Title

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

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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 svFc 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-ELDT1 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-

phages have both monocyte and embryonic microglia signatures. These results suggest that monocyte-derived brain-engrafted macrophages represent a novel therapeutic avenue for the treatment of brain radiotherapy-induced cognitive deficits.

EXTH-09. FIRST-IN-HUMAN DOSING CONSIDERATIONS OF A BISPECIFIC ANTIBODY FOR TREATING GLIOBLASTOMA

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Current therapy for glioblastoma (GBM) is incapacitating and limited by non-specific toxicity to the surrounding brain. We have developed an immunotherapeutic approach that selectively targets GBM by redirecting the patients' own T cells towards the tumor in an antigen-specific manner using a bispecific antibody. Our novel bispecific antibody ("BRITE") binds GBM-specific surface marker EGFRvIII and the CD3 receptor on T cells, resulting in crosslinking and tumor-specific cell lysis. We previously showed in patient-derived and syngeneic murine glioma models, that treatment with BRITE leads to long-term survival in mice with glioblastoma. A pharmacokinetic analysis in a CD3 humanized mouse revealed that BRITE in plasma and whole blood has an initial half-life of ~8 minutes and a terminal half-life of ~2.5 hours. Given our preclinical success, we have initiated clinical trial-enabling studies, including studies of BRITE toxicology and GMP manufacturing of drug product. A crucial consideration for our proposed Phase 1 dose escalation trial is the starting dose in humans. To this end, we utilized the FDA-recommended minimum anticipated biological effect level (MABEL) of BRITE – a holistic approach that considers all available *in vitro* and *in vivo* data – to calculate the first-in-human starting dose of BRITE.

EXTH-10. THE ACTIVATION AND SENSITIZATION OF GLIOBLASTOMA CELLS VIA COLD ATMOSPHERIC PLASMA TREATMENT

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BACKGROUND: Treatment of glioblastoma multiforme (GBM) continues to remain a challenge using conventional treatment. Through an in vitro study we assessed the efficacy of our novel cold atmospheric plasma technology (CAP) to sensitize GBM cells to temozolomide (TMZ). METHODS: The CAP jet is formed through the discharge (Pk-Pk: 5.8 kV) between a ring grounded cathode and a central anode and with He flow through a glass tube. The discharge process is driven by an AC high voltage (3.16 kV) with a frequency of 12.5 kHz. Human glioblastoma (U87MG) cells were cultured in DMEM supplemented by 1% (v/v) penicillin and streptomycin solution and 10% (v/v) FBS. CAP was delivered to U87 cells in a 96-well plate for 1 min in combination with 10 and 15 μM H2O2. The cell viability was measured by using the MTT assay. We then tested TMZ concentrations of 10 and 50 uM. Cell viability was monitored with the Cell Titer Glo 2.0. luminescent assay. All experiments were performed in triplicate and were independently repeated at least 3 times. RESULTS: We identified an activation state of U87MG cells after the plasma treatment. This activation state resulted in GBM cells sensitized to reactive species identified by decreased cell viability after treatment with H2O2 as compared to the H2O2 treatment alone ($p < 0.005$). In addition, the plasma-activated cells were sensitized to TMZ. Cells treated with CAP in combination with TMZ displayed decreased cell viability at TMZ concentrations of (10 uM) ($p < 0.05$) and (50 uM) ($p < 0.005$) as compared to TMZ alone. CONCLUSIONS: This study demonstrates the activation phenomenon on GBM cells via direct CAP treatment. Due to this activation, the GBM cells were sensitized to both H2O2 and TMZ identified via decreased cell viability. Future work looks to assess this effect of cell activation/sensitization with chemotherapy plus radiation treatment.

