HGG-13. BRAIN DISTRIBUTION AND CLEARANCE OF ALISERTIB IS LIMITED BY PGP AND BCRP EFFLUX PUMPS AND DEPENDENT UPON DELIVERY METHOD

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Diffuse midline gliomas (DMGs) harboring the H3K27M mutation are highly aggressive, uniformly fatal brain tumors that primarily occur in children. The blood-brain barrier (BBB), including drug efflux pumps, prevent numerous drugs from reaching CNS tumors at cytotoxic concentrations. Alisertib is an aurora kinase inhibitor that was previously identified in a drug screen as a compound of interest. However, its ability to penetrate the BBB is not well established. The goals of this study were two-fold: 1) determine the CNS distribution and clearance rates of alisertib following systemic delivery and if the BBB efflux pumps, Pgp and BCRP, alter distribution and clearance. 2) Compare alisertib distribution and clearance following convection-enhanced delivery to systemic results. WT and Pgp/BCRP knockout mice and Sprague-dawley rats underwent tail vein injection (mice and rats) or convection-enhanced delivery to the brainstem (rats only) of alisertib and sacrificed at 0, 2, 4, 8, 12, 16 and 24hours. The plasma and brain were collected and analyzed for alisertib concentration by HPLC-MS/MS. We found that in both mice and rats, alisertib concentration in the plasma and brain decreased biexponentially. Overall, Alisertib was found to be 3.14% brain penetrant. In Pgp/BCRP knockout mice, alisertib concentration in the plasma and brain decreased biexponentially, but was detected at higher concentration at all time points in the brain compared to WT mice. This resulted in a higher brain penetrance (17.26%). Differences based on anatomical brain region were significant in those rats which received convection-enhanced delivery. Alisertib was found in higher concentration in the pons and cerebellum compared to systemic delivery, but lower concentrations in the cortex and plasma. These results suggest that drug clearance may be a general mechanism limiting efficacy of drugs of interest and should be carefully considered during preclinical evaluation.

HGG-14. ACT001 – A PROMISING THERAPEUTIC FOR DIFFUSE INTRINSIC PONTINE GLIOMAS

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Diffuse Intrinsic Pontine Gliomas (DIPGs) are a subset of Diffuse Midline Gliomas (DMG) and are the most devastating of all brain tumors. There are no effective treatments and all children die of their tumor within 12-months. We performed a high-throughput drug screen with 3,570 biologically active, clinically approved compounds against a panel of DIPG cultures. Parthenolide, a compound derived from the herb *T.parthenium*, was found to be one of the most effective drugs tested, demonstrating significant anti-tumor activity. However, parthenolide also affected healthy cell viability and showed no *in-vivo* efficacy. ACT001 is a novel agent in clinical development that is a fumarate salt form of dimethylamino-micheliolide, which is semi-synthesized from parthenolide. ACT001 is blood-brain-barrier permeable and exerts an anti-tumor effect via inhibition of NF-κB and STAT3 pathways. ACT001 demonstrated potent anti-tumor activity against a panel of DIPG-neurospheres, with minimal effect on normal cells and inhibited colony formation *in-vitro*. To determine whether this activity could be replicated *in-vivo*, we tested ACT001 in a DIPG-orthotopic model. ACT001 was well tolerated and significantly improved survival of tumor-bearing animals, extending survival by 33% in ACT001 treated mice. We have initiated a Phase 1 paediatric trial of ACT001 for children with relapsed/refractory solid or CNS tumors, with an expansion cohort planned for patients with DIPG/DMG. Eleven patients have been enrolled, and the dose escalated from dose level-1 at 188mg/m2 bd to dose level-4 at 700mg/m2 bd. To date, no dose limiting or Grade 3/4 toxicities have been observed. At the highest dose level, clinical activity has been demonstrated in two patients, one with DIPG with a reduction in tumor burden, and another with DMG with H3K27M mutation with an objective radiographic and clinical response. These combined preclinical and clinical results suggest that ACT001 is an active novel therapy for patients with DIPG/DMG.

