Dowsett M. Aromatase inhibitors in breast cancer. The New England journal of medicine. 2003;348(24):2431-42. Epub 2003/06/13.
20. Eiermann W, Paepke S, Appfelstaedt J, Llombart-Cussac A, Eremin J, Vinholes J, et al. Preoperative treatment of postmenopausal breast cancer patients with letrozole: A randomized double-blind multicenter study. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 2001;12(11):1527-32. Epub 2002/02/02.
21. Smith IE, Dowsett M, Ebbs SR, Dixon JM, Skene A, Blohmer JU, et al. Neoadjuvant treatment of postmenopausal breast cancer with anastrozole, tamoxifen, or both in combination: the Immediate

Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen (IMPACT) multicenter double-blind randomized trial. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(22):5108-16. Epub 2005/07/07.
22. Cataliotti L, Buzdar AU, Noguchi S, Bines J, Takatsuka Y, Petrakova K, et al. Comparison of anastrozole versus tamoxifen as preoperative therapy in postmenopausal women with hormone receptor-positive breast cancer: the Pre-Operative "Arimidex" Compared to Tamoxifen (PROACT) trial. Cancer. 2006;106(10):2095-103. Epub 2006/04/07.
23. van de Vijver MJ, He YD, van't Veer LJ, Dai H, Hart AA, Voskuil DW, et al. A gene-expression signature as a predictor of survival in breast cancer. The New England journal of medicine. 2002;347(25):1999-2009. Epub 2002/12/20.
24. van 't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature. 2002;415(6871):530-6. Epub 2002/02/02.
25. West M, Blanchette C, Dressman H, Huang E, Ishida S, Spang R, et al. Predicting the clinical status of human breast cancer by using gene expression profiles. Proceedings of the National Academy of Sciences of the United States of America. 2001;98(20):11462-7. Epub 2001/09/20.
26. Isaacs C, Stearns V, Hayes DF. New prognostic factors for breast cancer recurrence. Seminars in oncology. 2001;28(1):53-67. Epub 2001/03/20.
27. Slodkowska EA, Ross JS. MammaPrint 70-gene signature: another milestone in personalized medical care for breast cancer patients. Expert review of molecular diagnostics. 2009;9(5):417-22. Epub 2009/07/08.
28. Manjili MH, Najarian K, Wang XY. Signatures of tumor-immune interactions as biomarkers for breast cancer prognosis. Future oncology (London, England). 2012;8(6):703-11. Epub 2012/07/07.
29. Cardoso F, Piccart-Gebhart M, Van't Veer L, Rutgers E. The MINDACT trial: the first prospective clinical validation of a genomic tool. Molecular oncology. 2007;1(3):246-51. Epub 2007/12/01.
30. Cardoso F, Van't Veer L, Rutgers E, Loi S, Mook S, Piccart-Gebhart MJ. Clinical application of the 70-gene profile: the MINDACT trial. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2008;26(5):729-35. Epub 2008/02/09.
31. Pearlin LI, Mullan JT, Semple SJ, Skaff MM. Caregiving and the stress process: an overview of concepts and their measures. Gerontologist. 1990;30(5):583-94. Epub 1990/10/01.
32. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W and Smyth GK (2015). “limma powers differential expression analyses for RNA-sequencing and microarray studies.” *Nucleic Acids Research*, **43**(7), pp. e47
33. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences of the United States of America*. 2005;102(43):15545-15550. doi:10.1073/pnas.0506580102.

34. Ind M, Blok E, Kuppen P, et al; Estrogen receptor pathway activity score to predict clinical response or resitance to neoadjuvant endocrine therapy in primary breast cancer. *Molecular Cancer Therapeutics 2020, 19:680-9*