**LIST ALL AUTHORS and AFFILIATIONS – underline presenting author**

Patrick Kellish¹, Daniil Shabashvili¹, Masmudur Rahman¹, Connor Hartzell¹, Mary Reinhard¹, Grant McFadden², Frederic Kaye³, Maria Zajac-Kaye³

1) Department of Anatomy and Cell Biology, University of Florida. 2) The Biodesign Institute, Arizona State University. 3) Animal Care Services and Veterinary Pathology, University of Florida. 4) Department of Medicine, University of Florida.

**TITLE**
Oncolytic Virotherapy for SCLC; Priming Immunity for More Effective Checkpoint Inhibition

**HYPOTHESIS:**
We hypothesize that MYXV oncolytic virotherapy will selectively target the malignant SCLC lesions and promote tumor specific immune responses. Combining treatment with immune checkpoint inhibition will enhance this effect by neutralizing immunosuppressive responses resulting in decrease tumor burden and increased survival.

**BACKGROUND/AIMS:**
Small cell lung cancer (SCLC) is an aggressive subtype of lung cancer with few treatment advances over the past 3 decades and poor survival. Oncolytic virotherapy employs a viral vector that has selective cytotoxicity for tumor cells and non-toxic for normal cells and tissues. Tumor specific viral infection and replication stimulates an initial innate immune response where acquired tumor antigens are presented to T-cells. Antigen primed T-cells then drive a tumor specific adaptive immune response. Combining an oncolytic virotherapy with immune checkpoint inhibitors that block inhibitory immune checkpoint molecules can enhance antigen presentation, T-cell activation, and increased T-cell survival in the tumor microenvironment. Myxoma virus (MYXV) has been widely tested in Australia to control rabbit populations with no toxicity to humans. MYXV selectively infects and replicates in mouse and human SCLC tumors cells with no cytotoxicity to normal tissues and is a promising oncolytic virotherapy agent that has not been tested in human lung cancer or in clinical trials. We have observed that untreated SCLC tumors in our genetically engineered mouse (GEM) model are devoid of immune cells, utilizing MYXV to first populate the tumor microenvironment with immune cells is crucial for observing maximal therapeutic effects from currently approved immune checkpoint inhibitors.

**METHODS:**
To study MYXV infection and viral replication in vitro, we utilized human and mouse SCLC cell lines with MYXV engineered with fluorescent reporters, evidence of viral replication was confirmed by transmission electron microscopy and the ability of infected cells to generate infectious viral progeny. Using an optimized conditional GEM model (Ad-Cre mediated p53/Rb1/p130 null) we examined the effects of intrapulmonary MYXV treatment on SCLC tumors at 5 and 30 days post treatment. Survival analysis was determined following intranasal MYXV administration at 100 days post-Ad-Cre mediated tumor induction. We also tested direct intratumoral MYXV injections in patient derived xenografts (PDX) and subcutaneous syngeneic allograft tumors in immunocompetent mice.

**RESULTS & CONCLUSIONS**
We have demonstrated efficient MYXV infection, viral replication, and cytotoxicity in both human and mouse SCLC cell lines in vitro while not detecting productive infection or cytotoxicity in non-tumorigenic control cell lines BEAS-2B and NHBE. Following intranasal MYXV instillation we observed active MYXV replication localized exclusively within lungs of tumor bearing GEM model mice at 3 days, and at 7 days post treatment MYXV replication was no longer detected. Additionally, in non-tumor bearing mice MYXV replication was not observed 3 days post treatment. Although the virus was no longer detectable 7 days after treatment we observed high levels of proliferating T-cells within the lungs 30 days after treatment, indicating T-cells are recognizing antigens and expanding. In our GEM model for SCLC, animals treated with only 2 intrapulmonary doses of MYXV (n=27) resulted in a statistically significant prolongation of survival compared to PBS control mice (n=31) (p=0.0016). When MYXV is given in combination with a low dose of cisplatin (n=29) the survival benefits are enhance compared to MYXV alone (p=0.021). Direct intratumoral MYXV injections performed on PDX tumors in immunodeficient mice showed efficient infection and viral replication in all patient samples, resulting in tumor necrosis. In immunodeficient NSG mice, detectable viral replication is observed for at least 15 days following MYXV injection. Direct intratumoral MYXV injections performed on allograft tumors in syngeneic immunocompetent mice showed extensive necrosis accompanied by immune cell infiltration into the tumor (confirmed by CD45 IHC), and viral replication was undetectable 7 days’ post treatment. We have successfully shown MYXV recruits infiltrating immune cells to SCLC tumors that are typically devoid of immune cells. We are now pursuing combining MYXV with immune checkpoint inhibitors α-PD-1 and α-CTLA-4 to enhance this immune response to provide maximal therapeutic effects.