Research, Melbourne, Australia, [8]Centre for Molecular Medicine Norway (NCMM), Nordic EMBL Partnership, University of Oslo, Oslo, Norway

Diffuse midline gliomas (DMGs) remain incurable cancers and new treatments are urgently needed. One promising new therapeutic avenue for these cancers is targeting of metabolic vulnerabilities including a heightened dependence on mitochondrial metabolism. We and others have shown that the oral, brain-penetrant imipridone drugs ONC201 and ONC206 target mitochondrial metabolism in cancer cells. In particular, ONC201 and ONC206 hyper-activate the mitochondrial protease ClpP, impair mitochondrial oxidative phosphorylation (OXPHOS), activate the integrated stress response (ISR) signaling pathway, and induce apoptosis in DMG preclinical models. We validated ClpP as a key target of ONC206 by showing that CRISPR/Cas9-mediated *CLPP* knockout significantly decreased ONC206 sensitivity in DMG cells. We further showed that imipridone-mediated ClpP activation resulted in significant degradation of the chaperone protein ClpX. Moreover, ONC201 and ONC206 treatment inhibited mitochondrial respiration, decreased mitochondrial membrane potential and triggered extensive mitochondrial structural damage, including disintegration of mitochondrial cristae. Time-course RNA sequencing of five DMG cell lines treated with ONC201 and ONC206, alone or in combination, revealed robust ATF4 and CHOP upregulation, indicating potent activation of ISR signaling. Notably, ATF4/CHOP upregulation was strongest in ONC201/6 combination-treated cells, indicating synergy between the two drugs. We further explored drug combinations by testing ONC201 together with ONC206, Panobinostat, JQ1, and Osimertinib to identify synergistic combination treatments. The strongest synergistic effect was found over a broad IC_{50} range for ONC201 and ONC206. Finally, we showed that ONC201 and ONC206 significantly prolonged survival of mice bearing brainstem DIPG xenografts. Ongoing studies include assessment of the *in vivo* efficacy of ONC201 and ONC206 across different CNS tumor models, as well as investigation and validation of clinically relevant biomarkers of response to treatment. In summary, our preclinical data strongly support the utility of the mitochondrial targeting agents ONC201 and ONC206 for the treatment of DMG and other malignant brain tumors.

HGG-33. EXPLOITING METABOLIC DEFECTS WITH NAMPT INHIBITORS IN DIPG

Ranjithmenon Muraleedharan[1], Collin Heer[2], Ranjini Sundam[1], and Charles Brenner[3]; [1]Yale University, New Haven, CT, USA, [2]University of Iowa, Iowa City, IA, USA, [3]City of Hope National Medical Center, Duarte, CA, USA

Diffuse intrinsic pontine glioma (DIPG) are universally lethal pediatric brain tumors with limited treatment options. We recently performed synthetic lethal drug screen with a panel of DNA repair and metabolic inhibitors *in vitro*, in patient-derived DIPG cells and isogenic cell lines engineered to contain key DIPG-associated mutations. Nearly 80% of DIPGs harbor a recurrent H3K27M mutation in H3.3 (H3F3A) or H3.1 (HIST1H3B) histones. This has prompted us to consider H3K27M mutation-induced exploitable defects for a therapeutic gain. This screen identified synthetic lethal interactions between H3K27M mutations and the nicotinamide phosphoribosyl transferase (NAMPT) inhibitor, FK866. The association between H3K27M mutations and NAMPTi sensitivity was validated in follow-up studies using isogenic WT and H3K27M-mutant expressing pairs of human immortalized astrocytes and neural progenitor cells (NPCs). In addition, we tested the effects of FK866 in a panel of unique DIPG patient-derived tumor neurospheres in short-term viability assays. We found that FK866 induced depletion of NAD and exhibited toxicity towards H3K27M mutant DIPG cell lines with an IC_{50} of ~2.5 nM. These findings were consistent across three structurally unique NAMPT inhibitors. Finally, we found that inducible expression of mutant H3K27M results in a gradual, 50% decrease in cellular NAD levels over the course of five days, suggesting that H3K27M mutations may induce an inherent NAD metabolic defect that is exploited by NAMPTi's. We now seek to understand the mechanistic basis for the observed metabolic defect and NAMPTi sensitivity associated with mutant H3K27M. In addition, we aim to identify specific NAMPTi and DNA damaging agent combinations which maximally exploit this H3K27M-associated NAD metabolic defects.

HGG-34. A DNA DAMAGE REPAIR SIGNATURE IN PRIMARY AND RECURRENT PEDIATRIC HIGH-GRADE GLIOMAS: PROGNOSTIC AND THERAPEUTIC VALUE

Natacha Entz-Werle[1,2], Laetitia Poidevin[3], Petr Nazarov[4], Benoit Lhermitte[5], Marie Pierre Chenard[5], Olivier Poch[3], Monique Dontenwill[1], and Eric Van Dyck[4]; [1]UMR CNRS 7021, Illkirch, France, [2]Pediatric oncohematology, university hospital of Strasbourg, Strasbourg, France, [3]ICube, Strasbourg, France, [4]Luxembourg Institute of Health Department of Oncology, Luxembourg, Luxembourg, [5]Pathology Department, University Hospital of Strasbourg, Strasbourg, France

