

EXTH-04. BLOCKADE OF NRF2/GLUTATHIONE METABOLISM AS A SYNTHETIC LETHALITY APPROACH FOR IDH1-MUTATED GLIOMA

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BACKGROUND: Mutations in isocitrate dehydrogenase (*IDH1/2*) are frequent genetic abnormalities in human malignancies. *IDH1/2*-mutated cancers are a recently defined disease entity with distinctive patterns of tumor cell biology, metabolism and resistance to therapy. Molecular targeting approaches against this disease cluster remain limited. **METHODS:** We investigated the redox homeostasis in *IDH1* mutant-transduced cells and patient-derived brain tumor initiating cells. The importance of antioxidant genes was confirmed through COX regression analysis on a large cohort of lower grade glioma. We investigated the biologic impact of Nuclear factor erythroid 2-related factor 2 (NRF2) on the glutathione *de novo* synthesis in *IDH1*-mutated cells. Finally, we evaluated the value of targeting NRF2/ glutathione metabolic pathway as a potential synthetic lethality approach for *IDH1*-mutated cell *in vitro* and *in vivo*. **RESULTS:** We discovered that acquisition of cancer-associated *IDH1* mutants results in constitutive activation of NRF2-governed cytoprotective pathways through decoupling of NRF2 from its E3 ligase Kelch-like ECH-associated protein 1. NRF2 mediated the transcriptional activation of *GCLC*, *GCLM* and *SLC7A11*, which strengthens the glutathione *de novo* synthesis, and relieves the metabolic burden derived from *IDH1* mutant neomorphic activity. Blockade of the NRF2/glutathione metabolic pathway synergizes with the elevated intrinsic reactive oxygen species, which results in overwhelming oxidative damage in *IDH1*-mutated cells, as well as a substantial reduction in tumor cell proliferation and xenograft expansion. **CONCLUSION:** Our findings suggest that blockade of the NRF2/glutathione synthetic pathway is a novel targeting strategy for *IDH1*-mutated malignancies.

EXTH-05. THERAPEUTIC IMPLICATIONS OF TTFIELDS INDUCED DNA DAMAGE AND REPLICATION STRESS IN NOVEL COMBINATIONS FOR CANCER TREATMENT

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TTFields are low-intensity, intermediate frequency, alternating electric fields which are applied to tumor regions using non-invasive arrays. TTFields is approved for the treatment of glioblastoma and mesothelioma with clinical trials ongoing in other cancer types. The mechanism of action for TTFields includes interference with mitosis, reduced DNA double strand break (DSB) repair capacity and the frank induction of DNA DSBs. The mechanism by which TTFields induces DNA DSBs appears to be through the enhancement of DNA replication stress with continued TTFields exposure. The induction of DNA DSBs appears to be as a result of significantly reduced expression of the DNA replication complex genes MCM6 and MCM10 as well as the Fanconi's Anemia (FA) pathway genes. TTFields treatment increases the number of RPA foci, decreases nascent DNA length and increases R-loop formation which are markers of DNA replication stress. These results suggest that TTFields-induced replication stress is the underlying mechanism and cellular endogenous source of DNA DSB generation via replication fork collapse. The current study suggests that TTFields exposure causes a conditional vulnerability environment that renders cells more susceptible to chemotherapeutic agents that induce DNA damage and/or cause replication stress. Supporting this is the synergistic cell killing seen with TTFields exposure concomitant with cisplatin, TTFields plus concomitant PARP inhibition with or without subsequent radiation, or radiation given at the completion of a TTFields exposure. Finally, TTFields-induced mitotic aberrations and DNA damage/replication stress events, although intimately linked to one another as one can expose the other, are likely initiated independently of one another as suggested by the gene expression analysis of 47 key mitosis regulator genes. These results establish that enhanced replication stress and reduced DNA repair capacity are also major mechanisms of TTFields effects, effects for which there are therapeutic implications.

