The Breast Cancer Personalized Nutrition study (BREACPNT):

A phase 2 single-blinded randomized study of algorithm-based personalized nutrition intervention compared to standard diet intervention in patients treated with endocrine therapy for early stage, hormone receptor positive breast cancer

Study Protocol

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1. Purpose

The Breast Cancer Personalized Nutrition (BREACPNT) study will evaluate the effect of a microbiome based personalized diet intervention on control of weight gain, glycemic response, disease outcome and various biomarkers in hormone receptor early breast cancer patients receiving adjuvant endocrine treatment.

2. Background

Weight gain is a common incident in breast cancer survivors [1]. As many as 50–96% of women experience weight gain during treatment. Weight gain in breast cancer survivors is complex and influenced by many factors such as tumor type, socio-demographic characteristics, and menopausal status [2,3]. Most breast cancer patients (~75%) are diagnosed with hormone receptor-positive (HR+) tumors and receive endocrine treatment for a period of at least 5 years. Endocrine treatment was identified as a risk factor for weight gain in several studies [2]. Weight gain during endocrine therapy was highest in women who were premenopausal or had previous chemotherapy [4].

Weight gain may decrease adherence to long-term hormonal therapy and increase risk for metabolic syndrome and cardiac disease. Importantly, post-diagnosis weight gain has been implicated as a risk factor in breast cancer recurrence and survival. Hence, weight management for breast cancer survivors is important for increasing adherence to therapy and lowering recurrence risk [5].

The essential role of the gut microbiota in modulating immune and metabolic functions in health and disease is increasingly recognized. Dysbiosis, a disruption in the balance of gut bacterial communities, is associated with many conditions [6]. The entire bacteria population in the digestive tract (microbiome) consists of ~1,000 species with a genetic repertoire of ~3 million different genes. The homeostasis of intestinal microbiota can be influenced by internal factors, such as genetic, age-related and hormonal, as well as by external factors, such as nutrition, stress, lifestyle and antibiotics [7]. The microbiome is directly affected by our diet and directly affect the body's response to food [8,9]. Particularly in breast cancer (BC), diet plays an important role in creating a microbiome

environment involved in estrogens metabolism [10]. High systemic estrogen levels contribute to breast cancer risk in postmenopausal women. Estrogen levels in the blood are regulated in part via enterohepatic recirculation, involving bacterial enzymatic pathways and deconjugation[11]. Indeed, profiling gut microbiota in postmenopausal breast cancer patients revealed altered composition and estrogen-independent low diversity of their gut microbiota compared to healthy controls [12]. Thus, the gut microbial community may affect estrogen-related breast cancer [13].

A recent study linked the gut microbiome diversity to weight gain [14] and microbiome alterations were found to contribute to post-dieting weight regain [15]. In addition it was found that the increase in breast cancer risk with increasing BMI among postmenopausal women is associated with an increase in estrogens, particularly bioavailable estradiol [16]. The Personalized Nutrition Project, conducted at Eran Segal's group in the Weizmann Institute of Science, recently showed in an unprecedented scale of 800 people that individuals vary greatly in their glycemic response to the same food [17]. Most importantly, it emphasized the involvement of functional microbial pathways and bacterial taxa in host glucose metabolism. This unique dataset yielded an algorithm capable of accurately predicting personalized postprandial glycemic response (PPGR) to arbitrary meals. The algorithm's predictions are based on many personal measurements, including blood tests, personal lifestyle and gut bacteria profiles.

Continuing studies demonstrate that short-term dietary interventions change the microbiome and are beneficial to the host in maintaining glucose levels. Moreover, Low glycemic index (GI) diets may be important in weight management [18]. In a small-scale pilot study using this algorithm, personally tailoring dietary interventions to healthy and pre-diabetic people, showed a significantly improved PPGRs accompanied by consistent alterations to the gut microbiota (personal communication) . These results suggest that individually tailored dietary interventions help maintain normal blood glucose levels and influence microbiome diversity, which, in turn, can control weight changes.

