# SILBIOTECH, INC.

**Lab Address:** 4851 International Blvd, #102
Frederick, MD 21703
**Clinical Director:** John Gillespie, M.D

**Office Address:** 15807 Chagall Ter. North Potomac, MD 20878
**Phone:** 301-787-1216, **Fax:** 301-987-9724
**Email:** info@bbdrisk.com, **Website:** www.BBDRisk.com

## DESCRIPTION OF THE BBDRisk Dx® TEST

• The BBDRisk Dx® test profiles four cancer biomarker oncoproteins, Matrix Metalloproteinase-1 (MMP-1), Carcinoembryonic Antigen-related Cell Adhesion Molecule 6 (CEACAM6), Hyaluronoglucosaminidase 1 (HYAL1), Highly Expressed in Cancer Protein 1 (HEC1), in breast hyperplasias utilizing formalin fixed paraffin embedded (FFPE) tissue by immunohistochemistry.

• Based on the above four marker profile, a comprehensive 'Cancer Risk Score' will be calculated in the range of 0-10. The Risk score is then compared with the plurality of risk scores in risk score data base (created with the validation study data) to classify the patient as 'Low Risk' (Risk Score =< 0.5), 'Intermediate Risk' (Risk Score (>0.5 and =<5.4) or 'High Risk' (Risk Score > 5.4) for developing future breast cancer and determine the cancer rate for a given risk score (please see the sample Test Results reports of low, intermediate and high risk results attached).

• The test Results Report will provide a 'Cancer Risk Score' for the patient based on her personalized tumor cancer marker profile and an overview of the 'Cancer Rate' over 5-10 years among women who have a similar 'Risk Score'.

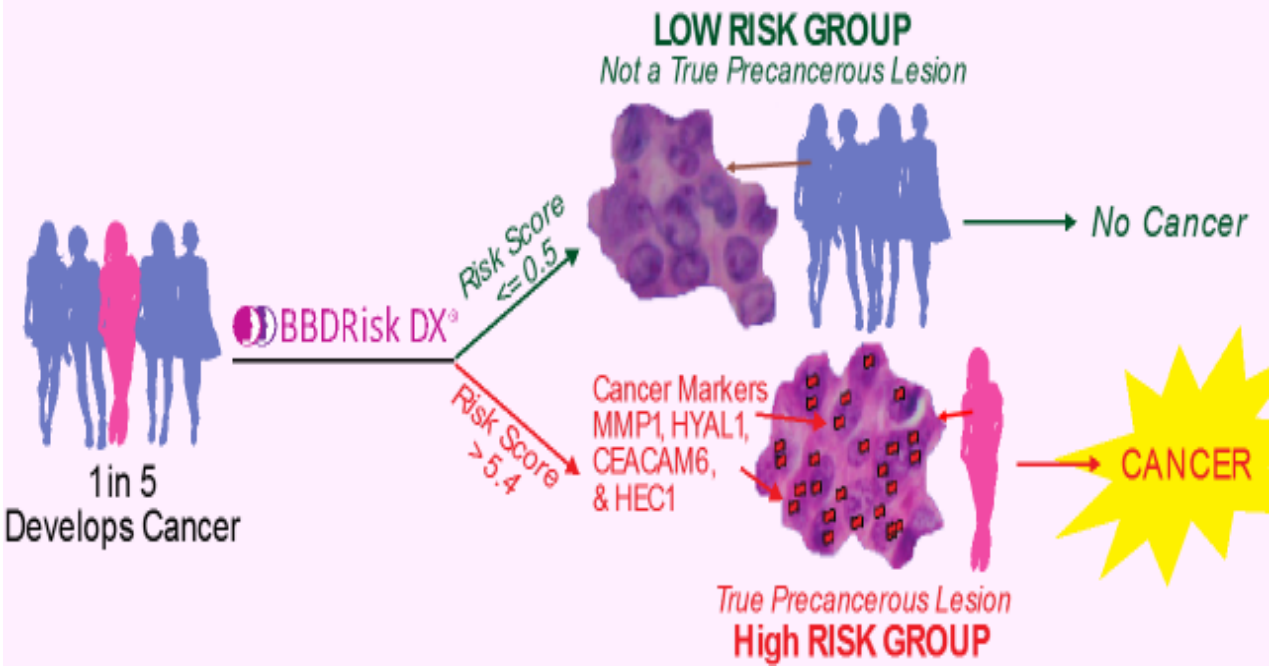
• Based on the Risk Score and the cancer rate for a given risk score, the patient can discuss her therapeutic options with her healthcare provider and choose the therapy that would be best suitable for her current health condition or no treatment at all if the risk will be low.