EXTH-06. DOWN-REGULATION OF PD-L1 VIA FKBP5 LOWERED BY A CYCLOOXYGENASE-2 INHIBITOR IN GSCs AND GBM CELLS MAY BE ATTRIBUTABLE TO ENHANCE ANTITUMOR EFFECTS OF IMMUNOTHERAPY

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BACKGROUND: Antitumor therapies targeting programmed cell death-1 (PD-1)/its ligand-1 (PD-L1) are influential at present stage. However, in glioblastoma (GBM), the expression of PD-L1 is variable and the role of anti-PD-1 antibody therapy is still unclear. The high expression

of PD-L1 affects cell proliferation and invasion in GBM cells. As COX-2 modulates PD-L1 expression in cancer cells, we tested our hypothesis that a COX-2 inhibitor, celecoxib may play a role on anti-PD-1 antibody treatment via down-regulation of PD-L1. **METHODS:** Six weeks old male C57BL/6 mice subjected to intracranial injection of mice glioma stem cells (GSCs) were randomly divided into four treatment groups; vehicle control (VC), celecoxib, anti PD-1 antibody or the combination of celecoxib and anti-PD-1 antibody groups and examined antitumor effects. To verify the mechanisms underlying antitumor effects, mice GSCs and human GBM cells were used. **RESULTS:** Compared to each single treatment in the glioma model, the combination therapy of anti PD-1 antibody and celecoxib significantly decreased the tumor volume and improved the survival period. Importantly, the high expression of PD-L1 in the glioma model, mice GSCs and human GBM cells was decreased by celecoxib. Interestingly, the reduction of PD-L1 was associated with post-transcriptional regulation of co-chaperone FK506-binding protein 5 (FKBP5) by celecoxib. The combination therapy of anti PD-1 antibody with celecoxib could be a promising therapeutic strategy targeting PD-L1 in GSCs and GBM. **CONCLUSIONS:** Down-regulation of PD-L1 via FKBP5 by celecoxib may play a role on the antitumor effects under the overwhelmed expression of PD-L1.

EXTH-07. OPTIMIZATION OF TARGETING ELTD1 IN GLIOBLASTOMA USING A MOLECULAR TARGETING APPROACH

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The standard of care for glioblastoma multiform (GBM), an aggressive form of cancer, has not significantly increased the prognosis for patients. ELTD1 (epidermal growth factor, latrophilin, and 7 transmembrane domain containing protein 1), a biomarker for angiogenesis, was found to be highly expressed in human high-grade gliomas. Novel treatments targeting ELTD1 with polyclonal (pAb) and monoclonal (mAb) antibodies were effective as a potential cancer therapy in a G55-xenograft mouse model. While our studies have demonstrated that the blood brain barrier (BBB) was leaky around the tumor region, other studies have shown that the BBB is not equally disrupted in GBM patients, therefore suggesting that the mAb may have difficulty crossing the BBB and infiltrating the tumor due to its size. To overcome these limitations, this study focused on the optimization of targeting ELTD1 by using an optimized scFv antibody fragment derived from our mAb against ELTD1. Immunocompromised mice were intracerebrally injected with human-G55 cells. Morphological MRI was used to monitor and calculate tumor volumes. Treatments using IgG, anti-ELTD1 mAb or fragment upon tumor detection. Vascular perfusion images were obtained to examine vascular alterations. Molecular targeting imaging (mtMRI) was conducted to assess the binding specificity of our antibodies against the tumor region. Targeting ELTD1 with varying antibodies (anti-ELTD1 mAb and scFv fragment) resulted in increased survival and decreased tumor volumes in a G55 xenograft GBM mouse model. Additionally, through the use of mtMRI, we determined altered levels of binding specificity against the tumor region using three different anti-ELTD1 attached probes (monoclonal and scFv fragment antibodies). Our data suggest that the optimization of an anti-ELTD1 therapy could be used to better target angiogenesis in glioblastomas.

EXTH-08. REPLACEMENT OF MICROGLIA BY BRAIN-ENGRAFTED MACROPHAGES PREVENTS MEMORY DEFICITS AFTER THERAPEUTIC WHOLE-BRAIN IRRADIATION

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Microglia have a distinct origin compared to blood circulating myeloid cells. Under normal physiological conditions, microglia are maintained by self-renewal, independent of hematopoietic progenitors. Following genetic or pharmacologic depletion, newborn microglia derive from the local residual pool and quickly repopulate the entire brain. The depletion of brain resident microglia during therapeutic whole-brain irradiation fully prevents irradiation-induced synaptic loss and recognition memory deficits but the mechanisms driving these protective effects are unknown. Here, we demonstrate that after CSF-1R inhibitor-mediated microglia depletion and therapeutic whole-brain irradiation, circulating monocytes engraft into the brain and replace the microglia pool. These monocyte-derived brain-engrafted macrophages have reduced phagocytic activity compared to microglia from irradiated brains, but similar to locally repopulated microglia without brain irradiation. Transcriptome comparisons reveal that brain-engrafted macro-