proved treatment, with survival just 9-11–months post-diagnosis. ONC201 shows preclinical and emerging clinical efficacy in early-stage clinical trials, extending survival of DIPG patients by ~9-11–months compared to historic controls. However, patients invariably develop resistance, with some patients completely refractory to treatment. Using a multi-omics approach, including pharmacology, proteomics, genomics, epigenetics, in vitro and in vivo modeling, across ten international laboratories, we have uncovered the inherent mechanisms of resistance to ONC201. We find ONC201 elicits antagonism of the Dopamine receptor D2 (DRD2), whilst also causing mitochondrial degradation through potent agonism of the Mitochondrial protease CLPP, that drives proteolysis of the electron transport chain (ETC) protein Succinate dehydrogenase A (SDHA) and degradation of critical mitochondrial tricarboxylic acid (TCA) cycle regulator Isocitrate dehydrogenase 3B (IDH3B). Loss mitochondrial respiration increased hypoxia and reduced α-ketoglutarate, inhibiting lysine demethylation, increasing methylation of H3K4me3 and H3K27me3, thus altering the epigenome of primary DIPG cells. Loss of SDHA caused oxidation of succinate forming superoxide driving redox regulated PI3K/AKT signaling, counteracted using the PI3K/AKT inhibitor paxalisib. The combination of ONC201 and paxalisib synergically extended survival of two aggressive DIPG PDX models (SU-SIPG-VI vehicle=73 vs. combination=100-days, p=0.0027; SF8626 vehicle=36 vs. combination=43-days, p=0.0002). Compassionate access to this combination (n=2 patients; immediately post-RT and following re-RT) resulted in reductions in tumor volume and complete resolution of disease symptoms, extending overall survival (e.g., diagnosis patient MR axial scan=1554 mm2 , following eight months on the combination, current tumor volume=464 mm2 (<70%), patient remains on treatment). Our findings harness the powerful anti-DMG/DIPG pharmacokinetic/dynamic properties of ONC201 and paxalisib, a combination that is currently in clinical trials (NCT05009992).

DIPG-08. THE DEVELOPMENT OF ACT001 AS A NOVEL THERAPEUTIC FOR DIFFUSE INTRINSIC PONTINE GLIOMAS

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Diffuse Intrinsic Pontine Gliomas (DIPG) are a subset of Diffuse Midline Gliomas (DMG) and are the most devastating of all brain tumours. There are currently no effective treatments. ACT001 is a novel anti-cancer agent in clinical development that is blood-brain-barrier permeable. Previous studies undertaken in adult glioblastoma suggest that ACT001 exerts an anti-tumour effect via induction of oxidative stress and inhibition of NF-kB and STAT3 pathways. In DIPG, we have found that ACT001 demonstrated potent cytotoxic activity against a panel of DIPG neurospheres and also inhibited colony formation. Flow cytometric analysis confirmed the induction of apoptosis *in vitro*. Interestingly, we also observed increased expression of some markers of the ROS-dependant NRF2 endogenous antioxidant pathway within DIPG neurospheres suggesting an alternative mechanism of inhibition of DIPG cell proliferation involving ROS generation. In vivo testing of ACT001 in a highly aggressive DIPG-orthotopic model showed that ACT001 was well tolerated and significantly improved survival. ACT001 treatment of HSJD-DIPG007 tumour-bearing animals extended median survival from 59 to 78 days (p=0.0005). ACT001 treated mice had significantly decreased proliferating DIPG cells, analysed by Ki67 staining (p=0.01), and significantly increased H3K27me3 staining (p=0.0479). Given the significant effects on H3K27me3 we evaluated two potential drug combinations with known, clinically available epigenetic modifers. Combination of ACT001 with the FACT inhibitor CBL0137 or the HDAC inhibitor SAHA each significantly and synergistically decreased colony formation. These combinations are currently being evaluated *in vivo* in orthotopic models of DIPG. ACT001 is currently in a Phase 1 paediatric trial for children with DIPG/DMG. Clinical activity has been demonstrated in DIPG/DMG patients, including a reduction in tumour burden and clinical response. These combined preclinical and clinical results suggest that ACT001 is a viable therapy for patients with DIPG/DMG and preclinical combination therapy testing may guide further clinical trials.

