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Gertsen Blinded Validation Project

Summary

OTraces in collaboration with the Gertsen Institute in Moscow performed a blinded validation study on OTraces breast cancer detection products, the CDx Chemistry System for processing automated tests for detection of cancer; BC Sera Dx Immunochemistry test kit for the measurements of proteins that are predictive as a group of the presence on breast cancer in women; and the OTraces Cancer Correlation Computation System for scoring blind samples for breast cancer risk. The study was conducted in two phases; Phase I) used the semi-automated OTraces LHS Chemistry System as the instrument test platform; Phase II used the OTraces CDx Chemistry System, a faster fully automated chemistry processing system. The goal of the market clearance trial was to find a replacement for the current screening mammography method for breast cancer screening (as a primary cost reduction to the Russian health care system). The replacement test must demonstrate performance equivalent to screening mammography. Accuracy of results for the blinded samples were greater than 96% for both sensitivity and specificity and the blinded samples predictive power compared well to the training set internal predictive power of 97%. Of note was that all stage 0 and stage 1 samples, both the training set and blinded samples were called correctly.

Also a different and separate model was constructed to predict cancer stage. This model produced a predictive power of 99% with only one sample scored as stage 1 when actually it was stage 2.

Measurement Systems

BC Sera Dx Test Kit

The OTraces, Inc. BC Sera Dx Breast Cancer detection Kit measures 5 low abundance proteins. 4 of these are focused on the microenvironment around the tumor (TME) and its actions to grow and the immune systems actions to kill the tumor. The fifth is a tumor marker that has no immune or other functional aspect in the tumors growth. We monitor proteins secreted by tumor cells into interstitial fluid and that promote anti-tumor actions within the TME. We measure serum level concentrations of these proteins. The computational methods yield the signature of the TME actions in the blood, thus we use serum as a proxy for TME actions. The actions of the tumor are in improving circulatory access; angiogenesis, circulation in surrounding tissue, IL-8, and vascularization in the tumor bulk, VEGF. We also observe the systemic response of patients, *i.e.* the changes in immune response to the disease. The hypothesis is that there are specific changes in levels of immune regulators, *e.g.* cytokines. Briefly, they are a pro-inflammatory cytokine, IL-6, and an anti-tumoral and apoptotic response of cytokine, TNFα. The test panel also uses one tumor marker, kallikrein 3 (PSA).

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This kit is designed to process 120 cancers scores and includes all both the capture and labeled antibody sets, calibrators, diluents and wash solutions and solid devices needed to process samples such as microtiter plates.

The test kit was manufactured by Maxim Biomedical, an FDA certified "Good Manufacturing Practice (GMP)" facility. Maxim Biomedical is a contract manufacturer of microtiter plate based immunoassays. They are the supplier to such mainstream In-vitro-diagnostic suppliers as Abbott and Roche for microtiter plate based assays.

CDx Instrument System

This fully automated testing instrument is manufactured to company specifications by Hamilton Company, based upon the MicroLab Starlet system. The CDx system is designed to optimize the performance and predictive power of the OTraces' cancer screening reagent test kits. The system allows completion of one full run of all 5 immunoassays in the test panel in one shift, with no operator intervention after initial setup. In production configuration, the system will complete 80 cancers scores per day. When OTraces reaches full population screening level, production throughput will need to increase to at least 300 cancer scores per day. The Hamilton group of systems in this category is scalable so increasing this important parameter can be done when needed. The system is fully automated with full walk away processing.

OTraces also has developed the semi-automated LHS chemistry system and some samples in this study were processed with this system, however, it was deemed by the Gertsen Institute to be of insufficient automation to service their needs.

Computation Software

The concentration measurements are processed through complex mathematic algorithms producing a score, arbitrary, from 0 to 200. 0 to 100 being not cancer and 101 to 200 being cancer, though medical needs may indicate a shift to a lower trip point to maximize sensitivity, for example. This score is not related to the stage or seriousness of the disease state but the probability of it being accurate. The scoring algorithm is based upon OTraces proprietary proteomic noise suppression and the spatial proximity correlation method. The algorithms are located on OTraces servers and the instrument is connected and operated through OTraces internet based operating system. All data transferred over the internet is encrypted and secure in accordance with FDA and HIPAA requirements. The process of converting this data into highly accurate test results is, as noted, performed remotely, using algorithms and software centrally stored in U.S. based servers by Bio-IT Solutions, Inc. exclusively and on OTraces' behalf. The OTraces servers are deployed by Razorwire Solutions and co-located in Virginia and California.

