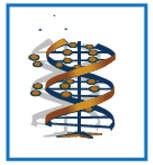
# Nutritional Strategies to Prevent Recurrence of Breast Cancer Among Breast Cancer Patients Using Physiological and Genomic Data

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## Introduction:

A major concern among women diagnosed with breast cancer is whether the disease will recur. Conventional medicine can provide breast cancer survivors only statistically probabilities based on population based studies given a particular treatment protocol (radiation, chemotherapy, and/ or SERMs, etc).

Tired of the one-size fits-all approach, oncogenomics research has helped the oncologist optimize a particular treatment given the unique genomics of the tumor (radiation versus chemotherapy). Yet, the genetic uniqueness of the patient is often overlooked.

For example, while Tamoxifen maybe recommended as the best SERM (based on oncogenomics testing) we at the Institute for Individualized Medicine evaluate whether the breast cancer survivor has a SNP (single nucleotide polymorphism) associated with cytochrome P450 2D6, which precludes the effective biotransformation of Tamoxifen to Endoxifen, the biological active SERM. This may explain the variable results associated with the use of Tamoxifen in women diagnosed with breast cancer.

If the breast cancer tumor is ER +, we also use functional genomic testing and nutrigenomic interventions to both understand the genomic influence on estrogen metabolites (2-OH estrone, 4-OH estrone, 16- alpha OH estrone, 2-methoxy estrone, 4-methoxyestrone, and 3,4 estrone quinone) and how best to either upregulate or down regulate gene expression along the estrogen "super highway", thereby decreasing estrogen and/or estrogen metabolites and their possible influence on estrogen sensitive tissues in the future.

Case histories are presented which integrate genomic data (DetoxGenomic Profile) and physiological data (Salivary Female Hormone Profile, Urinary Estrogen Metabolites) to personalize and individualize prevention programs using specific nutritional ingredients to decrease the risk of ER+ breast cancer recurrence. Understanding the origins of the disease as well employing nutrigenomic interventions can psychological boost the spirit of the patient, giving her a locus of control and allaying her fears about the statistical probabilities associated with breast cancer recurrence. Furthermore, the pharmacogenomic application of the genomic data can be used, prior to the administration of the drug, to identify whether it will have biological activity, cause possible adverse drug reactions, induce potential drug-drug, or drug-botanical interactions.

## Materials and Methods:

Breast Cancer Patients: Each woman completed an 18-page medical history questionnaire prior to any genomic, saliva or urine testing. The questionnaire asked for a detailed family health history, emotional and mental history, dietary and lifestyle choices and current signs and symptoms. Each woman considered for this study had to be in good health (other than being diagnosed with ER+ breast cancer), willing to alter their lifestyle, dietary and nutritional supplements based on their genomic results and do several laboratory tests (see below) during the course of this investigation. Three women were identified to take part in this study; they had similarities in age (53 to 55 years), diagnosis (ER+ breast cancer), histopathology (no node involvement), surgical procedure (radical mastectomy), no radiation or chemotherapy treatment based on oncogenomics (low Oncotype Dx score, Redwood City, California), either had used or were currently nutritional supplements (multiple vitamin-mineral, calcium and magnesium, vitamin C and vitamin E) and little or no OTC medications other than NSAIDs. Each also felt the need to understand the origin of the disease and be responsible and proactive in keeping the risk of recurrence to a minimum.

Lab tests: Each woman completed the following blood, saliva and urine tests according to the protocol outlined below.

