yses show decreased expression of cyclin B1 and activation of cyclin-dependent kinase 2 (CDC2), two proteins that modulate G2 progression. Taken together this data opens up another avenue for understanding the mechanisms underlying the KD and its impact on radiation therapy, ultimately helping us better understand how to implement it for clinical use.

EXTH-17. CDK7 IS A NOVEL THERAPEUTIC TARGET IN HIGH GRADE GLIOMA

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Cyclin dependent kinase 7 (CDK7), is a kinase that facilitates cell cycle progression and stabilises RNA polymerase II-based transcription. THZ1 is a novel irreversible inhibitor of CDK7 whose mechanism(s) of action are largely unknown. We now show that low nanomolar THZ1 has profound antitumor activity against high grade glioma (HGG) patient-derived cell lines and *ex vivo* HGG tissue slices. CDK7 appears indispensable for gliomagenesis as THZ1 perturbed the transcriptome and disabled CDK activation, leading to cell cycle arrest and DNA damage. THZ1 halted transcription of mitochondrial ribosomal genes, reducing mitochondrial translation and oxidative respiration. It also inhibited the expression of RTKs such EGFR and PDGFR, reducing signalling flux through downstream pathways. Finally, THZ1 disrupted nucleolar, Cajal-body and nuclear speckle formation resulting in reduced cytosolic translation and aberrant mRNA splicing. These studies validate CDK7 as a therapeutic target in HGG and provide new mechanistic insights into the antitumor activity of THZ1.

EXTH-18. REPURPOSING IBUDILAST IN COMBINATION WITH TEMOZOLOMIDE FOR GLIOBLASTOMA

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AIMS: Recurrence in patients with Glioblastoma (GBM) is inevitable, even in patients with O-6-Methylguanine-DNA Methyl Transferase (MGMT) methylation. We identified increased expression of the inflammatory cytokine, Macrophage Inhibitory Factor (MIF) and its receptor CD74 in patients with recurrent tumors. High levels of MIF and CD74 were associated with poor overall survival in glioblastoma patients. This study aims to determine efficacy of Ibudliast (AV411; 3-isobutyryl-2-isopropylpyrazolo-[1,5-a] pyridine) to block MIF expression and decrease tumor burden. Ibudilast is an anti-inflammatory drug that was developed for the treatment of bronchial asthma. METHODS: The patient derived cell lines (PDCLs) RN1 (MGMT unmethylated), BAH1 (MGMT methylated), and HW1 (MGMT methylated) were treated in vitrowith different concentrations of ibudilast in combination with temozolomide (TMZ). Patient derived xenograft (PDX) models of GBM were developed and also treated with ibudilast in combination with TMZ. Overall survival was calculated. RESULTS: Regardless of MGMT status, significant synergism between ibudilast and TMZ was observed in the PDCLs. Efficacy was associated with significantly decreased expression of its targets, MIF and CD74. Downstream proteins such as Src and Akt were also significantly inhibited. The combination induced apoptosis. RN1 and HW1 tumors were established intracranially in Balb/c nude mice. Significant increases in survival times of the mice were recorded when treated with the combination. CONCLUSION: Ibudilast in combination with TMZ resulted in significant blockage of MIF expression, increased apoptosis and longer survival in vivo. A phase I/II clinical trial for recurrent GBM patients is warranted.

EXTH-19. A COMBINATION OF PALBOCICLIB AND RADIOTHERAPY IMPROVES SURVIVAL IN GLIOBLASTOMA

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AIMS: New treatments are urgently needed for patients diagnosed with MGMT unmethylated glioblastoma. Palbociclib (Pfizer) is a CDK4/6 inhibitor. The aim of this study was to assess efficacy of palbociclib as a monotherapy and in combination with radiotherapy on glioblastoma patient derived cell lines (PDCLs) and xenografts that were MGMT unmethylated and RB1 proficient. METHODS: A panel of 6 PDCLs were treated with different concentrations of palbociclib alone, and with radiotherapy (4Gy). We assessed the effect of treatment using clonogenic assays (the ability to form tumor colonies). We also measured expression of RB1, CDK4, CDK6, H2AX and cleaved-PARP (a measure of apoptosis). The PDCL, RN1 was intracranially injected into the brains of Nod Skid gamma mice and treated in vivo with palbociclib (75mg/kg; gavage) and Radiotherapy (4Gy). RESULTS: Palbociclib alone was effective in inhibition of tumor cell proliferation in all 6 PDCLs with concentrations ranging from 11uM to 22uM. We found significant synergy when palbociclib was administered with radiotherapy. Protein expression levels of RB1, CDK4 and CDK6 were significantly inhibited with the combination treatment and H2AX and cleaved PARP were induced. In vivo, significantly longer survival was observed in mice treated with the combination of palbociclib and radiotherapy. The median survival was 100.5 days (combination treatment) compared to 93 days (control); 83 days (radiotherapy only) and 92 days (Palbociclib only) (p value=0.0488). CONCLUSION: The combination of palbociclib and radiotherapy significantly improves overall survival in our xenografts models. In mice, the combination is safe and crosses the blood brain barrier. A phase I/II clinical trial for glioblastoma patients with MGMT unmethylated and RB1 proficient tumors is currently in development.