3. Hypothesis Objectives and Endpoints

Study Hypothesis

Algorithm based personalized diet will be superior to standard low fat diet for controlling weight gain and glycemic response in breast cancer patients treated with endocrine therapy.

Primary Objective

To evaluate the efficacy of a personalized diet compared to a standard low fat diet to control body mass as measured by changes in body mass.
Endpoint: Body weight changes will be defined as the net body weight gained/lost in the 6 months' intervention period.

Secondary Objective
1. To evaluate the efficacy of the personalized diet compared to a standard low fat diet to control glycemic response.
   Endpoint: glycemic response control measured by the area under the glucose curve (AUC) during continuous glucose monitoring (CGM) period.

Exploratory objective

1. Evaluate disease outcomes as measured by disease free survival, Breast cancer recurrence in study subjects. Endpoint: 5 years Disease free survival (DFS), 5 years Breast cancer specific recurrence.
2. To investigate microbiome composition and modulation during the diet intervention period and assess if there are differences in modulations between the personalized diets as compared to the standard diet.
3. Investigate the mutual effects of gut microbiome and blood metabolites during the diet intervention period and search for possible biomarkers for dietary treatment efficacy.
4. Investigate inflammation parameters and immune profiles of patients (lymphocytes, T-cell receptor repertoire, antibodies profiling using phage display libraries) of HR positive patients undergoing intervention and their correlations to microbiome modulations.
5. Test whether patients have better compliance and adherence to algorithm-based personalized diets, compared to standard diets advised for weight control. The compliance to the diets will be measured by: Compliance questionnaire, 3-day dietary log, Number of study meetings attended.
6. Test whether HR-positive breast cancer patients have better adherence to hormonal treatment following weight-control diets. This compliance will be tested for 5 years post treatment.

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4. Study Design

This is a phase 2 randomized trial in hormone receptor positive breast cancer patients receiving adjuvant endocrine therapy. Figure 1 illustrates the study schedule.

