decreased for another 24h after eot and then started to recover, the combination of TTFIELDS and IN3 resulted in a decrease of cell numbers by an additional 92% at 72h after eot of TTFIELDS ($p = 0.0086$). All cells recovered in instances when IN3 treatment ended after 72h. The combination treatment of TTFIELDS plus IN3 induced an early stage of apoptosis in 44% of the cells, compared to 14% with TTFIELDS alone ($p = 0.0002$) and 4% with IN3 alone ($p < 0.0001$). CONCLUSIONS: TTFIELDS are an approved new treatment modality for GBM. A combination of physical damage of the spindle apparatus by TTFIELDS and chemical inhibition of the SAC led to earlier and prolonged effects, which significantly augment TTFIELDS efficacy. This work was supported by the Familie Mehdorn Stiftung.

P08.18 REPLICATION STRESS AS A DRIVER OF GENOMIC INSTABILITY IN MALIGNANT GLIOMAS

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Glioblastoma (GBM, WHO grade IV astrocytoma) is among the deadliest of solid tumors with median survival rates of only approximately 15 months. In spite of concerted efforts, molecular mechanisms/genes causing high recurrence and treatment resistance are poorly understood. The tumor suppressor BRCA1 plays a critical role in maintaining genomic stability, in general, and replication stress (RS), in particular. Numerous reports have demonstrated that wild type BRCA1 loss impairs the growth of several cancers (breast, ovarian, lung, prostate and colon). Our data show considerably higher level of RS in malignant gliomas compared to other solid tumors (breast, prostate ovarian) and identify a novel role for BRCA1, where high BRCA1 expression negatively associates with glioma patient survival. BRCA1 plays an unexpected tumor-promoting role in GBM, safeguarding a protective response to supraphysiological RS levels via transcriptional regulation of RRM2 expression. shRNA-mediated knockdown of BRCA1 or chemical inhibition of RRM2 both induce GBM cell death due to accumulation of RS-induced DNA damage *in vitro* and extend survival of tumor bearing mice *in vivo*, highlighting the clinical relevance of our findings. Acknowledgements: This work was supported by Danish Cancer Society Foundation.

P08.19 RESISTANCE TOWARDS THE MDM2 INHIBITOR IDASANUTLIN IS MEDIATED VIA THE NFKB PATHWAY AND IGFBP1 UPREGULATION IN GLIOBLASTOMA

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BACKGROUND: Dysregulation of p53 pathway is common in glioma. Frequent causes are p53 mutations or overexpression of the oncogene MDM2 in p53 wildtype tumors. Idasanutlin is a MDM2 inhibitor, which is currently under investigation for multiple myeloma and acute myeloid leukemia, but of principal interest also for other p53 mutated solid tumors. Therefore, preclinical data and molecular understanding about treatment effects and resistance mechanisms are important. METHODS: Treatment effects of idasanutlin monotherapy and combination with radiotherapy were analyzed on cell viability, proliferation and clonogenicity in p53 wildtype cell lines (U87MG, A172) and glioma-initiating cells (GICs, S24, T1) at radiation doses of 2 and 4 Gray (photons). Toxicity assays were performed on freshly isolated murine astrocytes and cerebellum granule neurons. Idasanutlin resistant cells were generated by treatment of U87MG cells with increasing doses of idasanutlin for 3 months. Resistance mechanisms were analyzed by microarray analysis and confirmed *via* qRT-PCR, immuno blots and treatment with the nuclear factor (NF)kB inhibitor JSH-23 and transient knockdown of insulin-like growth factor binding protein (IGFBP1) *via* siRNA. RESULTS: Idasanutlin reduced cell viability and clonogenicity in p53 wildtype glioblastoma cell lines and GICs. Combined treatment with radiotherapy showed synergistic effects on clonogenicity and proliferation at clinically relevant doses without toxicity on normal brain cells. Long-term treatment with idasanutlin resulted in resistance against the drug in U87MG cells and led to a more clonogenic, invasive and proliferative phenotype of the cells. Microarray analysis revealed an activation of NFkB and ERK1/2 pathway and an upregulation of IGFBP1 expression in idasanutlin resistant cells. While radiotherapy did not overcome resistance conferred by idasanutlin, inhibition of NFkB pathway by JSH23 treatment and transient knockdown of IGFBP1 by siRNA partly restored sensitivity towards idasanutlin. Transient knockdown of IGFBP1 additionally reduced the invasiveness of idasanutlin resistant cells. CONCLUSIONS: Combination of idasanutlin

treatment and radiotherapy showed synergistic effects on glioblastoma cells in a clinically relevant setting. However, long-term treatment with idasanutlin resulted in resistance against the drug mediated by activation of NFkB pathway and upregulation of IGFBP1. Targeting these pathways may provide strategies to overcome idasanutlin resistance.

