DDIS-06. ONC206, AN IMIPRIDONE FAMILY MEMBER, SUPPRESSES GLIOMA STEM CELL MAINTENANCE

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INTRODUCTION: Imipridones selectively target G protein-coupled receptors (GPCRs) that control critical signaling pathways in various cancer cells. Aberrant overexpression of GPCRs has been implicated in tumorigenesis. ONC201, a first generation imipridone that directly antagonizes dopamine receptor D2 (DRD2), continues to be evaluated in clinical trials for advanced cancers. The immediate downstream mechanism(s) of DRD2 inhibition and resulting anti-cancer activity remains an area of active study. **METHODS & RESULTS:** Here, we show that ONC206, an analog of ONC201 that shares the same imipridone core chemical structure and selective antagonism of DRD2, potently inhibits patient-derived glioma stem cell (GSC) populations. In silico analysis of a glioma patient database led us to investigate DRD2 signaling in glioma; alteration of DRD2 mRNA expression was directly connected to global gene expression change in glioma patient database. CellTiter-Glo cell viability assay showed that exposure to ONC206 in a dose dependent manner preferentially eliminated GSCs, compared to differentiated glioma cells. Protein array of stem cell markers revealed treatment of ONC206 down-regulated protein expression of oncogenic stem cell markers in the GSCs. Further, in vitro limiting dilution assay and sphere formation analysis showed that ONC206 prevented tumor sphere formation and tumor growth. **CONCLUSIONS:** These observations indicate that ONC206 exhibits promising anti-glioma activity and suggest that elucidating the downstream effects of antagonizing DRD2 signaling with ONC206 in gliomas is warranted.

DDIS-07. TG02, AN ORAL CDK INHIBITOR, INDUCES MULTIPLE CELL DEATH PATHWAYS IN HUMAN GLIOMA CELL LINES AND GLIOMA-INITIATING CELLS

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TG02 is an orally bioavailable, multikinase inhibitor which potently inhibits cyclin-dependent kinases (CDK) 1, 2, 5, 7 and 9. CDK9-dependent depletion of short-lived oncoproteins such as MCL-1 and MYC has been proposed as its primary mechanism of cytotoxicity. Here we report that TG02 exhibits strong anti-tumor activity in both human long-term cell lines (LN-18, LN-428, D247MG, LN-319, A172, U87MG, T98G, LN-308, LN-229) and in glioma-initiating cell lines (T-325, T-269, ZH-161, ZH-305, S-24) regardless of MGMT promoter methylation status. This activity was observed in acute growth inhibition assays at EC50 concentrations between 42.5 nM and 187.5 nM and in clonogenic survival assays at EC50 concentrations between 14 nM and 73.3 nM. Baseline expression of direct TG02 targets (CDK-9, CDK-5) and indirect TG02 targets (MCL-1, MYC) was detected in all cell lines by immunoblot, but did not correlate with sensitivity to TG02. Similarly, p53 mutation or PTEN inactivation status showed no association with TG02 sensitivity. Under hypoxic cell culture conditions, TG02 was less active against the glioma-initiating cell lines, ZH-161 and ZH-305, but not against the long-term cell lines, LN-229 and LN-308. Blocking the extrinsic cell death pathway by gene transfer of the viral gene product, cytokine response modifier (crma)-A, abrogated CD95 ligand-induced apoptosis, but had no effect on cell death induced by TG02. High concentrations of TG02 induced annexin V positivity on the cell membrane and minor caspase 3 cleavage, but co-exposure to the pan-caspase inhibitor, zVAD-fmk, did not attenuate TG02-induced cell death. High concentrations of TG02 also induced autophagy as assessed by LC3 cleavage, but inhibition of autophagy by 3-methyladenine reduced only caspase cleavage, but not cell death. These data suggest that TG02 is a highly potent anti-glioma agent in vitro that may induce cell death in a non-apoptotic, non-autophagic manner. Early clinical trials of TG02 in glioblastoma are ongoing.