Computation of Cancer Score, Brief Description

The Cancer score is produced in a multistep process. It designed to determine an accurate signature of an active tumor microenvironment (TME) in serum. The noise suppression is intended to suppress actions

of these proteins caused by conditions other than the question of interest; which is does the patient under test have, or not have an active tumor microenvironment. In other words the serum based concentrations are used as a proxy for the TME actions, via these methods[1].

1) Concentration is converted into a new independent variable for the correlation process called Proximity Score. This new independent variable:

a) Dampens or reduces noise inherent in all proteomic concentration measurements caused by conditions that inevitably up or down regulate the biomarkers of choice that are unrelated to the condition of interest, in this case breast cancer.

b) This suppression is confined to zones that are defined by the signature of the TME is serum determined by the mean values of the active proteins for samples with and without the TME for the disease of interest. Thus this damping is anchored on the mean values of cancer and not-cancer for each cohort. There are 4 possible zones defined by 1) less than mean value of the not cancer state; 2) between the mean value of the not cancer state and the mid-point in concentration between the mean values for not cancer and cancer states; 3) above the afore mentioned mid-point and less than the mean value of the cancer state; and 4) finally above the mean value of the cancer state. Each zone represents a separate suppression zone.

c) In the translation from concentration to Proximity Score, age drift in these mean values is neutralized. This is done by further anchoring on the mean values at each year of age of the target patient samples. Thus the translation is a family of equations, one for each year of age where the equations are associated with each defined zone.

2) These new variables, Proximity Score, are then plotted in multidimensional orthogonal space (one for each biomarker). Each unknown samples is then scored by computing its proximity to the training set data points that are also plotted within the multi-dimensional space. In this case we use 5 orthogonal axes. Unknown samples that "see" only not cancer in nearby locations will be scored 0 and likewise those that "see" only cancer will be scored 200 and so on. Scores in between are weighted by the count of each state in proximity. The score thus does not indicate severity of the disease but probability of being correct. For determining severity see below on the cancer stage prediction. The correlation method is called Spatial Proximity.

The Cancer Score

The cancer is scored with an arbitrary numbering system from 0 to 200. Typically 100 to 200 is considered breast cancer, however, good medical practice may indicate additional diagnostic procedures are necessary at scores below 100. The score is not an indicator of the severity of the breast cancer, but the probability that the prediction is correct. The score is also not directly related to numerical probability. It is an indication of location density of cancer vs. non-cancer data points in a complex 5 dimensional

[1] Diagnostic Proteomics – A New Approach – White Paper – Ver. 05-21-2020C.pdf, available from OTraces, Inc.