P08.20 THE SIGNIFICANCE OF TISSUE FACTOR SUPPRESSION IN MEDIATING THE LESS AGGRESSIVE, AND LESS THROMBOGENIC, PHENOTYPE OF IDH1 MUTANT GLIOMAS

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Isocitrate dehydrogenase 1 mutation (IDH1^{mut}) occurs in 20-30% of gliomas and induces DNA hypermethylation. IDH1^{mut} is associated with a much better prognosis than IDH1 wild-type (IDH1^{wt}) gliomas, yet the basis for this remains unclear. We recently discovered that IDH1^{mut} gliomas are much less likely than IDH1^{wt} gliomas to cause venous thromboembolism (VTE), which is a serious complication in glioma patients. Our data strongly suggest that suppression of Tissue Factor (TF), the primary initiator of coagulation, is a key reason for this. The TF gene, *F3*, was hypermethylated in patient-derived IDH1^{mut} cells (TB09, BT142, GBM164) but not in IDH1^{wt} cells (GBM6, GBM12, GBM43), with correspondingly lower TF protein expression and TF procoagulant activity (PCA) in the IDH1^{mut} cells. Treatment with decitabine, a demethylating agent, increased TF mRNA in IDH1^{mut} cells, but not in IDH1^{wt} cells. Exogenous treatment with the metabolic product of IDH1^{mut} enzyme, D-2-hydroxyglutarate, also directly suppressed TF PCA, as did forced expression of IDH1^{mut} in IDH1^{wt} cells. In an *in vivo* model of thrombosis, wherein the inferior vena cava (IVC) was surgically stenosed to slow blood flow and increase risk of thrombus formation, mice engrafted with IDH1^{mut} gliomas produced fewer and smaller IVC thrombi than mice with IDH1^{wt} gliomas (0.86 ± 0.86 mg vs 10.3 ± 3.6 mg, $P=0.02$). Furthermore, patients with IDH1^{mut} gliomas had lower preoperative circulating TF PCA compared to patients with IDH1^{wt} gliomas (0.49 ± 0.19 vs 1.2 ± 0.21 pg/mL, $P=0.036$), and elevated TF PCA correlated with an increased risk of subsequent VTE ($P=0.025$). In addition to coagulation, TF is known to directly enhance the malignancy of many cancers via activation of protease-activated receptor 2 (PAR2). In our IDH1^{wt} glioma cells, TF knockdown greatly reduced *in vitro* cell proliferation, invasion, and colony formation. TF knockdown also reduced the *in vivo* growth of IDH1^{wt} gliomas, and increased the median survival of engrafted mice by 86% (41.5 vs 77 days, $P=0.001$). Likewise, the potent and selective PAR2 antagonist, GB83, inhibited IDH1^{wt} cell proliferation by 54% ($P<0.0001$) and reduced cell invasion by 25% ($P<0.05$). Our data therefore suggest that suppressing TF expression and PCA is a critical component of the less thrombogenic, and less malignant, IDH1^{mut} phenotype. Targeting TF, and its activation of PAR2, may therefore represent a novel strategy to make IDH1^{wt} gliomas behave more like IDH1^{mut} gliomas.

P08.21 DISCOVERY OF CT-179--A SMALL MOLECULE INHIBITOR OF THE TRANSCRIPTION FACTOR OLIG2 WITH POTENT ANTI-TUMOUR ACTIVITY IN HIGH-GRADE GLIOMA

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OLIG2 is a protein transcription factor that is expressed exclusively in progenitor cells in the brain and spinal cord during development. Typically, OLIG2 is not active in the adult brain and is not found outside the central nervous system; however, it is re-expressed in most patients with high-grade glioma (HGG). The expression of OLIG2 potentially drives the growth, invasiveness, and resistance to radiation of HGG cells and tumours in animal models. CT-179 is a small molecule (M.W. 397) that, on the basis of homology modelling studies, is a putative disruptor of OLIG2 homodimerization. We found that, at low nanomolar concentrations, CT-179 profoundly inhibited the growth of, and induced apoptosis in, a range of patient-derived HGG cell lines. Approximately half of the HGG cell lines showed substantial degradation of OLIG2 protein, and this was associated with arrest of the cells in the G2/M phase of the cell cycle. We are currently exploring whether this leads to mitotic catastrophe. Additionally, CT-179 caused significant downregulation of receptor tyrosine kinases that are associated with HGG tumorigenicity, including epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR). Orally administered CT-179 (20 mg/kg) crossed the blood-brain barrier of intact mice and reached levels sufficient to inhibit HGG cells. Indeed, the growth of established orthotopic HGG xenografts in mice was significantly inhibited by orally or intraperitoneally administered CT-179, leading to increased survival. CT-179 is one of the few small molecules that has been identified to directly inhibit the function of a transcription factor. Our experiments showing the profound anti-tumour activity of CT-179 in HGG models pro-