200 HR+ breast cancer patients, eligible for adjuvant endocrine therapy will be recruited to the study. Upon recruitment, subjects will provide a stool sample for microbiome analysis and will undergo continuous glucose monitoring for 2 weeks. Thereafter, patients will be randomly assigned in a 1:1 ratio to receive a personalized diet recommendation or a standard low-fat diet for 6 months. The algorithm is based on patients' microbiome analyses and glucose monitoring results. Patients will be monitored by continuous glucose monitoring (CGM) at least 2 times during the 6 months' intervention period. At the end of the 6 months' period patients will undergo a second course of CGM for 2 weeks and provide a second stool sample for microbiome analysis. Patient clinical records will be followed 2-3 times yearly for 5 years for DFS and BC recurrence.
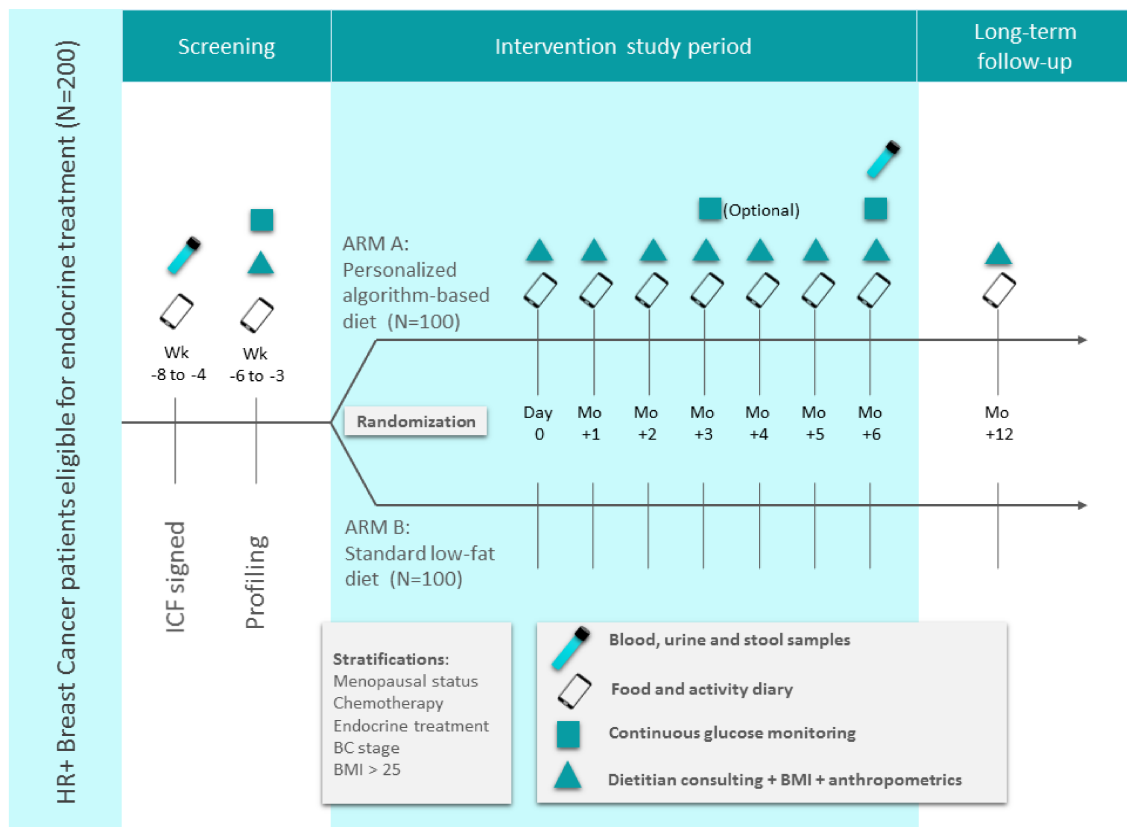

Figure 1: Study design and schedule

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Inclusion Criteria:

- Female patients, Age 18-70
- Patients diagnosed with stage 1-3 breast cancer, who underwent surgery, have finished their neo/adjuvant chemotherapy and radiotherapy if these were indicated and are treated with adjuvant endocrine therapy (either Tamoxifen or Aromatase inhibitor +/- GNRH agonists).
- Patients are at least 60 days after finishing their last non-endocrine oncology treatment (i.e. definitive surgery, radiation or chemotherapy – whichever is last), have received at least 30 days of endocrine therapy (tamoxifen or aromatase inhibitor) but no more than 24 months.
- Patients treated with neoadjuvant endocrine therapy are eligible provided they had undergone surgery, are at least 60 days post their last non endocrine therapy (definitive surgery or radiation and chemotherapy, if these were indicated), are continuing their endocrine therapy but did not receive more than 24 months post-surgery.
- Are willing to work with smartphone application

Exclusion Criteria:

- Oral Antibiotics/antifungal use in the previous 3 months to profiling stage (these patients will be able to join the study at a later point)
- Use of anti-diabetic and/or weight-loss medication
- BMI<18.5
- People under another diet regime and/or a dietitian consultation/another study?
- Pregnancy, breast feeding
- HIV carriers, Cushing syndrome, Chronic Kidney Disease, acromegaly, hyperthyroidism, liver cirrhosis
- Psychiatric disorders (Schizophrenia, Bipolar Disorder)
- Known diagnosis of IBD (inflammatory bowel diseases)
- Patients that underwent Bariatric surgery
- Known Alcohol or substance abuse
- Known Diagnosis of diabetes
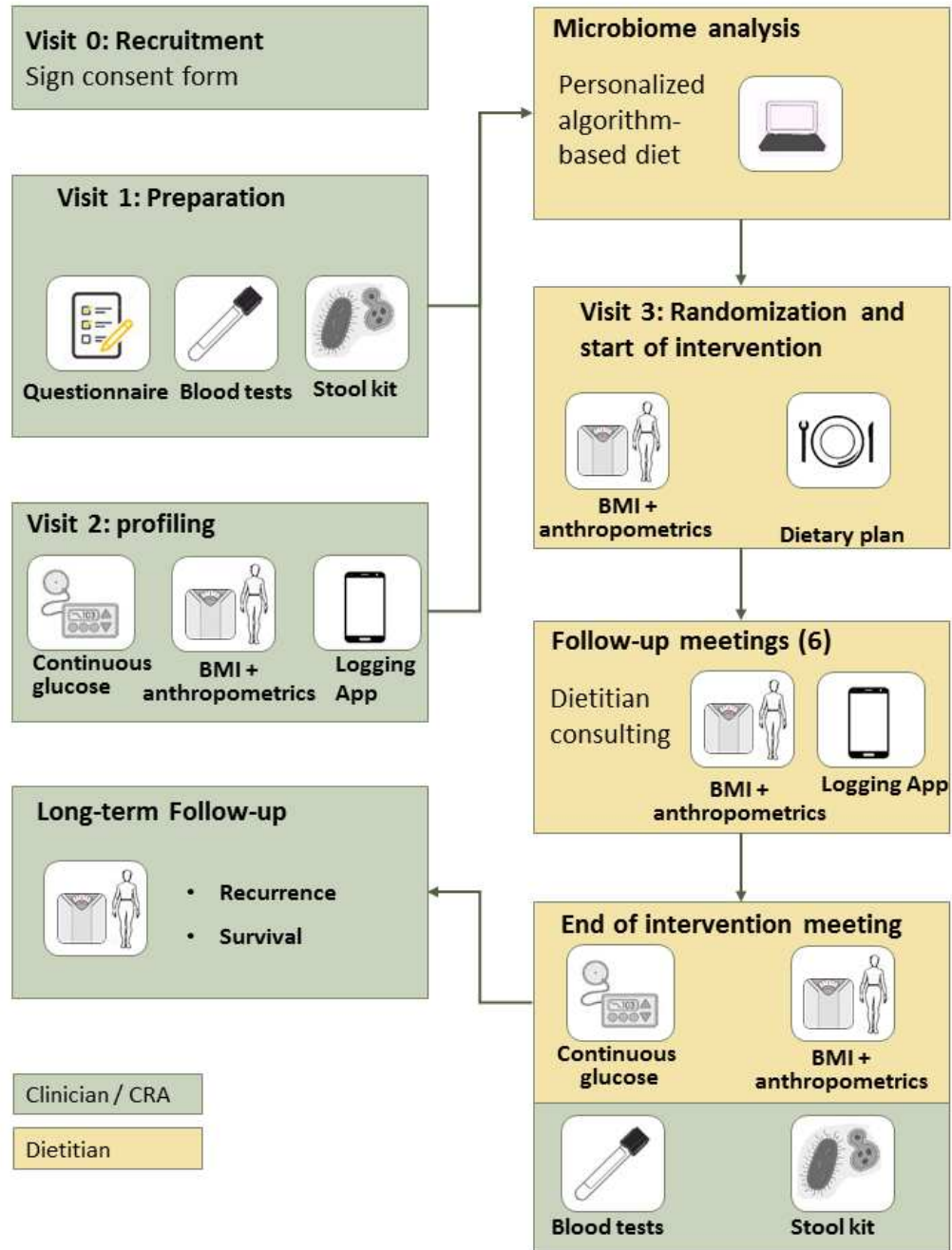
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Figure 2: Study scheme

Patient Recruitment

Breast cancer patients will be recruited to the study through their regular clinic visit at the Breast Oncology Unit at Sheba Medical Center. Patients eligible for the study will sign an informed consent. This recruitment process will be ongoing until the designated number of study patients is reached.