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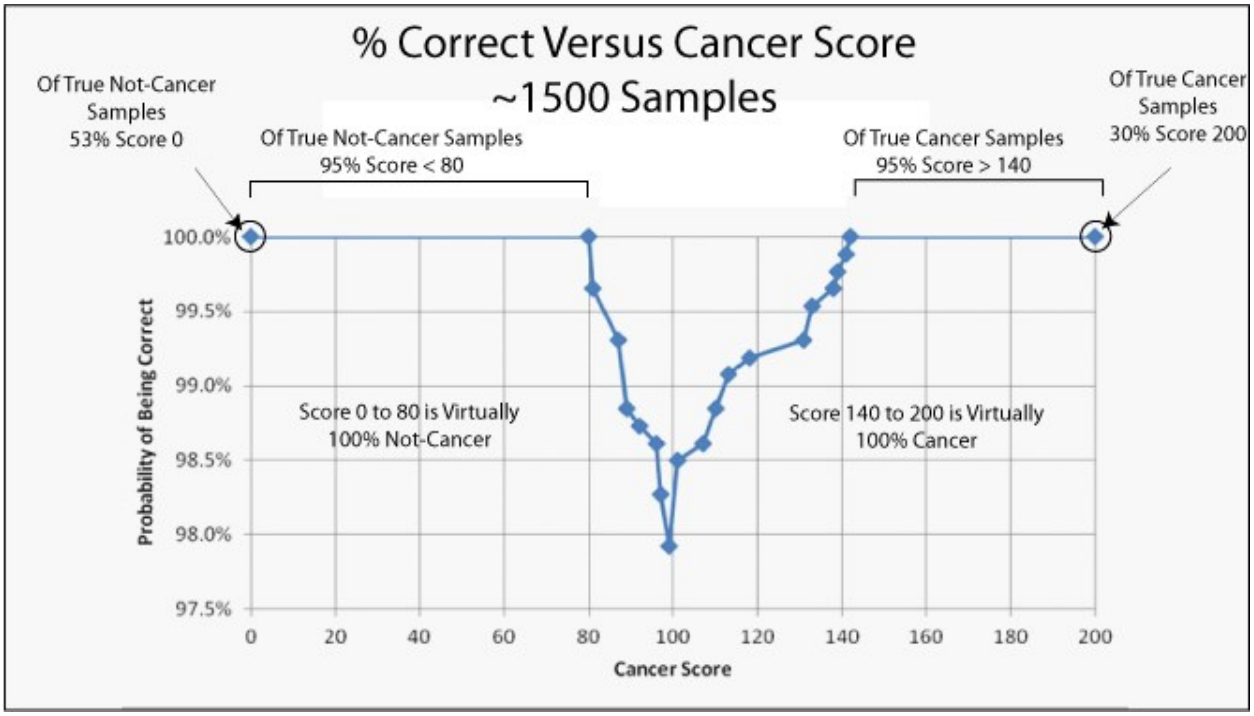
topology from which the scoring is computed. The relationship between score and probability is shown in the figure below. Scores above 140 are virtually 100% cancer based upon over 1500 validated samples. No false positives have been found at scores above 140. Likewise scores below 80 are virtually 100% not-cancer. At scores below 120 and above 80 the accuracy is about 99% to 97%.



Blinded Validation Trial Design

The trial design was done by Gertsen Institute to suit their needs. The only input from OTraces was the design of the training set for model construction (see below). The Gertsen Institute made no provision for excluding possible undetected true positive samples in the not cancer cohort (for cost reasons). Their thinking was that any true positives included in the not cancer cohort would be included as a false positive results (~0.5%). Since the samples were drawn anonymously no provision was made to follow up. OTraces was simply required to accept as a false positive possible any unknown true positive results.

The goal of the blinded trial was to achieve predictive power equivalent or better than screening mammography. Thus 90% for sensitivity and specificity were the accepted performance goals for the BC Sera Dx test kit.

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Sample Collection Criterion and Methods

Criteria for the Cancer (malignant) cohort group:

1) Mammogram positive BIRAD IV or higher.

2) Biopsy Positive - blood drawn one week after biopsy, to mitigate possible inflammatory reaction from the biopsy.

3) Population statistics should mimic a general population presenting for screening mammography. Including cancer stage and percentage presenting asymptomatic (stage 0, I, II, III and IV).

4) Age Distribution --- 35 to 75. Age distribution was broken into 4 groups (35-44, 45-54, 55-64 and 65 and up) and evenly distributed across each group, for the training asset only. The validation set was not constrained.

5) Cancer Stage - The malignant cohort contained 5% - Stage 0, 39% - Stage 1, 46% - Stage 2 and the remaining were stage 3 and 4 samples. All of the stage 0 samples and about 40% of the stage 1 samples were asymptomatic.

6) Inclusion Criterion – Biopsy positive women aged 35 to 75.

7) Exclusion criterion – none(**see section below on exclusion criterion)

8) Atypical ductal hyperplasia samples were not included in the breast cancer positive cohort.

Description of the cancer sample set actually collected.

1) 203 samples were collected in total, Phase I and II, of which 98 were used for the training set and the remained for the validation set.
2) As noted samples were draw one week after biopsy, the blood samples were processed into serum with the normal clinical lab protocol and then the serum was cryo-preserved. Treatment of the patient was initiated immediately after the blood sample draw.
3) Samples were processed on the OTraces Immunochemistry analyzers in one large batch about 6 months after samples collection began.
4) Diagnostic information presented below was determined from pretreatment imaging, biopsy and post treatment overall analysis.
    a. The cancer sample cohort consisted of:
        i. 8 Stage 0, all of which were diagnosed with Ductal Cancer In Situ (DCIS). All were diagnosed as 0 lymph duct involvement and no metastasis.

ii. 74 were diagnosed as stage 1, 49 of which had no lymph node invasion and 36 had one lymph node invasion and 1 had 2 lymph node involvements. One showed metastasis.
iii. 90 were diagnosed as stage 2, 73 of which had no lymph node involvement and the remaining had one lymph node involvement. One showed metastasis.
iv. 24 were diagnosed as stage 3, of which 16 had 2 lymph node involvements, and 5 had 3 lymph node involvements. None showed metastasis.
v. 7 were diagnosed as stage 4, 1 of which had no lymph node involvement, 3 had 1 1 lymph node involvement, and the remaining had 2 lymph node involvements. One showed metastasis.

b. There were 5 false negative cancer score calls in the cancer cohort, all were stage 2, in both the training set and the validation set.