DetoxGenomic Profile. SNPs (single nucleotide polymorphisms) were identified among 8 cytochrome P450 genes responsible for the following enzymes: CYP1A1, CYP1B1, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4. Multiple SNP locations were evaluated for CYP1A1, CYP1B1, CYP2C9, CYP2C19 and CYP3A4. The cytochrome P450 enzyme system uses oxygen to modify toxic compounds, drugs or steroid hormones. SNPs in genes coding for cytochrome P450 enzymes can increase or decrease the activity of that enzyme. For example a SNP on CYP1B1 up-regulates the conversion of estrone to 4-OH estrone, an undesirable estrogen metabolite know to induce estrogen sensitive cancer, whereas a SNP on CYP1A1 down regulates estrone's conversion to 2-OH estrone, a estrogen metabolite considered to be protective. SNPs were also evaluated in Phase II detoxification enzymes associated with methylation (Catechol-O-methyl transferase or COMT), 7 genes associated with acetylation (N-acetyl transferase), 3 genes linked to glutathione conjugation (Glutathione s-transferase or GST) and 3 gene locations for superoxide dismutase (SOD). COMT and GST facilitate estrogen metabolite detoxification and therefore SNPs can increase an individual's risk for estrogen induced cancers.

Briefly, a 10 ml blood sample was collected in an EDTA test tube and sent via overnight carrier to Genova Diagnostic Laboratory (Asheville, NC) where the samples were centrifuged and prepared for genomic analysis using Third Wave Invader DNA assay. This assay detects polymorphisms in a patient's DNA sample using a solution hybridization method in which two oligonucleotides hybridize in tandem with specific DNA sequences. Subsequent Cleavase and hybridization reactions result in generation of fluorescent signal. The biplex format of the assay enables simultaneous detection of all variants in a single reaction tube. The sensitivity and specificity of this assay is 99.7 %

Salivary Female Hormone Profile: Eleven saliva samples (3 ml each) were collected in individual plastic vials between 7 am and 9 am during a 30-day period; for the most part, they were collected every other day. All samples were frozen until the final sample was collected then shipped in a refrigerated Styrofoam box via an overnight carrier to Genova Diagnostic Laboratory (Asheville, NC). Free estradiol and progesterone levels for each vial were determined using RIA. Free testosterone was determined only on the last vial collected using RIA.

Urinary Estrogen Metabolite Profile: Prior to and then 3 months following nutrigenomic intervention urine was collected during a 24 hr period in two 1 liter plastic containers with ascorbic acid preservative after which a 120 ml homogenous aliquot was transferred to a cup with screw lid, then shipped overnight to Genova Diagnostic Laboratory (Asheville, NC). The following levels of estradiol, estrone (E1), estriol and estrogen metabolites (2-OHE1, 4-OH E1, 16α-OHE1, 2-methoxy E1 and 4-methoxyE1) were determined by GS-MS.


## ABSTRACT:

The greatest fear among postmenopausal breast cancer patients with hormone receptor positive tumors and have been treated with either chemotherapy and/or radiation is recurrence of the disease. Currently, the drug of choice used by conventional medicine to decrease the risk of recurrence is Armidex, a potent and selective non-steroidal aromatase enzyme inhibitor. In postmenopausal women, the principal source of circulating estrogen (estradiol) is conversion of androstenedione to estrone by aromatase in peripheral tissues with subsequent conversion to either estradiol to 2-hydroxyestrone, 4-hydroxyestrone or 16-α-hydroxyestrone, the latter two being potent carcinogens. However, Armidex does have potential side effects: osteoporosis, depression, hot flashes, vaginal dryness, short-term memory problems and emotional ups and downs.

Within conventional medicine, questions about the mechanism(s) underlying the development of breast cancer in each patient are never asked. Nor does conventional medicine inquire how genes, environment and/or nutrition might influence these mechanisms. As a result, most treatment protocols are based on medicine-of-the-averages.

At the Institute for Individualized Medicine, we evaluate specific physiological data (Urinary Estrogen Metabolites) and functional genomic information (single nucleotide polymorphism(s) for genes that encode for enzymes involved in the biotransformation of estrogen metabolites some of which are carcinogenic) to identify the possible underlying mechanism(s) associated the development of breast cancer for each patient. Based on this information, a personalized and individualized program is developed using specific nutritional ingredients, which can either "turn on" or "turn off" gene expression to decrease the risk of breast cancer recurrence.

Numerous case histories will be presented showing how the integration of these data (physiological and functional genomic information) can empower the patient, giving her a locus of control and allaying her fears about the recurrence of breast cancer.

