The Detection of Disseminated Pineal Parenchymal Tumor of Intermediate Differentiation (PPTID) in Cerebrospinal Fluid (CSF) Using Cell Capture and Immunocytochemistry

Kaitlyn Armijó1, Nathan Swedd1, Steven Hiara2, Tyger Piccher1, Deanne Fisher1, Kelly Kreitzburg1, Barbara Blouw1, Mikayla Martin2, Leslie Chen1, Michael Dugan1, Santosh Kaveri1
1Biocert, Inc., Department of Translational Neurosciences, Pacific Neuroscience Institute/Providence Saint John’s Cancer Institute

Background
- Pineal parenchymal tumors of intermediate differentiation (PPTID) account for 21% to 54% of pineal parenchymal tumors and may be complicated by cerebrospinal dissemination (most commonly at time of recurrence; up to 4-10 years post resection).
- The integral membrane protein synaptophysin is expressed in neuroendocrine cells and virtually all neuronal nuclei that contribute to synaptic transmission.
- PPTID frequently demonstrates synaptophysin independently of other neural differentiation markers.
- Cerebrospinal fluid (CSF) cell capture assays have demonstrated utility for assessing disseminated intracranial neoplasms.
- This study explores the use of cell capture technology paired with synaptophysin immunocytochemistry for assessing CSF specimens in a patient with PPTID.

Methods
- Reagent Testing:
  - The synaptophysin antibody (SYN) was titrated and compared on SCLC-21H and SK-MEL-28.
  - SCLC-21H cells were captured (CNSide technology, Biocert, Inc.) and immunocytochemistry was performed with SYN, and channels were digitally imaged.
- Case Study:
  - 30-year-old female patient with PPTID diagnosed two years prior was suspected of intracranial dissemination.
  - Five (5) CSF specimen collections were obtained over the course of 165 days.
  - Approximately 7 mL of CSF was submitted for each cytology and cell capture.
  - Cytology was performed at Providence Saint John’s Health Center.
- Cell capture and immunocytochemistry (CNSide platform, Biocert, Inc.) compared multiple capture antibody cocktails termed “1986,” “1822,” and “gTP1.”
  - 1986 is a cocktail of antibodies including EpCAM, TROP2, HER2, EGFRI, MET, MUC1, FOLR1, SUSD2, CD318, and NCAD.
  - 216 is a cocktail of antibodies including CD56, MCEP, HNF1, and AC8.
  - gTP1 is proprietary to Biocert, Inc.
- Cells of interest (COI) were defined immunophenotypically as synaptophysin positive and CD45 negative.
- Patient was started on intrathecal (IT) chemotherapy on day 21; treatment was discontinued on day 164 for clinical worsening.

Conclusions
- A modified CNSide assay may offer a novel way to assess response to treatment in a primary brain tumor arising from the pineal gland.
- Some PPTID patients will develop cerebrospinal dissemination and the means of evaluating this condition are currently very limited.
- Craniospinal control in this setting can affect quality of life and overall survival.
- Cell capture technology in conjunction with appropriate immunocytochemical assays demonstrated the potential to assess CSF samples in a PPTID patient with a suspected disseminated neoplasm.
- Further investigation into cell capture methods, immunohistochemical candidates, and clinical outcomes is warranted.

Mean fluorescence intensity (MFI) distributions among cells of interest for synaptophysin and antibody capture cocktail immunoreactivity on a single CSF sample (day 164)

Cells of interest are immobilized and interrogated immunophenotypically using both inclusion (i.e., synaptophysin) and exclusion (i.e., CD45) markers.

Mean fluorescence intensity (MFI) distributions among cells of interest for synaptophysin and antibody capture cocktail immunoreactivity on a single CSF sample (day 164)

Average synaptophysin MFI among this population remained consistent between antibody capture cocktails; however, gTP1, 1986, and 1822 cocktails demonstrated varying signal intensities among cells of interest.

Mean fluorescence intensity (MFI) distributions among cells of interest for synaptophysin and antibody capture cocktail immunoreactivity on a single CSF sample (day 164)

CNSide Workflow and Results

Cell capture and immunocytochemical workflow

Cells of interest per mL vs. time in a single patient with PPTID utilizing different capture cocktail formulations

CNSide Workflow and Results

Mean fluorescence intensity (MFI) distributions among cells of interest for synaptophysin and antibody capture cocktail immunoreactivity on a single CSF sample (day 164)