Characterization of HER2 Amplification in the Cerebrospinal Fluid of Patients with Leptomeningeal Disease in Stage IV Patients with Breast Cancer

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Leptomeningeal Disease (LMD) is a devastating complication that occurs in approximately 5% of patients with breast cancer.

Survival with LMD is 3 months or less when untreated.

LMD is diagnosed via clinical and radiographic evaluation as well as by cytology analysis of the Cerebral Spinal Fluid (CSF).

These methods are unable to identify actionable mutations in the CSF, which limits the choice of therapy.

CNSide assay is a commercially available dual platform that captures CSF tumor cells and detects actionable mutations in the supernatant of the CSF.

Recently, a single institution experience as well as a case study demonstrated improved overall survival in HER2 positive patients (primary tumor) that were diagnosed with LMD and treated with Intrathecal Trastuzumab.

Previously it was shown that HER2 can be detected on CSF tumor cells using Immunocytochemistry.

Currently, several clinical trials are exploring the efficacy of anti-HER2 directed Intrathecal therapy for patients with HER2 amplification as detected in the primary tumor.

This work shows the use of CNSide in characterizing the CSF for HER2 amplification, and other actionable mutations in patients with breast cancer with a suspicion of LMD.

**Methods**

- **CSF of sixty-three (n=63) patients with breast cancer and a suspicion of LMD was collected in CEE-Sure™ tubes.**
- **CSF Tumor Cells were captured using a combination of a 10 antibody capture cocktail and a Streptavidin coated microfluidic channel.**
- **Captured cells were stained via Immunocytochemistry for CD45, Cytokeratin and DAPI and subsequently processed for HER2 FISH analysis.**
- **CSF Supernatant was processed using the CNSide™ assay platform.**

**Detection of HER2 by FISH on CSF tumor cells**

HER2 switch from primary tumor to LMD detected on CSF tumor cells

**Comparison biomarkers in primary tumor vs CSF**

**Conclusions**

- **HER2 amplification was detected in 35 of 63 (56%) breast cancer patients with LMD.**
- **CNSide identified HER2 heterogeneity compared to the primary tumor in 24 of 63 (38%) of patients with LMD.**
- **HER2 amplification by FISH was identified in one patient who was HER2 negative by NGS.**
- **Cepi7 polyclonal was identified in 10 of 63 (16%) of patients.**

**Polysomy of Chromosome 17 in patients with equivocal HER2 FISH results on the primary tumor**

<table>
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<th>Patient No.</th>
<th>Assay Result of Chromosome 17</th>
<th>Percentage of cells (of 200 evaluated)</th>
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<tbody>
<tr>
<td>8</td>
<td>Tetrasomy</td>
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</tr>
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<tr>
<td>17</td>
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<td>57%</td>
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**References**

5. PETC1000097
8. Dobs et al. JCO (2014)