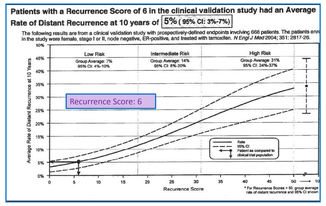

### Breast Cancer and SNPs Associated with Phase I and Phase II Detoxification

| | Px D | Px L | Px M |
|---|---|---|---|
| **Phase I** | | | |
| CYP1A1 | ↓ | | |
| CYP1B1 | | ↑ | ↑ |
| **Phase II** | | | |
| COMT | Homozygous | Heterozygous | Homozygous |
| GSTM1 | Absent | Absent | Absent |
| GSTP1 | Wild type | Heterozygous | Homozygous |

### Recurrence Score for Patient Diagnosed with Estrogen Receptor + Breast Cancer

Patients with a Recurrence Score of 6 in the clinical validation study had an Average Rate of Distant Recurrence at 10 years of 5% (95% CI: 3%-7%)

The following results are from a clinical validation study with prospectively-defined endpoints involving 668 patients. The patients enrolled in the study were female, stage I or II, node negative, ER-positive, and treated with tamoxifen. N Engl J Med 2004; 351: 2817-26.




### Phase I DetoxGenomic Profile: ER + Breast Cancer Patient

**Cytochrome P-450**

| Result | Gene | internet information |
|---|---|---|
| ✓ | CYP1A1 * | www.genovations.com/gdgen01 |
| ● | CYP1B1 * | www.genovations.com/gdgen02 |
| ● | CYP2A6 | www.genovations.com/gdgen10 |
| ● | CYP2C9 * | www.genovations.com/gdgen05 |
| ✓ | CYP2C19 * | www.genovations.com/gdgen06 |
| ✓ | CYP2D6 | www.genovations.com/gdgen03 |
| ✓ | CYP2E1 | www.genovations.com/gdgen04 |
| ✓ | CYP3A4 * | www.genovations.com/gdgen07 |

### Phase II Detoxification Profile: ER+ Breast Cancer Patient

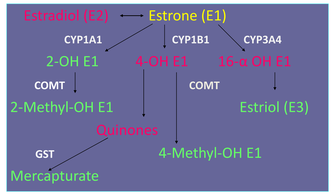
**Methylation**

| Result | Gene | SNP Location | Internet Information | Affects |
|---|---|---|---|---|
| ++ | COMT | V158M | www.genovations.com/gdv158m | Liver/Gut |

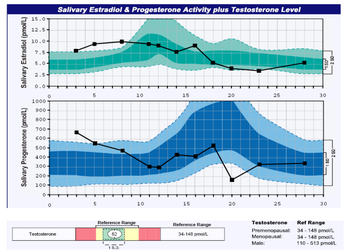
**Glutathione Conjugation (Glutathione s-transferase)**

| Result | Gene | Location | Internet Information | Affects |
|---|---|---|---|---|
| ABSENT | GSTM1 | 1p13.3 | www.genovations.com/gdgstm1 | Liver/Kidney |
| - - | GSTP1 | I105V | www.genovations.com/gdgstp1 | Brain/Skin |
| - - | GSTP1 | A114V | www.genovations.com/gda114v | Brain/Skin |

### Estrogen Metabolism
(Phase I and II Detoxification Enzymes)




### Salivary Estradiol & Progesterone Activity plus Testosterone Level




### Estradiol and Estrogen Metabolites (Urine): ER+ Breast Cancer Patient Prior to Nutrigenomic Intervention

| | |
|---|---|
| Estrone (24hr urine) | 2.87 |
| Estradiol (24hr urine) | <dl |
| Estriol (24hr urine) | <dl |

| | |
|---|---|
| 2-Hydroxyestrone (24hr urine) | 1.15 |
| 2-Methoxyestrone (24hr urine) | 2.12 |
| 16α-Hydroxyestrone (24hr urine) | <dl |
| 4-Hydroxyestrone (24hr urine) | 2.72 |
| 4-Methoxyestrone (24hr urine) | <dl |
| 2-Hydroxyestrone/16α-Hydroxyestrone Ratio (24hr urine) | >2.35 |
| 2-Methoxyestrone/2-Hydroxyestrone Ratio (24hr urine) | 1.84 |
| 4-Methoxyestrone/4-Hydroxyestrone Ratio (24hr urine) | <dl |