Screening and profiling stage (-3 Months to Day -1_)

During this stage consenting patients will:

1. Meet a study coordinator and complete questionnaires regarding their medical background, nutritional habits and lifestyle activities (filled online using the REDcap software, using a dedicated tablet computer). Patients will provide blood samples for CBC, blood chemistry including liver function, lipid profiling, HBA1C, TSH, CRP, LH FSH and future exploratory analyses, and urine samples for estradiol derivatives. All patients will receive a code from the software and their data will be anonymized.

2. Patients will be asked to log a three-day food diary using a designated mobile phone application.

3. Patients will receive a designated stool kit to collect the stool sample at home. In the next meeting (profiling stage) patients will return the stool sample which will be used for microbiota profiling.

4. Meet with a certified dietitian to build a menu for the "profiling period" based on the three-day food diary of dietary habits provided by the patient. This meeting will include anthropometrics measurements (weight, height, waist and hip circumference) and connection to the glucose measurement device (Abbott Freestyle Libre). The CGM kit includes a sensor affixed to the back of the arm, which continuously monitors glucose levels in the interstitial fluid, translates and records blood glucose levels. Patients will be connected to a CGM for two weeks and will be asked to follow their diet plan as given to them by the dietitian, according to their regular habits and lifestyle. During the two weeks of connection patients will be instructed to use a dedicated application, in which they will

log in real-time their food diary, exercise, sleep, wake up, special events. Patients will return the CGM kit via a courier service from the patients' home upon measurement completion. The stool sample will be processed for microbiome profiling. The resulting data and data provided by the CGM kit will be analyzed to provide a personal profile for each patient.

Randomization and Intervention

Following the profiling stage: patients will be randomly assigned to one of two arms of the study. Approximately 100 subjects will be randomized to each arm. Patients will be blinded to the arm to which they were assigned .Randomization will be done by a computer program, taking into account the following stratification factors:

1. Menopausal status at study entry
2. Previous chemotherapy
3. Endocrine treatment type (Tamoxifen/Aromatase inhibitor)
4. Breast Cancer stage
5. BMI above/below 25

The intervention arm will be an 'algorithm-based' arm in which patients will receive personally tailored dietary recommendations. The prediction algorithm is based on gradient boosting regression model and is capable of accurately predicting personalized postprandial glycemic responses to arbitrary meals based on microbiome, CGM data from the profiling period (two weeks of CGM connection data) and other clinical data such as blood tests and lifestyle features. This model predicts PPGRs using the sum of thousands of different decision trees. Trees are inferred sequentially, with each tree trained on the residual of all previous trees and making a small contribution to the overall prediction. The features within each tree are selected by an inference procedure from a pool of 187 features representing meal content (e.g., energy, macronutrients, micronutrients); daily activity (e.g., meals, exercises, sleep times); blood parameters (e.g., HbA1c%, HDL cholesterol); CGM-derived features; questionnaires; and microbiome features (metagenomic relative abundances and KEGG pathways)[17]. The algorithm was developed using a standard leave-one-out scheme to rank every meal of each participant in the profiling period (i.e.,

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the PPGR to each predicted meal will be hidden from the predictor). The model was validated in an independently collected 100-person cohort . [17]

The control arm will receive nutritional recommendations according to the standard Israeli dietary approach Mediterranean-style low-fat diet. In order to provide patients with diets that support their energetic needs and meet the recommendations for weight loss in people with overweight or obesity, the daily caloric target for each patient (in both arms) will be calculated as average between:

1. Estimated Energy Requirements (EER) calculated with the use of Mifflin equation for Resting Energy Expenditure (REE) [19]. The result from this equation will be divided by 0.7 (as REE represent ~70% of total energy expenditure).