Criteria for the Not Cancer cohort group:

1) These samples were drawn from the general population and were not chosen to have specific abnormal or non-malignant breast conditions. They were not characterized for immune related conditions. As best as possible the makeup of this group mimicked women who would present for their annual mammogram.

2) Age Distribution --- 35 to 75. Age distribution was broken into 4 groups (35-44, 45-54, 55-64 and 65 and up) and evenly distributed across each group, for the training asset only. The validation set was not constrained.

3) Inclusion Criterion – women aged 35 to 75.

7) Exclusion criterion – none(**see section below on exclusion criterion)

Exclusion Criterion

Note that it is critical to have representation in the training set for the "noise" from non-malignant conditions that may effect biomarker serum concentration levels. This method suppresses information in serum contraction measurement that is unrelated to the condition of interest in this case breast cancer. Though "Proteomic Noise Suppression" reduces this effect it cannot completely eliminate it. Thus the training set must see the residual effect of this "noise". Thus we do not exclude any non-malignant conditions from the training set. Though prudence indicates that certain conditions that are not likely to be seen at an annual health screen (e.g. such as recent healing severe trauma, or current ongoing cancer treatment) be excluded from a clinical trial under regulatory authority scrutiny. Thus the clinical trial may logically exclude such conditions from being included, but the training set will have proper representation.

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Description of the not cancer sample set actually collected.

1) 205 samples were collected in total, Phase I and II, of which 100 were used for the training set and the remained for the validation set.
2) The blood samples were processed into serum with the normal clinical lab protocol and then the serum was cryo-preserved.
3) Samples were processed on the OTraces Immunochemistry analyzers in one large batch about 6 months after samples collection began.
4) Samples were drawn from women presenting for an annual screening mammogram.
5) Non-malignant conditions noted - 15% Hypertension, 5% Rheumatoid Arthritis, and under 1% Pancreatitis, Stomach Ulcer, Anxiety, Hypothyroidism, Obesity, Peptic Ulcer, Aortic Aneurism, Aortic Sclerosis, ED, Diabetes, Coronary Artery Disease, Temporal Lobe Seizures, Cardiomyopathy, Ventricular Tachycardia, Sleep Apnea.
6) Non-malignant breast conditions noted – 27% lymphadenopathic (shotty) breast, 6.0% breast fibro adenoma, 3.5% mastopathy, all at approximately 1% or less, mastopatia, 2% fibro-fatty breast, 1.5% lactocele.
7) Two samples had a history of treated thyroid cancer.
8) There were 5 false positive cancer score calls in the not cancer cohort, one was fibro adenoma and one was lymphadenopathic (shotty) breast, and the rest were normal donors, in both the training set and the validation set.

Note the not cancer cohort could potentially contain ~0.5% undetected breast cancers. OTraces deemed accepting these as statistically false positives acceptable by the trial design and by default. Since the "putatively healthy" samples also could include immune related conditions that could impact the test these would also show up in the false positive cohort as true false positive results.

Sample Size

408 samples (~200 not cancer and ~200 with breast cancer) were processed in two phases. The sample set was split into two sets of approximately 200 (100 not cancer 98 with breast cancer, biopsy) for the training set and the rest were blind samples.

OTraces required that the training set be equally split cancer/not cancer to assure that the training set model not be biased. The slightly off equal 100/98 was due the Gertsen Institute providing actual diagnosis in the unequal numbers. OTraces accepted this slight skew. The training set requires the diagnosis up front for model processing. The blind samples had no restrictions by OTraces, except the ages need to conform to the model, 35 to 75. The blind sample makeup of not cancer vs. breast cancer was of course unknown at the time of analysis. These samples were measured by the clinical chemistry laboratory at the Gertsen Institute in Moscow on the OTraces CDx Instrument utilizing the BC Sera Dx breast cancer test kit. OTraces supplied both the instrument and the reagent test kits and supplied operator training to Gertsen

technicians. OTraces was not allowed in the clinical laboratory during the test runs. OTraces did have a qualified technician, fluent in Russian language, near the site during the test running.