• For example, standard screening for women whose BBDRisk Dx® results indicate a "Low Risk" (Score=< 0.5) of developing cancer. If BBDRisk Dx® results indicate that a patient has Elevated Risk of developing breast cancer (score >5.4), risk-reducing medications (tamoxifen, raloxifen, aromatase inhibitors) and/or prophylactic mastectomy that are currently FDA approved for women with atypias will reduce the risk by delaying the onset of cancer, or preventing the development of cancer over the long term. If the score is >0.5 and =< 5.4 (intermediate risk), increased surveillance.

• Thus the BBDRisk Dx® test provides important personalized information on the risk level based on the tumor biology of a patient in terms of a Risk Score when deciding on undertaking prophylactic endocrine treatments and or surgery (please see flow diagrams below).

**Illustrations of how BBDRisk Dx® Test works.** Please see the Flow Diagrams below:

# BBDRISK DX® CLINICALLY VALIDATED GENOMIC TEST FOR MANAGING BREAST ATYPICAL HYPERPLASIA




# Integration of BBDRisk DX® Test in Clinical Oncology Practice to Prevent Breast Cancers

Radiologist identifies a suspected mass by Mammography/MRI and reports to primary care physician. The patient referred to a Surgeon for biopsy.

Radiologist → Surgeon → Pathologist

Pathologist reviews histology of the mass and diagnoses as Atypical Hyperplasia or Usual Hyperplasia or Papilloma or any combination.

Surgeon biopsies the mass and sends the tissue to a Pathologist

Mail

BBDRisk Dx® Test ordered by Surgeon, or Oncologist or Primary Care Physician or Gynecologist and tissue sample mailed to Silbiotech by Pathologist.

BBDRisk DX®

Multimarker BBDRisk Dx® Test on hyperplastic tissue

| Risk Score ≤ 0.5 Low Risk | Risk Score 0.5 and ≤ 5.4 Intermediate Risk | Risk Score > 5.4 High Risk |
|---|---|---|
| Standard Screening | Increased Surveillance | Tamoxifen or Raloxifen or AIs and/or Mastectomy → Prevention Treatments |

Stop ~20% – 25% of overall sporadic breast cancers/year → STOP

## Documentation showing clinical validation of the BBDRisk Dx test system, and markers used to perform testing.

The following clinical validation data published in peer reviewed journals for the BBDRisk Dx test are submitted:

**Article #1.** I. Poola, Q. Yue, J. Gillespie, PS. Sullivan, et al (2019) Breast Hyperplasias, Risk Signature and Breast cancer. **Cancer Prevention Res.** 12,471-480 **PMID**: 31239263

• This is a clinical validation study of the four BBDRisk Dx® Test analytes (cancer markers) using hyperplasia samples (n= 440) with up to 19 years of clinical follow up information.

• The data published in this article established the validity of the four analytes of BBDRisk Dx® Test assay, MMP-1, CEACAM6, HYAL1 and HEC1, for predicting future breast cancer development with very high (91%) accuracy, specificity, sensitivity, PPV and NPV. The validation data published in this article provided direct evidence for assaying the above four cancer analytes for the BBDRisk Dx® Test service.

• This study also established an algorithm for translation of the above four cancer marker expression data into a comprehensive Risk Score from 0-10 with higher risk score corresponding to higher risk of subsequent cancer development. The algorithm for calculation of breast cancer Risk Score of a new patient sample for the BBDRisk Dx® Test service is published in this article.