### Estradiol and Estrogen Metabolites (Urine): ER+ Breast Cancer Patient After Nutrigenomic Intervention

| | |
|---|---|
| Estrone (24hr urine) | 20.04 |
| Estradiol (24hr urine) | 5.15 |
| Estriol (24hr urine) | 0.34 |

| | |
|---|---|
| 2-Hydroxyestrone (24hr urine) | 2.99 |
| 2-Methoxyestrone (24hr urine) | 1.34 |
| 16α-Hydroxyestrone (24hr urine) | 2.53 |
| 4-Hydroxyestrone (24hr urine) | 0.70 |
| 4-Methoxyestrone (24hr urine) | 0.79 |
| 2-Hydroxyestrone/16α-Hydroxyestrone Ratio (24hr urine) | 1.17 |
| 2-Methoxyestrone/2-Hydroxyestrone Ratio (24hr urine) | 0.45 |
| 4-Methoxyestrone/4-Hydroxyestrone Ratio (24hr urine) | 1.13 |

### Nutrigenomic Intervention

| Supplement | Amt/Day | Reason |
|---|---|---|
| Dim Pro | 1 | Up-regulates CYP1A1 |
| SamE | 1 | Cofactor for COMT |
| N-acetyl-cysteine | 1 | Precursor to GSH |
| Selenomethionine | 1 | Cofactor for GSH |
| Ester C | 2 | Anti-oxidant |
| Nutri E | 1 | Anti-oxidant |

## LEARNING OBJECTIVES/GOALS:

1. To understand the underlying genetic predisposition(s) associated with breast cancer using functional genomics;
2. To identify for each breast cancer patients how her genetic predisposition(s) may have lead to the disease;
3. To use physiological data (Female Hormone Test, Urinary Estrogen Metabolites) to get a "snapshot" of steroid hormone levels and down stream estrogen metabolites;
4. To overlay physiological and functional genomic data to expand options beyond those offered by conventional medicine to prevent breast cancer recurrence;
5. To identify nutritional strategies that can influence gene expression and thereby decrease the risk of breast cancer recurrence.

## Conclusions:

1. By evaluating an individual's genomic information, a clinician can better understand the underlying genetic predisposition(s) associated with the disease process and then tailor specific prevention and/or pharmacological regimens specific to that unique individual.
2. While the genetics of the tumor are important to identify the proper pharmacological treatment, it is equally important to include the patient's pharmacogenomics to improve treatment outcomes.
3. "Snap shots" of physiological data (Salivary Female Hormone Profile, Urinary Estrogen Metabolites) highlight circulating levels of steroids (estradiol, progesterone and testosterone) and levels of specific estrogen metabolites (4-OH estrone, 16-alpha OH estrone, and 3,4 estrone quinone) along with their ratios, which can contribute to the disease process in women with ER+ breast cancer. These "snap shots" are useful tools to measure the effectiveness of nutrigenomic interventions (see # 4).
4. Nutrigenomic interventions specific to that patient's functional genomics (phase I and phase II detoxification pathways) and circulating levels of estradiol (salivary) and estrone metabolites (urine) are utilized to decrease the recurrence of ER+ breast cancer.
5. Breast cancer patients experience a psychological boost when given individualized and personalized treatment strategies based on her genomic map and physiological data compared to medicine-of-the-averages or one-size-fits-all treatments.
6. Functional genomic testing and evaluating a woman's steroid levels can be useful strategies in breast cancer prevention particularly in women taking birth control, HRT, bio-identical hormones and who have high circulating levels of estrogen (peri-menopause).