showed that single nucleotide polymorphism (rs4462560) of nei endonuclease VIII-like 1 (NEIL1) may serve as a predictor of acute RILI. However, the underlying mechanisms remain to be elucidated.

**Materials/Methods:** CRISPR/Cas9 was used to construct NEIL1 rs4462560 mutant-type (NC_000015.10: g.75355623C>G) in human A549 lung epithelial cells. Mutant-type and wild-type A549 cells were irradiated at the dose of 10 Gy, after which cell clonality was validated by colony formation assay, apoptosis was examined by flow cytometry, and radiation induced DNA double strand breaks (DSBs) were detected by immunofluorescence. Western blot analysis was performed to assess changes in protein expression levels of members of DNA repair genes, MAPK/JNK pathways, and epithelial–mesenchymal transition markers. Besides, SP600125, a JNK inhibitor, was used to validate the role of MAPK/JNK pathway played in the RILI susceptibility of mutant-type NEIL1 rs4462560.

**Results:** NEIL1 rs4462560 mutant-type A549 cells (E01) showed diminished apoptosis compared with NEIL1 rs4462560 wild-type A549 cells (E01). DNA repair related proteins, like XRCC1, PARP-1, hnRNP-u and WRN were much abundant in P8 cells. Both immunofluorescence and western-blot analyses detected continuous expression of γ-H2AX in E01 cells rather than P8 cells in 2 hours after 10 Gy irradiation. Furthermore, E01 cells showed higher protein levels of MAPK phosphorylation, JNK phosphorylation, and Vimentin, and lower protein levels of E-cadherin than P8 cells after 10 Gy irradiation, while the administration of SP600125 diminished these differences.

**Conclusion:** Our findings implicate that NEIL1 rs4462560 may affect DNA repair, cell apoptosis, and epithelial–mesenchymal transition via MAPK/JNK pathway. NEIL1 rs4462560 may play a crucial role in acute RILI, which will be validated by further in vivo study.


### 3156
**Effect Of Tumor Treating Fields And Radiotherapy Combination On Brain Tumor And Normal Brain Cell Lines**

**Materials/Methods:** Glioblastoma (U118MG and T98G), brain cancer stem cell (BCSC), brain stem cell (BSC), and brain endothelial primary cell (BEPC) lines were used. Using an arduino processor, a frequency field with different frequency (100-200-300-400-500 kHz), voltage (1-2-3-4 V) and duration (24-48-72 hours) was developed to apply TTF. Combination treatment increased apoptosis in BCSC lines both in annexin V and caspase 3 analyses (p<0.05), but this effect was detected only with annexin V in U118G cell line (p<0.05). No effect was determined for T98G and BEPC cell lines. Interestingly RT-induced apoptosis decreased with combination treatment in BSC cell lines. In cell cycle analyses none of the cell lines showed accumulation in G2/M phase with only TTF. With combined application, effect on cell cycle became prominent in tumor cell lines (U118MG and T98G), while no difference was observed in BCSC, BCS and BEPC cell lines. TTF alone did not produce genotoxicity through micronuclear level. Combined applications did not increase micronucleus rates produced by RT (p<0.05). In clonogenic analyses combined applications decreased % survival 1.72-fold in BCSC, 1.33-fold in U118MG when compared to RT alone group. Although no apoptotic effect was experienced in BSC cell line % survival decreased by 1.23-fold in clonogenic analysis.

**Conclusion:** Combination treatment produced synergistic effect in tumor and BCSC cell lines but this effect disappeared in normal brain cell lines indicating a therapeutic gain with combination treatment. This result should be supported by in vivo studies.

Results: Cells treated with increasing levels of 5-FdU exhibited decreased viability compared to cells that were not treated with 5-FdU. The effect on viability appears to be dose dependent. Cells that were treated with 5-FdU exhibited a decreased ability to grow neurospheres at 8 days compared to those not treated with the drug. There was a decrease in the number of neurospheres formed and also a decrease in the size of the neurospheres that were formed when the cells were treated with 5-FdU.

Conclusion: New treatments for GBMs are desperately needed. We show that 5-FdU decreases cell viability and stemness in patient-derived glioblastoma stem cells. Studies are ongoing to determine if the effect of 5-FdU on glioma stem cells is tolerable at the required 5-FdU concentration. 5-FdU represents a promising treatment strategy for GBM patients.


3158 TAK-243 Combined With Radiation And Other DNA Damaging Agents As A Novel Therapeutic Strategy For Small Cell Lung Cancer

S. Majeed,1 M. Aparnathi,2 L. Song,2 J. Weiss,2 A. Schimmer,1,3 M. Tsao,2 G. Liu,2 and B.H. Lok;2 1University of Toronto, Toronto, ON, Canada, 2Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada

Purpose/Objective(s): Small cell lung cancer (SCLC) (~ 15-17% of lung cancer) is an aggressive disease, with a dismal overall five-year survival of 7%. The current first-line therapy consisting of cisplatin and etoposide chemotherapy (C/E) +/- radiation (RT), has changed minimally in >30 years. TAK-243, an E1 ubiquitin activating enzyme (UAE) inhibitor, is a promising novel SCLC therapy. TAK-243 limits the formation of ubiquitin conjugates that mediate many cellular processes including DNA repair signaling. By dysregulating cancer-specific dependencies of UAE, TAK-243 may induce malignant cell death and potentiate DNA damage induced by RT and chemotherapeutic agents. Initial findings show TAK-243 is particularly effective for 2 SCLC cell-lines (EC50 of 0.006 - 0.011nM), compared to other cancers and normal tissues (n = 29, EC50 ranging from 0.012 to 1.31nM; Hyer et al., 2018). However, TAK-243 has not been comprehensively evaluated as a single agent or in combination with C/E and RT for SCLC.