Comments on the Trial Design

The goal of the Russian Ministry of Health was to find a replacement to screening mammography that is not viable in the newly created private market sector. Longitudinal studies of women who might be positive for the blood test and negative in imaging were not planned at this stage as research based statistics indicated the specificity of the blood test may be at least three times better than screening mammography. Thus striving for the best possible specificity was not of interest to the Russian Ministry of Health. Additionally the possibility of having 0.5% true positive in the false positive cohort was not deemed important to them and OTraces accepted this by default.

Breast Cancer Prediction

The cancer scoring performance is broken into three groups, 1) the training set, 2) Phase II Validation set (blind) and 3) the Phase I data set computed as blind. The table below shows the results.

The data was collected on OTraces Instruments and reagents run at the Gertsen Institute by Gertsen personnel. The cancer scores were computed on OTraces LIMS Servers located in the US. The Instruments are coupled to the server and the server performs the data computation, reporting and repository.

Phase I data was collected on the semi-automated OTraces LHS Chemistry System. This system was deemed not automated enough by the Gertsen Institute to serve the automation needs of their clinical lab, which is dedicated to clinical testing for cancer treatment and research. Phase II was conducted on the OTraces CDx Instrument system. This system is fully automated and serves the full walkway needs of the lab. The training set was developed in Phase II to set the model with the new equipment in the Gertsen Clinical lab using local Russian samples. Operations of both instruments used the same test kit, the BC Sera Dx kit. The results for these samples were supplied with actual diagnosis in order to train the breast cancer model. The Phase II Validation Set were supplied to OTraces server's blind and randomized. OTraces scored these samples and the Gertsen Institute returned the accuracy of this scoring to OTraces. The Phase I set was, as noted, run on the older semi-automated instrument. In both cases the same reagent test kit was used, the BC Sera Dx test kit. These samples were processed through the model to check the accuracy of these samples measured on different equipment. As can be seen the predictive power was about 96 to 98%.

The Breast Cancer Training Set Model has processed 209 blind samples from the Gertsen Phase I study (run as blinds) and the Gertsen Phase II study, (blinds) with a combined false negative and positive rate of 2%, or a predictive power of 98%. The overall results are shown in the table 1 below.

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Data Set	Diagnosis	Cohort Size	False Calls	Sensivity Specificity	Predictive Power	Number that failed Stability	Number Corrected	Uncertain
Training Set	Cancer	98	2	98.0%	97.0%	N/A	N/A	N/A
	Not Cancer	100	4	96.0%		N/A	N/A	N/A
Phase II Validation Set	Cancer	57	2	96.5%	98.2%	4	4	0
	Not Cancer	55	0	100.0%		0	0	0
Phase I Set	Cancer	48	1	97.9%	98.0%	6	6	0
	Not Cancer	50	1	98.0%		3	2	1

Table 1 Breast Cancer Scoring

The spatial proximity correlation method is topology based. Thus it can suffer from topology-based instabilities. The problem can be seen in the mind's eye by imagining a proteomics base test with two biomarkers. The topology in this case is a flat plane. If the resulting cancer score is impressed on a third vertical dimension. Areas with steep slopes in the third axis are susceptible to small measurement errors. These areas would deep valleys or a cone shaped topology on this third dimension. With 5 biomarkers the instability can be seen in the sixth cancer score dimension. OTraces uses a stability test to locate such data sets (samples). These are thus suspect. These cases may be seen to jump from low score e.g. 50 to score above 150 for example with noise injection. Typically 3 to 4 of these are seen in 100 data sets (see the right hand side of table 1 above). The vast majority of the data shows no shift in cancer score at all, even with +-10% noise injected into the raw concentration values. This is because much of these data sets are sitting on flat planes in the topology. These unstable data sets can be corrected. In these cases a secondary algorithm is used that is treats the concentration measurement different in the conversion into proximity score. This secondary algorithm uses ratios of the raw concentrations and it has 15 orthogonal dimensions. These unstable points are then scored with this secondary model created with these newer more complex independent variables. If the secondary model is stable for the unstable point using the primary model the secondary cancer score is reported. We will typically see 3 or 4 of these per one hundred samples. Usually two or three are corrected and the others are confirmed. We have never encountered a sample that when corrected was then found to be incorrect.

