Detection of mutant p53 positive glioblastoma cells in cerebrospinal fluid (CSF) using a microfluidic based immunoassay

Background
- Because tumors of the central nervous system (CNS) may shed cells that accumulate in cerebrospinal fluid, CSF tumor cells (CSF-TCs) can be used in lieu of brain biopsy to evaluate various cellular biomarkers.
- Tumor suppressor p53 expresses in a wide variety of tumors; cell-free p53 DNA has been observed in CSF.2,3 glioblastoma multiforme (GBM) patients with simultaneous EGFR and p53 mutations have worse outcomes than with tumors showing a mutually exclusive pattern.4
- Biocept investigated the presence of the mutant p53 in GBM tumor cells using antigen specific antibodies cocktail based and microfluidics technology.
- Biocept adapted enhanced cellular analysis methods, antibody capture, microfluidic channels, molecular evaluation by IHC and FISH, and digital imaging techniques originally developed to identify rare tumor cells in blood to evaluate tumor cells in CSF.
- Biocept’s CNSide cell capture technology successfully identified tumor cells in CSF from a GBM patient with confirmed mutant p53 gene (R273C).

Methods
- Flow cytometry was performed on continued cancer cell lines, with or without p53 mutation, to measure anti-mut p53 antibody specificity; detection was accomplished with labeling with PE-labeled anti-mouse IgG.
- Patient research use only (RUO) CSF samples and tumor cell lines were incubated in Biocept’s CSF preservation tube for at least 48 hours at room temperature before processing.
- Samples were labeled with capture antibodies targeting glioma surface antigens, followed by labeling with biotin anti-mouse IgG.
- CSF-TCs were enriched through Biocept’s streptavidin coated microfluidic device, followed by fixation, permeabilization and staining against CD45, mutant p53 or p53 and also labeling with fluorescently labeled streptavidin and DAPI.
- Analysis was performed by an automated imaging system.
- Cell capture and immunochemistry were performed with CNSide platform at Biocept, Inc., the glioma antibody capture cocktail (gTP1) used is proprietary to Biocept, Inc.

Conclusions
- A modified CNSide assay may enable recovery of glioblastoma cells from cerebrospinal fluid (CSF)
- Initial experiments distinguished cell lines with known inactive conformations of p53 using mut-p53 immunocytochemistry.
- The glioma antibody capture cocktail (gTP1) captured p53 mutated cells from CSF in a patient with glioblastoma in an early clinical feasibility study.
- Additional experiments are needed to optimize the gTP1 capture cocktail and evaluate the clinical utility of this assay.

Workflow and Results
- Biocept’s CNSide assay successfully identified tumor cells in CSF from a GBM patient with confirmed mutant p53 gene (R273C).
- The glioma antibody capture cocktail (gTP1) captured p53 mutated cells from CSF in a patient with glioblastoma in an early clinical feasibility study.
- Additional experiments are needed to optimize the gTP1 capture cocktail and evaluate the clinical utility of this assay.