DIPG-09. DIFFUSE MIDLINE GLIOMA-ADAPTIVE COMBINATORY TRIAL (DMG-ACT): A BIOLOGY-DRIVEN PLATFORM TRIAL IN PEDIATRIC AND YOUNG ADULT PATIENTS WITH DIFFUSE MIDLINE GLIOMA

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BACKGROUND: Despite advances in our understanding of the biology of diffuse midline gliomas (DMGs), little progress has been made in improving outcomes. Therapy development is limited by lack of preclinical data across multiple model systems and laboratories; limited knowledge about blood-brain barrier penetrance; and lack of multi-agent therapies. We aim to address these issues through DMG-ACT, where we translate robust preclinical data using ONC201 as the therapy backbone in a multi-arm, combination strategy within an innovative trial design. DESIGN: DMG-ACT is an open-label, multi-institutional trial of combination therapy for patients with DMG between 2 and 39 years of age. The trial utilizes a novel Bayesian drug combination platform design with adaptive shrinkage (ComPAS). ComPAS allows ongoing assessment of therapy efficacy with borrowing of data across different arms and the ability to eliminate ineffective drug combinations and add new promising combinations throughout the trial. ONC201 is the backbone therapy in each arm and given in combination with other agents that show additive or synergistic benefit in preclinical testing. Patients enter into one of three cohorts: newly diagnosed (Cohort 1), post-radiation (Cohort 2), and relapsed/progressive (Cohort 3). The cohorts offer a target validation option to assess intratumoral pharmacokinetics and pharmacodynamics of drug given prior to tumor biopsy. Cohort 1 and 3 offer radiation or re-irradiation with concomitant single agent therapy followed by maintenance combination therapy. The primary efficacy endpoints are median progression-free survival at 6 months (Cohort 1 and 2) and overall survival at 7 months (Cohort 3). Exploratory endpoints include intratumoral drug concentration, toxicity profile of combination therapy during radiation therapy, toxicity profile and efficacy of combination therapy, CSF and ctDNA analysis, and health related quality of life, cognitive, and patient/proxy-reported outcome measures. This trial was successfully launched in November 2021 with updates to be presented at the meeting.

DIPG-10. A PHASE I TRIAL OF PANOBINOSTAT FOLLOWING RADIATION THERAPY IN CHILDREN WITH DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG) OR H3K27M-MUTATED THALAMIC DIFFUSE MIDLINE GLIOMA (DMG): REPORT FROM THE PEDIATRIC BRAIN TUMOR CONSORTIUM (PBTC-047)

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INTRODUCTION: Panobinostat is an oral HDAC inhibitor with preclinical activity against DIPG. The phase I study in children with progressive DIPG (stratum 1) defined the maximum-tolerated dose (MTD) as 10 mg/m^2 administered 3x/week, 3 weeks on/1 week off. Herein, we report results of stratum 2, involving children with non-progressive DIPG/DMG using an alternative schedule. Primary objectives were to describe the toxicity profile and define the MTD; secondary objectives were to describe progression-free survival (PFS) and overall survival (OS). PATIENTS AND METHODS: Patients with non-progressive DIPG or H3K27M-mutated thalamic DMG were eligible >14 days following standard radiation therapy only. Panobinostat was given every other day, 3x/week, on alternate weeks. Patients who received at least one dose of panobinostat were evaluable for toxicity. Four dose levels (DL) were evaluated: DL1 (16mg/m^2/dose), DL2 (22 mg/m^2/dose), DL3 (28 mg/m^2/dose) and DL4 (36 mg/m^2/dose). Dose escalation was determined by a continuous reassessment method. Correlative studies included pharmacokinetics obtained on course 1, day 1, and day 3 prior to subsequent dosing. RESULTS: Thirty-four eligible patients (median age, 7.6 [3-16] years) were enrolled with 29 evaluable for dose finding; DL1, n=3; DL2, n=10; DL3, n=11; DL4, n=5. The primary toxicities were myelosuppression and gastrointestinal. Eight DLTs occurred: DL2, Grade 3 thrombocytopenia (n=1); DL3, Grade 4 neutropenia (n=3), Grade 4 neutropenia and Grade 4 thrombocytopenia, (n=1); DL4, Grade 2 nausea (n=1), Grade 3 increased ALT (n=1), Grade 4 thrombocytopenia (n=1). Median PFS from drug initiation was 4.4 (1-11.2) months; median OS from diagnosis was 11.7 (4.5-25) months. These did not significantly differ from the PBTC historical cohort (PFS, p-value 0.4967; OS, p-value 0.6457). CONCLUSION: The MTD of panobinostat administered on this schedule to children with non-progressive DIPG/DMG is 22 mg/m^2/dose. The primary DLT was myelosuppression. There was no significant improvement in PFS or OS in this cohort.