Materials/Methods: SCLC cell-lines were treated with incremental doses of TAK-243 (0-1nM) and viability was determined by a resazurin conversion assay after 6 days. EC50 values were compared. To evaluate combination effects, C/E were administered (1:1 ratio, 0-1nM) to cell-lines together with a fixed cell-line specific TAK-243 dose (EC30). Area under the curve (AUC) was compared between TAK-243+C/E and C/E-alone groups. TAK-243 (0-1nM) was administered with RT (2-8 Gy) in a similar manner and cell viability was measured after 9 days. Dose modification factor (DMF), the ratio of RT dose required between control and drug-treated groups, was calculated at survival fraction 0.37. TAK-243 single agent therapy (20mg/kg, BIW) was evaluated in a patient-derived xenograft (PDX) SCLC model (SCRX-Lu149). Subcutaneous tumors were established, and tumor volumes were compared between experimental and control conditions. Kaplan-Meier survival analysis and significance testing using the Log-Rank Test were completed.

Results: Single-agent therapy, EC50 values of SCLC cell-lines (n = 11) ranged from 0.003-0.12nM in vitro. TAK-243-treated animals experienced significantly increased freedom from volumetric endpoint (p-value = 0.028) when evaluated in vivo. TAK-243+Chemotherapy Treatment revealed a positive combination effect for the majority of SCLC cell-lines (n = 5, ΔAUC from -13-47 units), suggesting potential chemosensitivy. TAK-243+Radiotherapy, Potential radiosensitization was observed in all SCLC cell-lines (n = 5) as tested by the resazurin assay (DMF from 1.25-1.35).

Conclusion: Cell-line and PDX models of SCLC are sensitive to TAK-243. With TAK-243, lower doses of C/E and RT are required for most cell-lines. Use of TAK-243 may be a novel strategy to improve SCLC therapies. These findings will be validated using clonogenic survival assays and interrogated in vivo, which may provide a basis to move TAK-243 to the clinic.

Author Disclosure: S. Majeed: None. M. Aparnathi: None. L. Song: None. J. Weiss: None. A. Schimmer: Consultant; Novartis Pharmaceuticals, Jazz Pharmaceuticals, Otsuka Pharmaceuticals. Director of the Research; Princess Margaret Cancer Centre. M. Tsao: Research Grant; Pfizer, Merck, AstraZeneca, Bayer. G. Liu: None. B.H. Lok: Research Grant; Pfizer. Honoraria; AstraZeneca.

3159 PDPPN+ Tumor Initiating, Treatment Resistant Glioblastoma Cells Promote Radiation Resistance Via PRC2

A.S. Modrek,1 E. Eskilsson,2 R. Ezilarasan,3 Q. Wang,2 L.D. Goodman,4 K. Bhat,5 T. Le,6 F.P. Barthel,7 M. Tang,1 J. Yang,1 L. Long,1 J. Gumin,1 F. Lang,8 R. Verhaak,9 K. Aldehyde,1 and E.P. Sulman1 1University of Washington, Department of Radiation Oncology, New York City, NY, 2MD Anderson Cancer Center, Houston, TX, 3Nanjing Medical University, Nanjing, China, 4Baylor College of Medicine, Houston, TX, 5University of Texas Medical School, Houston, TX, 6Jackson Labs, Bar Harbor, ME, 7University of Michigan, Ann Arbor, MI, 8National Cancer Institute, Bethesda, ME

Purpose/Objective(s): Tumor recurrence following radiation therapy for patients with malignant gliomas leads to nearly universally fatal outcomes. Treatment resistant glioma stem cells are thought to propagate and drive growth of these tumors, but their markers and our ability to target them specifically are not well understood. Transcriptome analyses suggest that expression of the type-I integral membrane glycoprotein podoplanin (PDPPN) may be a prognostic marker in astrocytic gliomas. Here, we aim to explore whether PDPPN is a glioma stem cell marker and understand its biological significance.

Materials/Methods: Transcriptome and clinical outcome analyses was carried out using standard bioinformatics tools on publically available TCGA datasets. Glioma stem cell cultures were cultured in serum-free media. PDPPN shRNA constructs were generated using standard cloning techniques and delivered via lentiviral transduction. Animal injection experiments were performed with a stereotactic injection apparatus and conducted with prior approval by the institutional review board. Glioma stem cell neurosphere formation assays were used to test response to irradiation, which was delivered in 2, 4 or 6 Gy doses prior to the assay.

Results: We demonstrate here that PDPPN expression is an independent prognostic marker in gliomas across multiple independent cohorts comprising both high- and low-grade gliomas. PDPPN expression is prominent in glioma stem cells and correlates with DNA hypermethylation-induced RT resistance. Enrichment analyses of PDPPN expressing GSCs revealed that PDPPN correlates with polycomb repressive complex 2 (PRC2) of which the catalytic subunit enhancer of zeste 2 (EZH2) controls DNA methylation and has been previously shown to protect GSCs from radiation-induced cell death.

Conclusion: PDPPN identifies the tumor-initiating, treatment-resistant (TITR) glioma cells responsible for radiation resistance and may serve as a novel therapeutic target.