Pediatric high-grade gliomas (pHGGs), including diffuse intrinsic pontine gliomas (DIPGs), despite their low incidence, are the leading cause of mortality in pediatric neuro-oncology. Frequently, pHGGs, harboring mainly histone mutations, are or become resistant to standard therapies like irradiation or chemotherapies. Recent insights showed in adult HGG the predominant role of DNA damage repair (DDR) as a way of prognostic classification. Given the recent evidence that transcription conflicts like in histone-mutated gliomas can induce replication stress and be linked to DDR abnormalities, this study is aiming to establish a DDR signature able to classify specifically pHGGs and to cluster among them poor responders to radiation. Transcriptomic analyses were performed to discriminate seven pHGGs comparatively to a cohort of 10 pilocytic astrocytomas with specific DDR deregulations. The specific transcriptomic signature obtained from this differential gene expression analysis was compared to the aHGG signature already established. To strengthen, refine and finalize the DDR signature able to classify and cluster the pHGGs, we explored both signatures and their common genes in already published transcriptomic analyses of DIPGs and sus-tentorial pHGGs. To check DDR protein expressions correlated to loss of trimethylation as well as histone and TP53 mutations, an immunohistochemical assessment of several DDR markers was performed on a collection of 21 pHGG diagnostic samples and 9 paired relapses. To validate the DDR functional inhibition, we used 3 patient-derived cell lines bearing H3.3K27M mutations. A finalized signature of 28 genes involved in DNA repair and cell cycle machineries was used to cluster in two groups the pHGG cohorts. The differential protein expression of PARP1, XRCC1, p53 and stem cell markers was linked significantly to the more resistant pHGGs and the rapid progressions after radiotherapy. Those DDR makers might be used as theranostic and therapeutic targets, which were screened in PDCLs with promising results.

HGG-35. IDENTIFICATION OF G PROTEIN-COUPLED RECEPTOR 17 (GPR17) UP-REGULATION IN PAEDIATRIC H3 K27M-MUTANT DIFFUSE MIDLINE GLIOMA AND EXAMINATION OF ITS ROLE IN DIFFUSE MIDLINE GLIOMA CELL LINES

Katie Loveson and Helen Fillmore; University of Portsmouth, Portsmouth, UK

Paediatric H3 K27M-mutant diffuse midline glioma (pDMG) is an incurable, aggressive childhood brain malignancy, that arises in a region- and age-specific nature. The underlying pathophysiology suggests dysregulation of postnatal neurodevelopmental processes causing aborted cell differentiation. The cell of origin is unclear, but data suggests an oligodendrocytic lineage (OPC), supported by the over-expression of transcription factors such as Olig1 and Olig2 in 80% of DIPG cases. In-depth bioinformatics and principal component analyses (PCA) of genes involved in brain development and pDMG support reports of OPC gene dysregulation and led to the identification of the G-protein coupled receptor 17 (GPR17) and its association with pDMG. GPR17, a rhodopsin-like orphan GPCR (unknown ligand), has the typical features of the GPCR superfamily, seven transmembrane domains (TM1-TM7), eight amphipathic helices, an extracellular N-terminal domain, and an intracellular C-terminus. GPR17 has been implicated in several normal physiological and pathological processes, such as oligodendrocyte differentiation, spinal cord injury and brain injury. GPR17 mRNA and protein expression was confirmed in all pDMG cell lines tested. Using a well-characterised agonist (MDL 299,51) and antagonist (HAMI3379) to modulate GPR17 function in pDMG cell lines resulted in phenotypic and genomic changes as well as in cell growth and migration. HAMI3379, a GPR17 specific antagonist resulted in a significant reduction in GPR17 mRNA and protein expression ($p<0.006$) and a significant reduction in migration ($p<0.0025$). When pDMG cells were pretreated with HAMI3379 in combination with known cytotoxic agents (Bleomycin, a radiation mimic, Panobinostat or Vincristine), there was a decrease in cell viability compare to cytotoxic agent alone. There are no current effective therapies for pDMG patients and the ability of blocking GPR17 function to enhance sensitivity to standard therapies is appealing and warrants further investigation.

HGG-36. ONC201 TARGETING IN DIPG

Alqassem Abuarqoub[1], Tatiana McAnulty[2], Eyal Amiel[1], and James Stafford[1]; [1]University of Vermont, Burlington, VT, USA, [2]The Pennsylvania State University, University Park, PA, USA

Diffuse intrinsic pontine glioma (DIPG) is a group of predominantly pediatric brain tumors with an average age of diagnosis of 6–7 years old, and a poor prognosis (median survival of ~1 year). Given the location of DIPG in the brainstem, surgical approaches are limited. Furthermore, the tumors have limited responsivity to traditional chemotherapy or radiotherapy, ergo new therapeutic options are needed. Recently, the drug ONC201 has emerged as a potential therapeutic option with outcomes sometimes surpassing progression-free and expected survival outcomes. However, the selectivity of its effect and mechanism in DIPG is still unclear. Here, we pursue a better understanding of ONC201 and its mechanism of action directly in DIPG patient-derived cell lines. First, we demonstrate that a range of DIPG cell lines are highly sensitive to ONC201 and compare this sensi-