vide the impetus needed for moving this molecule into the clinic as a novel therapy for patients with HGG.

P08.22 TARGETING GLIOBLASTOMA MITOCHONDRIAL METABOLISM TO INHIBIT CELL PROLIFERATION & TUMOR GROWTH

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BACKGROUND: Abrogating tumor mitochondrial metabolism by targeting the mitochondria and inhibiting the mTOR pathway is an effective mechanism to impede glioblastoma growth. Inhibiting the mitochondrial activity with an organic arsenide compound, PENAO, has been previously shown to decrease oxidative phosphorylation eventuating in the inhibition of glucose metabolism in patient derived glioblastoma cell lines. Temozolomide, an inhibitor of the mTOR pathway, has also been shown to interfere with glycolysis. We combined PENAO and temozolomide to determine if the drugs act synergistically to inhibit glioblastoma tumor metabolism and cell proliferation. **METHODS:** *In vitro* experimentation of PENAO and temozolomide as single agents, and in combination, were performed on glioblastoma patient-derived cell lines (PDCLs). Both agents were also assessed *in vivo* using an orthotopic mouse model in which the animals were implanted with the PDCL, RN1. Once tumor growth was confirmed, we randomized the animals into different treatment arms: (i) untreated control; (ii) PENAO alone (3mg/kg/day); (iii) low dose temozolomide alone (1mg/kg/day); (iv) high dose temozolomide alone (5mg/kg/day); (v) combined PENAO and low dose temozolomide; and (vi) combined PENAO and high dose temozolomide. **RESULTS:** A significant synergistic effect on tumor cell growth was observed *in vitro* using a panel of PDCLs treated with the combination of PENAO and temozolomide. Apoptosis, measured by FACS analysis for the expression of the marker, Annexin was observed in approximately 90% of the cell population when treated with the combined treatment of PENAO and temozolomide compared to 25% for PENAO alone and 44% for temozolomide alone. An orthotopic model was established by intracranially injecting the PDCL, RN1 in balb/c nude mice. Temozolomide was delivered by gavage while PENAO was continuously administered subcutaneously with an osmotic pump. While high dose temozolomide alone improved the overall survival times of mice (12-day advantage compared to the control) which is more than what was observed in the combination treatment of PENAO with temozolomide (5-day advantage compared to control). **CONCLUSION:** Highly synergistic effects observed *in vitro* were not translated to the *in vivo* model. Although the combination of PENAO and temozolomide 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 crossing the blood brain barrier were inadequate to deliver the synergistic effects observed *in vitro*. Future studies will investigate mechanisms of enhancing drug delivery of PENAO to the brain.

P08.23 A SYSTEMATIC REVIEW AND META-ANALYSIS OF TOPOISOMERASE INHIBITION IN PRE-CLINICAL GLIOMA MODELS

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BACKGROUND: Glioblastoma is the most common malignant primary brain tumor in adults. Pre-clinical animal studies make an important contribution to the development and planning of novel treatment designs for human clinical trials. We investigated the concordance of the efficacy of topoisomerase inhibition in pre-clinical animal models and human clinical trials. **METHODS:** We systematically searched Pubmed, Medline and Embase using pre-defined terms to identify publications describing pre-clinical animal studies investigating the use of topoisomerase inhibitors. We included studies assessing median survival and tumor volume and extracted data pertaining to study quality and design. Study quality was assessed using a 12-item checklist. We performed random-effects meta-analysis to assess treatment efficacy. Heterogeneity was assessed using the I^2 statistic and was further investigated using metaregression. We assessed for publication bias using Egger regression and Trim and Fill analysis. **RESULTS:** The systematic review identified 52 publications reporting a total of 61 survival and 29 tumor volume experiments. We included 47 and 16 respectively in the meta-analysis. Overall, topoisomerase inhibition prolonged median survival by a factor of 1.33 (95% CI 1.23 – 1.43) and reduced tumor growth by a factor of 3.21 (95% CI 1.99 – 5.18), with considerable heterogeneity in each dataset ($I^2=92.2\%$ and 95.5% respectively). Study quality was modest (median score 6/12), with infrequent reporting of both randomisation (54%) and blinding (8%). For survival studies, multivariable metaregression identified the glioma model used ($F=6.08$, $p=0.0005$) and the route of drug delivery ($F=8.61$, $p=0.0010$) as parameters associated with differences in efficacy. We found evidence of significant publication bias in both datasets. **CONCLU-**