We have one point that scored unstable in both models (see table). This sample also scored an average cancer score using both models and all the "unstable" cancer scores of 100.1, exactly in the center of the scoring range. This is the only such score we have encountered in about 2000 samples. The sample was from the Phase I data set and its actual diagnosis was not cancer. This score would be reported as uncertain with a rerun recommendation. No scoring number would be reported as it cannot be trusted. This rare result is, of course possible, with this sort of analysis and must be accounted for in the reporting instructions given to the medical community in the products package insert.

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This method for correcting unstable data is patented.

Prediction of Breast Cancer Staging from BC Sera Dx test data recovered from the Gertsen Validation Studies

OTraces also created a model for prediction of breast cancer stage. This is not part of the market clearance program required by the Russian Ministry of Health, but was done to assess the ability of these biomarkers to track the actions within the TME. The figure below "Average Up regulation by Breast Cancer Stage" clearly indicates that there is a strong predictive signal for not only predicting breast cancer but also stage. Additionally, the strong signals indicate that perhaps separation of very early stage zero breast cancer from later (stage 1 and up cancers) can be done.

This project is not part of the market clearance program required by the Russian Ministry of Health. However, keen interest was expressed in determining if stage could be predicted and this could be the basis for a second longitudinal study in Russia to determine monitoring and treatment methods for very early stage breast cancer perhaps before imaging can "see" it. This notion is based upon the dramatic response of the immune system to stage 0 breast cancer. Note that all of the stage 0 cancers were verified by imaging and biopsy for this work.

Stage Prediction Model

OTraces constructed a second model to predict cancer stage. Stage cannot be construed from the cancer prediction score. It is related to probability of being accurate. The cancer cohort contained 186 cancer subjects with stage information. Of these 29 were stage 3 (or 4), 86 were stage 2 and 71 were stage 1 or 0. Only 8 samples were diagnosed as Stage 0, which are not enough samples to develop a proper correlation algorithm so these were grouped with stage 1. The model correctly called the stage in 185 of these samples. One sample was called as stage 1 whereas the Gertsen Institute diagnosed this sample as Stage 2.

OTraces plotted the average actions of these biomarkers in the transition from not cancer to cancer and its various stages, had 186 samples diagnosed with breast cancer with stage diagnosis from the biopsy. The figure below "Average Up regulation by Breast Cancer Stage" clearly indicates that there is a strong predictive signal for not only predicting breast cancer but also stage. Additionally, the strong signals indicate that perhaps separation of very early stage 0 breast cancer from later (stage 1 and up cancers) can be done. As indicated below there are not enough stage 0 breast cancer in the cohort to complete a stage 0/stage 1 and up model. However, a more complex model could be done as noted below.

A correlation Model for predicting the stage of breast cancer is not the same as the models used to predict the not cancer or breast cancer state. The mathematics of the Training Set Models is designed to separate training set data into two states, usually "STATE A" and "NOT STATE A" (e.g. breast cancer and not breast

cancer). As such the model cannot directly predict the cancer stage in breast cancer patients. The breast cancer versus not cancer score, from the cancer-scoring model will not accurately estimate cancer stage and it will not achieve high predictive power for staging. In order to use the correlation method to predict cancer stage of cancer samples from the BC Sera Dx test kit, OTraces constructs three models. These models follow the binary directive of the correlation model for "STATE A" and "NOT STATE A". Thus the three models are predictive for the groups of staging 1) Stage 1 versus Stage 2 and 3; 2) Stage 2 versus 1, and 3; and 3) Stage 3 versus Stage 1 and 2. These three models create a matrix of scores giving probability each sample falling on either side of the three cases. This matrix can then be de-convoluted to determine the predicted breast cancer stage.