EXTH-20. TARGETING GLIOBLASTOMA METABOLISM THROUGH mTOR AND MITOCHONDRIAL INHIBITION

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BACKGROUND: PENAO is a potent mitochondrial inhibitor, currently in a Phase 1 clinical trial in Australia as a pan-cancer treatment. Temsirolimus is an inhibitor of the mTOR pathway, a pathway that is highly upregulated in glioblastoma, and has also been shown to interfere with mitochondrial metabolism. The aim of this study is to combine both PENAO and temsirolimus, both potent inhibitors of mitochondrial metabolism, to synergistically impede glioblastoma growth, particularly in patients with an unmethylated *MGMT* promoter. METHODS: PENAO and temsirolimus were tested *in vitro* as single agents and in combination using patient-derived cell lines (PDCLs). Additionally, in an orthotopic mouse model in which the animals were implanted with the PDCL RN1, we combined PENAO (3 mg/kg/day) and temsirolimus (1 mg/kg/day or 5 mg/kg/day). RESULTS: *In vitro* drug cytotoxic assays using a panel of PDCLs showed promising synergistic effects with PENAO and temsirolimus. IC_{50} dose of PENAO and temsirolimus combined showed 11- to 20-fold decrease in cell viability. Annexin V-PI assay revealed that the drug combination induced apoptosis in ~90% of the cell population. Unfortunately, *in vivo* results showed no significant difference between treatment and control groups, and did not extend survival. CONCLUSION: Although the combination of PENAO and temsirolimus was highly effective in PDCLs *in vitro*, it did not translate into a significant survival improvement *in vivo*. Ongoing studies have identified that the concentration levels of PENAO reaching the brain were inadequate to deliver the synergistic effects observed *in vitro*. Future studies will investigate mechanisms of enhancing drug delivery of PENAO to the brain and mimic the synergistic effect we observed *in vitro*.

EXTH-21. GALLIUM MALTOLATE INTERFERES WITH IRON METABOLISM IN GLIOBLASTOMA

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BACKGROUND: Previously, we presented compelling data that gallium maltolate (GaM) has a profound antitumor effect on glioblastoma (GBM) in vitro and in vivo. We now provide evidence elucidating the mechanism of action by which GaM exerts its antineoplastic activity. METHODS: Interaction of transferrin with GaM was confirmed by UV-Vis (ultraviolet visible) spectroscopy. Cellular iron proteins and the effects of GaM were evaluated by Western blot and immunohistochemistry (IHC). The action of GaM on the iron-dependent M2 subunit of ribonucleotide reductase (RRM2) was analyzed by Western blot and electron paramagnetic spectroscopy (EPR) in human U87 and D54 GBM cells. RESULTS: Transferrin receptor (TfR) and RRM2 IHC revealed strong expression by GBM tumors, but not by normal brain cells in both patient surgical and U87 xenograft tissues. The keen iron requirement of GBM cells was reflected by the higher proportion of H-ferritin (metabolic) vs. L-ferritin (storage) staining we observed in GBM tumors. More intense TfR staining was noted in treated vs. untreated xenograft tumors. This finding is consistent with an upregulation of TfRs secondary to tumor iron deprivation produced by GaM treatment. Cellularity counts were comparable between GaM-treated and control tumors, but the proportion of dead cells was higher in treated ones. Moreover, the number of mitotic figures was significantly lower in treated tumors (p=0.043). Further analysis of the effect of GaM on RRM2 function showed that GaM formed complexes with transferrin and inhibited the tyrosyl radical of RRM2, and consequently RRM2 activity, without reducing RRM2 protein levels. CONCLUSION: We propose that GaM exerts its antineoplastic effect by forming identifiable complexes with transferrin, enabling it to be taken up by GBM cells via transferrin receptors, where it

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