DDIS-08. THE SMALL MOLECULE IMIPRIDONE ONC201 IS ACTIVE IN GLIOBLASTOMA WITH DRD2 PATHWAY DYSREGULATION

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ONC201, an imipridone that is a selective antagonist of the G protein-coupled receptors dopamine receptor D2 (DRD2) and D3 (DRD3), has exhibited tumor shrinkage and an exceptional safety profile in a phase II recurrent glioblastoma clinical trial (Arrillaga et al, 2017). In vitro and in vivo studies have previously demonstrated single agent ONC201 efficacy in glioblastoma models (Allen et al 2013). In vitro efficacy profiling of ONC201 in the Genomic of Drug Sensitivity in Cancer (GDSC) collection of cell lines confirmed broad anti-cancer efficacy with high sensitivity in human brain cancer cell lines. DRD2 is overexpressed in glioblastoma and DRD2 antagonism induces tumor cell apoptosis via the same signaling pathways that respond to ONC201. Investigation of The Cancer Genome Atlas (TCGA) revealed that DRD2 is highly expressed in glioblastoma relative to other dopamine receptor family members and that genetic aberrations were rare. High expression of DRD2 occurred in primary, rather than secondary, glioblastoma and was associated with a poor prognosis. Immunohistochemistry analyses of tissue microarrays revealed DRD2 overexpression in glioblastoma relative to normal brain. A linear correlation between DRD2 mRNA and ONC201 GI50 was observed among glioblastoma cell lines in the NCI60 panel. A significant induction of serum prolactin, a surrogate biomarker of target engagement, was detected in ONC201-treated glioblastoma patients. Interestingly, expression of DRD5, a D1-like dopamine receptor that counteracts DRD2 signaling, was significantly inversely correlated with ONC201 potency in the NCI60 and GDSC datasets (P < .05). Furthermore, a missense DRD5 mutation was identified in cancer cells with acquired resistance to ONC201. Resistance could be recapitulated with overexpression of the mutant DRD5 gene or, to a lesser extent, with the wild-type gene. In conclusion, the DRD2 pathway is a therapeutic target that is dysregulated in glioblastoma and contains biomarkers of tumor sensitivity to ONC201 that are under clinical evaluation.

DDIS-09. IDH1 MUTATION-INSPIRED A-KETOGLUTARIC ACID MIMICS FOR EPIGENETIC THERAPY OF HIGHER GRADE GLIOMAS

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Mutations at the active site of isocitrate dehydrogenase 1 gene (IDH1; R132H) occur at a high frequency (>70%) early in the oncogenesis of lower grade malignant gliomas, and result in a dramatic accumulation of the oncometabolite D-2 hydroxyglutarate (D-2HG), that effectively replaces the normal metabolite α -ketoglutarate (α -KG) in cell physiology. Surprisingly, IDH1 mutations bestow superior therapeutic responses to alkylating agents and better patient survival. D-2HG, effectively competes with α -KG and potently inhibits various dioxygenase reactions, including the TET1&2 DNA demethylases and histone demethylases thereby, re-shaping and reprogramming the epigenetic landscape, and consequent transcriptional silencing; the DNA repair protein O6-Iguanine DNA methyltransferase (MGMT), which confers drug resistance is one major target for such repression. As a novel and innovative strategy of turning the tide against GBMs by exploiting the mechanistic aspects of the oncometabolite, we hypothesized that D-2HG and α -KG derivatives that can replace the natural metabolite in epigenomic dioxygenase reactions will serve as potent anti-glioma drugs either by themselves or in combination with the alkylating agents. To this end, we synthesized a 2,4-dimethyleneglutaric acid (DMG), an α -KG mimic with methylene groups inserted at the C2 and C4 positions. The hydrophobic DMG ester, by itself, was cytotoxic with IC50 values up to 300 μ M against brain tumor cell lines, however, when combined at 100 μ M with TMZ resulted in >20-fold increased synergistic cell killing. DMG induced oxidative stress inhibited α -KG dehydrogenase, MGMT and TET1&2. We also observed a marked reduction in ATP levels and rapid metabolic flux changes indicating an altered cellular metabolism. Collectively, these data reveal that acute treatments of α -KG analogs can alter the cellular metabolic and epigenetic makeup in a manner ascribed to D-2HG, and open up much-needed novel and exciting avenues of oncometabolite-based therapies for brain tumors (supported by CPRIT grant RP170693 to KSS)

DDIS-10. COMPARISON OF CEST AND DWI TO EVALUATE EARLY RESPONSE OF NITROXOLINE TREATMENT IN TEMOZOLOMIDE-RESISTANT GLIOBLASTOMA MODEL

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Glioblastoma multiforme (GBM) is the most common malignant primary brain tumor with often recurrence. The currently available imaging param-