2. Average daily caloric intake obtained from patient's log in the app during the profiling stage. For overweight patients (BMI>25) a total of 500Kcal will be reduced from their reported caloric intake to allow weight loss as accepted according to the American Association of Clinical Endocrinologists guidelines.

The diet recommendations for both arms will be provided and explained by a certified dietitian which will meet the patients from both arms at Day 0 and monthly thereafter for a total of 6 scheduled monthly visits. Weight and other anthropometric measurements (height, waist and hip circumference) will be taken at this and all following meetings with the dietitian. Participants will be asked to document their food intake and daily activities including exercise and sleep using a dedicated smartphone app throughout the intervention period.

Intervention Meetings:

Patients from both arms will be invited during the intervention period to monthly follow-up meetings with a certified dietitian (total of 6 meetings). Meetings will include evaluation of patients' compliance to the dietary recommendations they received and additional advice will be provided if needed. During the follow up meetings anthropometric measurements will be taken (height, weight, hip and waist circumference). We will also follow up on patients via phone, email, text massage, in order to increase compliance. During the monthly meeting at the beginning of Month 4 of the intervention period patients will be

offered to be reconnected to CGM for 2 weeks (optional). Data from CGM connections will be analyzed at the end of the intervention

End of intervention

At the end of the 6 months intervention period, patients will be invited to a meeting in which they will undergo anthropometrics measurement, urine, blood samples and stool sample. Additionally, patients will be connected to CGM for 2 weeks (mandatory).

Patients will be followed up at 12 months following the start of the intervention. They will attend a follow up meeting with the study coordinator in which BMI, anthropometrics, and a 3 day food diary will be recorded.

Long term follow up

Long-term clinical follow-up will be collected from the electronic medical records for recurrence and survival calculation purposes for a period of up to 5 years post recruitment.

5. Data Acquisition, Storage and Analysis

All samples will be stored at the Breast Cancer translational Research laboratory at Sheba Medical Center. The samples will be stored at -80C, bacterial DNA samples will be stored at -20C. The samples will be stored encoded with no identifying information. Identifying details and codes will be kept in a file stored at Sheba medical center. Encoded stool samples will be transferred to the Segal laboratory at the Weizmann Institute of science. There, samples will be processed for bacterial DNA processing. All clinical data will be coded. Data will be transferred using the REDcap server and stored on Weizmann servers behind a protected firewall and be accessible only to the study team

Future research:

Samples will be stored for up to 10 years. All future use of stool and blood samples will be subject to IRB approval.

6. Safety Endpoints

No safety endpoints planned for this study

7. Statistical Considerations

Sample size determination. To estimate the required sample size, we performed power analysis while estimating effect size using the results of different studies describing controlled diet intervention aimed at weight control. Our goal is to detect a difference of at least 2 kg in net weight loss/gain (kg) between control group and experimental group. Based on the study of Shai et al. [20], the standard deviation of weight loss is 4.2 and the projected sample size with alpha of 0.05 and power of 0.8, with an estimated dropout rate of 10%, is 107 people for each arm totaling 214 individuals.

Primary, secondary and exploratory endpoint analysis (brief summary). All statistical analyses will be performed using Python 2.7. Continuous variables will be presented as mean±SD and dichotomous/categorical variables as proportions. The normality of the distribution of continuous variables will be tested by the Kolmogorov-Smirnov test. If normality will be rejected, non-parametric tests will be used. To test the association between continuous variables with normal distribution, the Pearson correlation coefficient will be performed and to test associations between continuous variables which do not distribute normally or for ordinal variable, the spearman correlation coefficient will be used. To compare parameters for continuous variables in 2 time points the paired-samples t-test will be performed (or Wilcoxson test for non-normally distribute variables), in dichotomous/categorical variables the McNemar test will be performed. To compare continuous variables in a number of time points ANOVA with repeated measures will be used. For comparison of dichotomous or categorical variables in number of time points the Cochran's Q test will be performed. P values < 0.05 will be considered significant.