Model Predictive Performance

This report documents the performance of the correlation computation method for predicting the stage of breast cancer for the positive samples from the concentration measurements from the cancer detection Gertsen studies. The two studies had 186 samples diagnosed with breast cancer. Of these 29 were stage 3 (or 4), 86 were stage 2 and 71 were stage 1 or 0. Only 4 samples were diagnosed as Stage 0, which are not enough samples to develop a proper correlation algorithm so these were grouped with stage 1. Also only one was diagnosed with stage 4 and this was grouped with the stage 3 diagnoses. This staging distribution is approximately that which would be seen in women presenting for annual screening. The cancer is usually detected before it reaches stage 3 and 4 and stage 0 is very rare as it cannot be seen easily in imaging (less than 2 mm size). These cancer samples were all positive in imaging and biopsy. When sufficient sample are obtained the staging algorithm will be able to separate stage 0 and 4 also, as a dramatic signal is seen across all stages (signal being a significant difference in age adjusted mean values for cancer and not cancer). Out of the 186 total samples diagnosed by biopsy to have breast cancer by the Gertsen Institute the staging correlation algorithm miss called one sample as stage 1 whereas the Gertsen Institute diagnosed this sample as Stage 2 (99.5% Predictive Power).

There were not enough samples to measure an independent validation sample set. Alternatively, the model was validated by a method called boot strapping. One sample is removed from the training set samples group and the model is then rebuilt. This removed samples in then scored by the new reduced model. This process is done for all samples in the training set one-by-one. The miss calls are then added up to score the internal predictive power of the training set. The results were consistent across the data set. The same single incorrect sample was consistently called as Stage 1 instead as stage 2, with 99% predictive power consistent across the data boot strapped data set.

The stage 0 and stage 4 condition can be de-convoluted in a similar way with sufficient samples.

Biomarker Behavior by Stage

The biomarkers have a pronounced amplified reaction to the tumor at the early stage 0 of the cancer indicating a likely strong early stage performance.

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Figure 1 below shows average biomarker concentration by stage (-1.0 is not cancer on the graph). Note that the 5 biomarkers strongly up regulate at the onset of stage 0 cancer. This indicates a clear differentiation from not cancer to nascent cancer and a strong likelihood of very strong early detection performance of the final scoring model when combined with a staging model.

As noted above all five biomarker sharply up regulate at the onset of stage 0 breast cancer, then 4 of the 5 settle back somewhat and VEGF continues to rise. One could speculate that the immune system is adjusting to the tumors presence and/or the tumor is disguising itself from the immune system; and that as the tumor grows it demands increasing vascularization. Note also that the average cancer score jumps sharply from 21 to 190 upon onset and then drops down a bit too about 175. As noted above this behavior is not related to the severity of the cancer but the collective proximity of training set data points to the location of the blind sample on the 5 dimensional topology of the training set model. A higher score means that the prediction of cancer has a higher probability of accuracy, not more severe. Stage 0 cancers tend to locate on the grid topology near clusters of training set cancers.

This behavior would tend to indicate a strong early stage detection capability, perhaps significantly earlier than can be detected by imaging.

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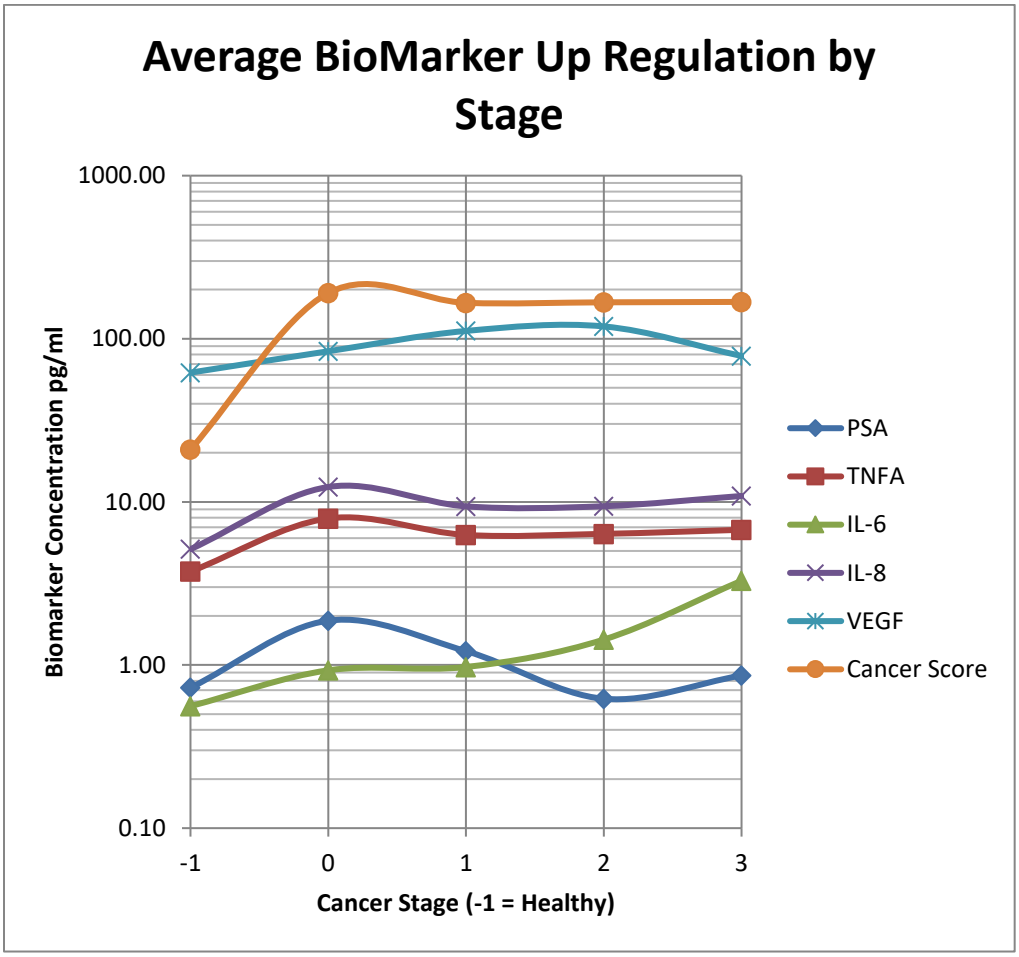