HGG-15. THE IMIPRIDONE ONC201 IN COMBINATION WITH THE ONCOLYTIC ADENOVIRUS DELTA-24-RGD HAS A SYNERGISTIC EFFECT IN PRECLINICAL MODELS OF PHGGS AND DMGS

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Pediatric High Grade Gliomas (pHGGs), including Diffuse Midline Gliomas (DMGs), are aggressive pediatric tumors with a poor overall survival. In the last years, ONC201 has emerged as a promising agent in the field of pediatric brain tumors. Another interesting approach is virotherapy; Delta-24-RGD, which is an oncolytic virus, has demonstrated safety and effectiveness in different preclinical models and in clinical trials. Therefore, in this work we set to evaluate whether the combination of ONC201 with Delta-24-RGD could result in an increased therapeutic benefit in pHGGs and DMGs. Given that ONC201 targets mitochondrial metabolism in a preclinical setting, we assessed potential negative interactions of the combination therapy. While ONC201 treatment resulted in decreased viral protein load (E1A and fiber), there was no significant negative impact on the viral replication (measured by hexon staining). ONC201 did not disrupt the activation of mTORC1 pathway by the adenovirus. Furthermore, Delta-24-RGD did not affect the decrease in basal oxygen consumption rate induced by ONC201. Our results suggested that ONC201 and Delta-24-RGD are not antagonistic. Evaluation of the *in vitro* cytotoxicity in different human pHGG (CHLA-03-AA and SF188) and DMG (TP-54 and SU-DIPG-IV) cell lines showed that the combination treatment was significantly better that either agent alone. *In vivo*, a single local injection of Delta-24-RGD followed by a weekly ONC201 of mice bearing CHLA-03-AA cell line orthotopically significantly increased the median overall survival (PBS: 48 days; ONC201: 54.5 days; Delta-24-RGD: 62 days; ONC201+Delta-24-RGD: 95 days (P=0.0008)) of these mice leading to 20% long-term survivors, free of disease. Currently, we are evaluating the effect of the combination in immunosuppressed and immunocompetent models of DMGs. In summary, our data indicate ONC201 in combination with Delta-24-RGD could be a potential therapeutic choice for patients affected by pHGGs and DMGs.

HGG-16. DISSECTING THE EFFECT OF ATM DELETION ON RADIOSENSITIVITY IN DIFFUSE MIDLINE GLIOMAS WITH H3K27M MUTATION

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Diffuse midline gliomas (DMGs) are responsible for a large proportion of childhood brain tumor deaths. Currently, radiation therapy is thought to be one of the most effective treatment options, but more than 90% of children still die within 2 years of diagnosis. DMGs are defined by somatic histone 3 K27M (H3K27M) mutations that have been shown to promote the G0/G1 to S cell cycle transition. The majority of DMGs also contain loss-of-function mutations in *TP53*. Prior research demonstrated that orthotopic xenograft and primary mouse models of non-H3K27M-mutated gliomas with inactivation of p53 are preferentially radiosensitized by inactivation of Ataxia Telangiectasia Mutated (ATM), a kinase that mediates DNA repair in response to DNA damage caused by radiation. The high frequency of mutations that deregulate p53 in DMGs raises the possibility that H3K27M-mutant DMGs may also be radiosensitized by ATM inhibition, representing a unique therapeutic opportunity. Here, we hypothesize that H3K27M-mutant DMGs that have loss of function of p53 will be radiosensitized by loss of ATM. To test this hypothesis, we used the RCAS-TVA viral gene delivery system to generate genetically-faithful primary mouse models of H3K27M-mutant DMG with *p53* deletion, and we used Cre recombinase to delete *Atm* in the tumor cells of these mice and generated littermate controls that retained *Atm*. Mice were imaged weekly via luciferase-based bioluminescence to track tumor development and irradiated with three daily fractions of 10 Gy after tumor detection. We subsequently quantified the survival of mice without neurological decline following irradiation. In separate cohorts, we collected primary tumors after irradiation to verify H3K27M expression and to assess cell cycle arrest and mechanisms of cell death. These studies will elucidate mechanisms by which ATM inactivation can radiosensitize H3K27M-mutant DMGs with nonfunctioning p53, which will guide the design of clinical trials testing ATM inhibitors in DMG patients.

HGG-17. FOCUSED ULTRASOUND-ENHANCED DELIVERY OF RADIOLABELED AGENTS TO DIFFUSE INTRINSIC PONTINE GLIOMA

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Diffuse intrinsic pontine glioma (DIPG) arising in the brainstem is the deadliest pediatric brain cancer with nearly 100% fatality and a median