SION: The meta-analysis showed that the use of topoisomerase inhibitors may be beneficial for the treatment of high grade glioma in adults similar to what has been observed in human clinical trials. However, it should be noted that the data is limited by risk of bias, relatively small numbers and covariance. We have nevertheless identified design features impacting perceived drug efficacy that should be accounted for in future experiment design.

P08.24 METALLOTHIONEIN 1E ENHANCES GLIOMA INVASION THROUGH MODULATION MMP-2 AND MMP-9 IN MOUSE BRAIN TUMOR MODEL

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Malignant glioma cells invading surrounding normal brain are inoperable and resistant to radio- and chemotherapy, and eventually lead to tumor regrowth. Identification of genes related to motility is important for understanding the molecular biological behavior of invasive gliomas. According to our previous studies, Metallothionein 1E (MT1E) was identified to enhance migration of human malignant glioma cells. The purpose of this study was to confirm that MT1E could modulate glioma invasion *in vivo*. Firstly we established 2 cell lines; MTS23, overexpressed by MT1E complementary DNA construct and pV12 as control. The expression of matrix metalloproteinases (MMP)-2, -9 and a disintegrin and metalloproteinase 17 were increased in MTS23 compared with pV12. Furthermore it was confirmed that MT1E could modulate MMPs secretion and translocation of NFkB p50 and B-cell lymphoma-3 through small interfering ribonucleic acid knocked U87MG cells. Then MTS23 and pV12 were injected into intracranial region of 5 week old male nude mouse. After 4 weeks, for brain tissues of these two groups, histological analysis, and immunohistochemical stain of MMP-2, 9 and Nestin were performed. As results, the group injected with MTS23 showed irregular margin and tumor cells infiltrating the surrounding normal brain, while that of pV12 (control) had round and clear margin. And regrowth of tumor cells in MTS23 group was observed in another site apart from tumor cell inoculation. MT1E could enhance tumor proliferation and invasion of malignant glioma through regulation of activation and expression of MMPs.

P08.25 IMAGING SCORING SYSTEM FOR PREOPERATIVE DIAGNOSES OF MOLECULAR STATUS IN GLIOMAS

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Gliomas are the most common primary brain tumors. There are no serum markers for the diagnosis of gliomas, and histopathological examination of surgically resected tissue is essential. Some recent studies have suggested that radiological examination may help to identify molecular signature of gliomas preoperatively. We intended to establish an imaging scoring system for preoperative diagnosis of molecular status in gliomas. We reviewed WHO grade 2 - 3 gliomas surgically treated at Keio University Hospital between April 2006 and March 2015. We analyzed an association between several radiological characteristics and genetic features of gliomas. The imaging findings were evaluated by two neurosurgeons and two radiologists independently, and the final judgement was made by consensus of more than 3 people. Calcification (CT), indistinct tumor border (T1), paramagnetic susceptibility effect (T1*), inhomogeneous tumor contents (FLAIR) and non temporal location were found to be more common in tumors with a -1p/19q codeletion. No imaging findings were associated with IDH mutation. Indistinct tumor border, surface localization (FLAIR), inhomogeneous tumor contents were also found to be associated with promoter methylation of the MGMT gene. Based on these findings, an imaging scoring system for prediction of molecular status was designed, which was now under validation with another patient cohort.

P08.26 ATP DEPLETION INDUCED BY DUAL INHIBITION OF ALDH AND MITOCHONDRIAL COMPLEX I MAKE THERAPEUTIC RESPONSE IN GLIOBLASTOMA

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INTRODUCTION: Deprivation of energy metabolism has been emerged as an effective therapeutic approach for various tumors. Cancer cells prefer cytosolic NADH supplied by aldehyde dehydrogenase (ALDH) for ATP production through oxidative phosphorylation (OxPhos). Dual inhibition of ALDH using KN817 and OxPhos using phenformin successfully induced ATP depletion and subsequent cell growth arrest in lung cancer. However, it remains to be elucidated how ATP depletion causes cell growth arrest. This study is