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NCCN Guidelines Panel: Breast Cancer Risk Reduction Panel

On behalf of Silbiotech, Inc., I respectfully request the Breast Cancer Risk Reduction panel to review the enclosed data on BBDRisk Dx® (Benign Breast Disease Risk Dx) Test for inclusion in the breast cancer risk reduction guidelines for patients diagnosed with high risk atypical-, and non-atypical hyperplasias.

Specific Changes: Recommend the tumor biology based BBDRisk Dx® MAAA (Multianalyte Assays with Algorithmic Analysis) Test as a routine component of risk evaluation of breast atypical hyperplasias (ADH and ALH) in page BRISK-3 and non-atypical (Usual-, and papilloma) hyperplasias and for choosing the candidates for risk reducing prophylactic endocrine therapy and or prophylactic mastectomy.

FDA Clearance: Not required because BBDRisk Dx® is a LDT. It is regulated by CLIA (#21D2137469)

Rationale: Clinical follow up studies have established that ~20-25% of atypical-, and ~10% of non-atypical (UDH, Papilloma) hyperplasia subjects subsequently develop breast cancer after 1-5 or more years. The clinical challenge has been how to stratify the 20-25% of atypical-, and ~10% of non-atypical high risk women from the low risk group to personalize risk reducing measures. Until now there were no molecular tools available for risk stratification of hyperplasia patients. Currently, prophylactic measures are administered without any molecular basis to willing patients. As a result, low risk women are unnecessarily over-treated and subjected to side effects of endocrine therapy or undergoing unnecessary mastectomies. On the other hand the patients who have the real risk but opt out of risk reducing therapies to avoid side effects are not getting the benefit of prevention measures

To fill the unmet need, Silbiotech has developed BBDRisk Dx® Test using State-of-the-Art molecular approaches for assessing a tumor biology based breast cancer risk and administering risk reducing measures. Briefly, the putative cancer predictive markers were first discovered by gene expression studies of atypical hyperplasias from patients who subsequently developed cancer in comparison with those who did not develop. Second, selected putative markers were validated using sets of Case (patients who subsequently developed cancer) and Control (cancer-free subjects) tissues for breast cancer risk prediction. Third, a multimarker (MAAA) test model was developed that predicts cancer with very high Accuracy, Sensitivity, Specificity, PPV and NPV. Finally, the validity of the developed MAAA test was established in independent sets of hyperplasia tissues with up to 19 years of follow up information on breast cancer development or non- development.

The following articles are submitted in support of this proposed change.

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 4 BBDRisk Dx® Test analytes that were previously discovered by gene expression (article #2) using hyperplasia tissues (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 the 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 of 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 derived from the data published in this article.

• In addition, this article describes 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 for future cancer development. The algorithm for stratifying the risk of a new patient as low, intermediate or high risk in the BBDRisk Dx® Test service is derived from the data 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 database is derived from the data published here.

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 molecular 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 3 markers along with another putative marker, HEC1, were further validated in a large study (n=440) and published in the above article #1 and are the analytes for BBDRisk Dx® Test assay.

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

• In this study, the validity of HYAL1 (discovered by gene expression studies, article #2 Nature Med. above), was established as a breast cancer predictive marker with very high sensitivity, specificity, PPV and NPV using 161 hyperplasia tissues and clinical follow up information on breast cancer development or non-development.

• The HYAL1 validation data published in this article was the basis for selecting this marker for further validation in a large study and published in article #1 and as one of the 4 analytes of the BBDRisk Dx® Test.

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.

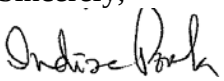
• This is a validation study of CEACAM6 (discovered by gene expression studies (article #2)) using 108 breast hyperplasia tissues with clinical follow up information on breast cancer development or non-development.

• This study established the validity of CECAM6 as a breast cancer predictive marker with very high sensitivity, specificity, PPV, NPV and accuracy.

• The data published in this article also established that CEACAM6 in combination with another cancer marker, MMP-1 (article #2 above), increased the Sensitivity, Specificity, PPV, NPV and accuracy of breast cancer prediction in both atypical and non-atypical groups of hyperplasia patients.

• The data published in this article on CECACAM6 individually and in combination with MMP-1 are the basis for selecting these 2 markers along with HYAL1 and HEC1 for further validation study that was later published in the above article #1 and selecting the combination of the above 4 markers as analytes of BBDRisk Dx® Test.

Sincerely,



Indira Poola, Ph.D