**Background**

Approximately 70,000 new cases of brain tumors will be diagnosed this year with glioblastoma (GBM) accounting for about 17% of those cases. Unfortunately, the median survival for patients with GBM is only 14.6 months due to the complexity of the tumor, the pattern of diffuse spread within the brain, and the lack of effective therapeutic options. Complex in vitro tumor models developed in 3D better mimic in vivo biology, and, when utilizing patient-derived cells, may offer robust platforms for new drug development and patient-specific drug response profiling. We have previously shown this to be true with both breast and ovarian cancer and we have adopted this approach to modeling GBM eX vivo. We hypothesize that primary derived GBM tissues and stem cells cultured in complex 3D microenvironments can recapitulate the intra-tumor heterogeneity and drug resistance similar to that found in the clinic. To this end, we have developed 3D models of GBM that incorporate tumor cells and endothelial cells within a complex extracellular matrix and cultured them for up to 2 weeks under perfusion flow. We have created these models using both cell lines and primary patient cells.

**Methods**

**Co-Culture Model Development**

Tumors were removed and enzymatically digested into single cells. The single cells were either cultured as 3D spheroids in KIYATEC’s EV3D™ DRP platform or allowed to develop into neurospheres. 3D spheroids were treated with 11 clinically relevant chemotherapies and targeted agents. Viability was assessed and dose response curves were generated using non-linear regression. Perfused 3D heterotypic Microtumors (pMT) were made of neurospheres (NS) and endothelial cells in ECM and cultured for up to 2 weeks in the 3DKUBE™ perfusion microreactor, which supports non-lytic analysis. Additionally, the impact of normoxic environmental (20% O₂) conditions and hypoxic environmental conditions (5% O₂) on the cellular microenvironment was examined by culturing 3D heterotypic Microtumors made of NS for up to 2 weeks in the 3DKUBE perfusion microreactor.

**Conclusions**

- Cell viability is affected by perfusion
- NS cultured with HBCs show a general increase in cellular metabolism
- NS grown under normal oxygen tension show general increase in cellular metabolism compared to hypoxia
- U87 NS grown in suspension culture lose CD133 expression, but show increased CD56 expression over time
- Drug response profiling is consistent with Temozolomide resistance and NGS sequencing results

This work is supported by NCI SBIR Contract #: HHSN26120130043

Acknowledgements: Chris Corless, MD, PhD at Knight Cancer Inst.