8. Possible Benefits

Patients will receive counseling and close monitoring by a certified dietitian throughout the study, regardless of the research arm to which they were assigned

Patients will have the opportunity to evaluate their blood glucose levels in response to food that they tend to eat, exercise, etc., throughout the CGM connection.

Patients will receive an analysis of their glucose response to the foods that they ate.

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Patients will have access to different nutritional tools that will be available to them on a secure website or on their mobile phone (App).

At the end of the study all patients will be given access to their personal tailored dietary recommendations, built for them by the study team based on their personal data, regardless of the arm they were assigned to during the study.

9. Possible Risks and Analysis of Risk/Benefit Ratio

When blood tests are taken, there is no risk except a slightly discomfort associated with the prick, hematoma or local infection it the prick area.

In order to monitor glucose levels patients will be connected to a continuous glucose monitor (CGM). The CGM includes a sensor that will be inserted using a small needle into the body. There is ultra-low risk of inserting the sensor including mild discomfort associated with inserting the sensor, a local infection in the prick area, a mild redness at the patch area. We consider this risk to be quite low. Continuous Glucose Monitoring may reveal a previously undiagnosed diabetes. These patients will be excluded from the trial and the patient and treating oncologist will be notified to provide appropriate therapy and inform the patient's general physician.

Caloric restriction will be provided only to patients who are overweight or obese (BMI>25) and not to patients with BMI at the normal range (18.5-25). Patients with BMI lower than the normal range will be excluded from the study.

10. Risk Management Procedures

Confidentiality

Patients will be identified by a numerical study ID. Only the designated research staff at Sheba Medical center will have access to the patient's fully identified medical information. The information that matches the code to the identifying information will be kept in a safeguarded database that is password protected.

11. Subject Payment/Costs

Subjects will not be directly remunerated for participation in the study. There is no cost to the subject for study participation.

12. End of Study definition

It is estimated that accrual will be completed in approximately 24 months
Time from initiation of intervention to last post intervention meeting – 12 months.
End of the study is the date of the last visit for the last patient which will be approximately 36 months from first patient intervention

Clinical endpoints will be collected up to 5 years after end of intervention.

13. Consent Procedures

Study purpose, methods, materials, risks, benefits, and alternatives will be provided in a detailed description in the consent form and will be discussed with the patient by the investigator or authorized designee. Patients will be told they are free to refuse to participate and may withdraw their consent at any time for any reason. The consent forms will be signed and dated by the patient before his or her participation in the study. The informed consent forms and process shall be documented in the patients' clinical records. A copy of the signed consent form will be provided to the   patient.

14. Privacy

If patients wish to review or discuss their results this information will be discussed in private consultation with the study team medical personnel.

15. Data Security

The collection and processing of personal data from subjects enrolled in this study will be limited to the data needed to investigate this study's hypothesis. Access to identifiable data will be limited to Sheba Medical Center designated personnel; patient level de-identified data will be available only to investigators authorized by the Principal Investigator.

Data files are stored on a password-protected computer/database and will be accessible only to the designated investigators and research staff. Only the research staff will have the link that can match the code to traditional identifying information. The data sets used

for analysis will be coded and not contain any traditionally used identifying information that could be used to identify the patient.

16. Study/Intervention Discontinuation

Patients will be discontinued from study intervention in the following circumstances:

1. The patient is enrolled in any other clinical trial involving any investigational product or any other type of medical research judged not to be scientifically or medically compatible with this study.

2. Investigator decision: the investigator decides that the patient should be discontinued from the study or study intervention if the patient, for any reason, requires treatment with a therapeutic agent that effects study indication/intervention or for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and good clinical practice (GCP).