EXTH-11. TREATMENT WITH DELTA-24-RGDOX OF SUBCUTANEOUS TUMORS RESULTS IN ABSCOPAL EFFECT ERADICATING INTRACRANIAL MELANOMAS

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Immune checkpoint blockade has revolutionized cancer therapy; however the therapeutic benefit is limited to only a subset of patients with immunogenic ("hot") tumors and is compromised by immune-related adverse events. We have reported the efficacy of oncolytic adenovirus Delta-24-RGDOX (DNX-2440) in syngeneic glioma mouse models. We hypothesized that localized treatment with the virus is effective against disseminated melanomas, including intracranial melanomas. We tested the hypothesis in the subcutaneous (s.c.)/s.c. and s.c./intracranial (i.c.) melanoma models derived from luciferase-expressing B16-Red-FLuc cells in C57BL/6 mice. First, through monitoring tumor growth with bioluminescence imaging, we found that, in both s.c./s.c. and s.c./i.c. models, three injections of Delta-24-RGDOX significantly inhibited the growth of both the virus-injected s.c. tumor and untreated distant s.c. or i.c. tumor, thereby prolonging survival. Next, through cell profiling with flow cytometry, we observed that the virus increased the presence of T cells and effector T cell frequency in the virus-injected tumor and mediated the same changes in T cells from peripheral blood, tumor-draining lymph nodes (TDLNs), spleens, and brain hemispheres with untreated tumor. Moreover, Delta-24-RGDOX decreased the frequency of exhausted T cells and regulatory T cells in the virus-injected and untreated i.c. tumors. Consequently, the virus promoted recruitment and/or *in situ* expansion of antigen-specific T cells in tumors expressing the target antigen. Therefore, we concluded that local intratumoral injection of Delta-24-RGDOX resulted in systemic immune activity against the disseminated tumors. Furthermore, we speculate that given the immunogenicity, cancer-selectivity and intratumoral administration of the virus, Delta-24-RGDOX is expected to have an improved safety profile when compared to immune checkpoint blockade treatment strategies. This is the first report demonstrating that local administration of oncolytic adenovirus results in eradication of intracranial tumors, suggesting Delta-24-RGDOX could be used to manage brain metastases of melanoma.

EXTH-12. RADIATION ENHANCES MELANOMA RESPONSE TO IMMUNOTHERAPY AND SYNERGIZES WITH BENZODIAZEPINES TO PROMOTE ANTI-TUMOR ACTIVITY

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Melanoma brain metastases (MBM) occur in ~50% of advanced melanoma patients. It is unclear if systemic therapies synergize with radiotherapy (RT) and what the impact of RT timing has on efficacy. We find that RT followed by ICI (immune checkpoint inhibitors) (RTàICI) improves MBM patient survival compared to other combination strategies, also shown here in a murine melanoma model. RNA-seq of MBM tumors in the RTàICI group exhibit overrepresentation of genes implicated in NFKB signaling. There is also expression of $GABA_A$ receptor subunits across both treatment groups. We show that melanoma cells express functional $GABA_A$ receptors and that benzodiazepines impair tumor growth. Combination of sub-lethal RT doses with benzodiazepine results in significant ipsilateral and out of field abscopal anti-tumor activity, which is associated with enhanced tumor infiltration with poly-functional CD8 T-cells. This study provides evidence that RT enhances MBM response to ICI and synergizes with benzodiazepines to promote anti-tumor activity.

EXTH-13. NEUROSURGICAL DELIVERY OF THE POLY ADP RIBOSE POLYMERASE-1 INHIBITOR OLAPARIB FROM A THERMO-RESPONSIVE BIODEGRADABLE PASTE POTENTIATES RADIOTHERAPY AND PROLONGS SURVIVAL IN HIGH-GRADE GLIOMA

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There has been considerable interest in repurposing the poly ADP ribose polymerase inhibitor and purported radiosensitiser olaparib (Lynparza), with a recent dose escalation study of olaparib plus temozolomide in recurrent GBM showing good tolerance (Fulton et al 2018). Due to systemic therapy-associated caveats such as dose-limiting toxicities and blood-brain-barrier penetration, here we assess localised post-surgical delivery of olaparib from our previously developed poly(*DL*-lactic-co-glycolic acid) (PLGA) and poly(ethylene glycol) (PEG) (PLGA/PEG) thermo-sensitive biodegradable paste. Metabolic and clonogenic assays revealed impaired proliferation and clonal growth respectively, upon acute exposure of high-grade glioma cells to olaparib (3–5μM), an effect dramatically potentiated with 3Gy radi-