Figure 1 Biomarker Behavior by Stage

Critique of the Model

The model consists of 31 stage 3 (or4) samples, 90 stage 2 samples and 82 stage 1 (or 0) samples. There were only 1 stage 4 sample and 4 stage 0 samples in the data set, not enough to train the model, thus these were grouped with the next stage 0 to 1 and 4 to stage 3. An ideal model would have about 100 samples of each cancer stage and it would be balanced in that each stage grouping would have an equal number of samples. Furthermore there are not sufficient samples to test the model on blind samples. However, the model internal outcome, one sample out of 186 incorrect is representative of the separability of these biomarkers when grouped by stage. Though this model will not be ideal for translation to blind samples because of the skew in group sizes, we can surmise that a model with no skew will be highly predictive.

The graph above showing average concentration behavior of these biomarkers is not a model and cannot be used to predict anything regarding staging. The Training Set Model is a highly complex 5 dimensional topology that has internal scoring for staging. This scoring is based upon proximity to the training set data placement in the topology. And the training set data, in order to not skew the predicted outcome, should be balanced. The four stage 0 samples though is sufficient to estimate the average concentrations of the 5 biomarkers at this stage of cancer thus the rapid up regulation from not cancer is real, and indicates strong predictive power in the transition from not cancer to stage 0.

The next step in this experiment would be to:

1) Collect sufficient samples to balance the model, with the focus on collecting at least 100 stage zero breast cancer found from imaging and verified by biopsy, as was done in this study. This collection process will be done on cancer positive patients found in general screening after-market launch. This will allow us to finalize this model. This model will be built a model strictly for the transition from not cancer to stage 0, and from stage 0 to higher stages.

2) Provide on an interim basis the cancer score for general diagnosis and the stage 0 prediction during the ongoing general screening operations. The hypothesis being that this model would be highly predictable for detecting very early stage breast cancer (possibly before imaging).

3) Collect from general screening patients with detected breast cancer, by the blood test and who are predicted to be stage zero but cannot be seem in imaging.

4) The next step is out of OTraces' purview but the plan would be to develop a monitoring and treatment plan for these patients.

It should be noted that it is thought that there are many undiagnosed women with stage 0 breast cancer, as it is very difficult to see in imaging and it can take two years for a detected stage0 breast cancer to progress to stage 1. Some of these women may convert to not cancer due to immune system actions but, of course this is unknown because the presence of this very early stage cancer is unknown.

We think predicting the variance from stage 1 through 4 is not as valuable as the oncologist will make this determination from the biopsy. However, calling stage 0 we think may be very valuable as will increase the diligence of radiologists reading images and these women may be ideal candidates for a breast cancer vaccine as, at stage 0, the immune system is at its strongest in its anti-tumor actions. Adapting current treatment method may result in simple monitoring of these patients with the blood test until then are negative or until they can be seen in imaging and then perhaps simple lumpectomy after it is located.