3. Patient decision: If the patient requests to be withdrawn from study intervention but agrees to stay in the study she will be evaluable for the endpoint if she attended at least one follow-up meeting post randomization. If the patient wishes to withdraw participation in the study she can do so at any time and in such a case data and samples will be destroyed

4. Disease recurrence.

5. Discontinuation of Inadvertently Enrolled Patients: If the investigator identifies a patient who did not meet enrollment criteria and was inadvertently enrolled, a decision on whether or not the patient may remain on intervention will be made and documented. Patients will be evaluable for the primary endpoint if they were randomized and attended at least one follow up meeting post start of intervention.

17. Monitoring

Sheba Medical Center will monitor the study. Source documents will be reviewed to ensure all subjects have properly signed and dated the informed consent forms. All information will be reviewed to ensure eligibility criteria as per the protocol, and supporting source data will be verified.

18. Record Retention

Research records with patient identification will be kept for 10 years after study completion. The collected data and related de-identified health information may be kept indefinitely. Record retention will comply with the specific requirements of the Sheba Medical Center IRB. No personal health information will be retained.

19. Publication

The results of this research will be presented at meetings or in publication. However, the subject's identity will not be disclosed in those presentations.

20. Facilities and Personnel

All study activities will occur within the patient's home and breast cancer institute clinic at Sheba Medical Center. All communications with patients will be through the Sheba Medical Center.

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Appendix 1: **Samples Collection, Storage and Analysis**

Blood: during the study we will collect blood samples 2 times : At the initial screening, and at the end of the intervention,at time points 0 and 6m. Two blood samples will be taken- one sample for immediate analysis and a second sample (blood and plasma) would be stored in deep freeze (-80°C) for future metabolomics testing (blood tests are detailed in schedule of activities table, see Appendix 2).

Stool: Patients will be asked to provide several stool sample at 2 time points throughout the study. Stool samples are required for the study, and will be collected at baseline and at the end of intervention. Stool samples will be stored at Sheba Medical center and transfered to Segal lab at the Weizmann Institute. The samples will be stored encoded with no identifying information. The samples will be stored at -80C, bacterial DNA samples will be stored at -20C. The samples will be stored for 10 years. Identifying details and codes will be kept by the principal investigator and designated personnel. All future use of stool samples will be subject to Helsinki approval.

Urine: urine samples will be taken from every patient at 2 time points, including at the beginning and at the end of the intervention, in order to characterize estradiol derivatives.

Appendix 2: Schedule of activities

Trial period	Screening	Profiling	Intervention						End of treatment	Followup	
Scheduling window	week -8 to -4	week -4 to -3	Day 0 (M1)	M2	M3	M4	M5	M6	M7	M12	Every 6 months (5years)
Signed ICF	X										
Review of eligibility	X										
Medical History	X										
Nutrition/Lifestyle/Medical Questennaire	X								X		
Weight		X	X	X	X	X	X	X	X	X	
Height		X	X	X	X	X	X	X	X	X	
Other Anothropometrics		X	X	X	X	X	X	X	X	X	
Provision of stool kit	X										
CBC		X							X		
Blood Chemistry including total serum protein and albumin and fasting glucose		X							X		
Lipid profiling		X							X		
Liver profiling (GGT, Bilirubin, Alkaline Phosphatase, AST,ALT)		X							X		
TSH		X							X		
CRP		X							X		
HbA1C		X							X		
Hormonal Profiling (LH, FSH)		X							X		
Urinalysis (estradiol derivatives)		X							X		
Whole blood sample for expolratory analysis		X							X		
Dietitian Consult		X	X	X	X	X	X	X	X	X	
Profiling stage menu		X									
Food and Activity Diary log in	X	X	X	X	X	X	X	X	X	X	
Continuous Glucose Monitoring Connection (2 weeks)		X				X (optional)			X		
Survival And Breast Cancer recurrence follow up										X	X (from EMR)
Hormonal Treatment Adherence Follow up	X								X	X	X (from EMR)
Diet Adherence follow up (weekly phone, text or email followup)			X	X	X	X	X	X	X		