• The samples size utilized for the validation of the test in this study has the power of more than 99% with significance level of 0.0001. Therefore, the sample size applied in this study was sufficient for establishing the clinical utility of the test

• In addition, this article describes the development of a model for risk stratification of patients based on the risk score into low (risk score =< 0.5), intermediate (risk score >0.5 and =< 5.4) and high (risk score >5.4) risk groups. The risk reporting algorithm for stratifying the risk of a new patient as low, intermediate and high risk in the BBDRisk Dx® Test service is published in this article.

• Finally, this study established the cancer rates at 5 years, 10 years, 15 years and beyond for the low (risk score =< 0.5), intermediate (risk score >0.5 and =< 5.4) and high (risk score >5.4) risk groups.

• The algorithm for calculation of cancer rate for a given risk score of a new patient in the BBDRisk Dx® Test in comparison with plurality of risk scores in the risk score data base that was established in this study.

## Other related Clinical Validation Data.

**Article #2.** I. Poola, R. L. DeWitty, et al (2005) Identification of MMP-1 as a putative breast cancer predictive molecular marker by global gene expression **Nature Medicine**, 11, 481-483. **PMID**:15864312

• This article describes the global gene expression study of breast atypical hyperplasias from patients who subsequently developed cancer in comparison with those who did not develop for a minimum of 7 years.

• This study describes the discovery of a number of putative breast cancer predictive markers by gene expression analyses (data are published in NIH GEO data base Access#GSE2429). The putative markers included several cancer promoting molecules among which are CEACAM6, HYAL1, MMP-1 and HEC1

• Of the several discovered putative markers, MMP-1 was validated in this study using 105 breast hyperplasia tissues and the clinical follow up data on breast cancer development or non-development

• The data published in this article established for the first time that the risk for sporadic breast cancers among hyperplasia patients can be predicted based on the expression of MMP-1 in hyperplasia tissue

• Two additional cancer markers discovered in this study, HYAL1 and CEACAM6, were further validated and published (article #3 and article #4 respectively presented here)

• The combination of the above three markers along with a fourth putative marker, HEC1 were further validated in a large study (n=440) and published in the above article #1 and are the analytes for the BBDRisk Dx® Test assay service.

**Article #3.** I. Poola, J.Abraham, J. et al (2008) Molecular risk assessment for breast cancer development in patients with ductal hyperplasia. **Clinical Cancer Research**, 14, 1274-1280. **PMID**: 18281563

• This is a clinical validation study using 161 breast hyperplasia tissues of patients and with clinical follow up information on breast cancer development or non-development.

• This study established the validity of a cancer marker, HYAL1, that was previously discovered by gene expression studies (article #2 Nature Medicine described above), as a breast cancer predictive marker with very high sensitivity, specificity, PPV and NPV using the above patient samples.

• The HYAL1 validation data published in this article was the basis for selecting this marker for further validation in a large study that was later published in the above article #1 and as one of the four analytes of the BBDRisk Dx® Test assay service.

**Article #4.** I.Poola, B. Shokrani, et al (2006) Expression of CEACAM6 in Atypical Ductal Hyperplasias is associated with development of breast cancer. **Clinical Cancer Res.** 12, 4773-4783 **PMID:** 16899629.

• This is a retrospective clinical study using 108 breast hyperplasia tissues with clinical follow up information on breast cancer development or non-development.

• This study established the validity of a cancer marker, CECAM6, that was previously discovered by gene expression studies (described here in article #2, Nature Medicine), as a breast cancer predictive marker with very high sensitivity, specificity, PPV and NPV using the above patient samples.

• The data published in this article also established that CEACAM6 in combination with another cancer marker, MMP-1, increased the Sensitivity and Specificity of breast cancer prediction in both atypical and non-atypical groups of hyperplasia patients.

• The data published in this article also established for the first time that the combination of multiple markers increases the accuracy of breast cancer risk prediction

• The data published in this article on CECACAM6 individually and in combination with MMP-1 are the basis for selecting these two markers along with HYAL1 and HEC1 for further validation in a large study that was later published in the above article #1 and selecting the combination of the above four markers as analytes of BBDRisk Dx® Test service.