

tivity to other patient-derived brain tumor and normal controls. Next, we directly show that the mitochondrial protease, ClpP is the primary target of ONC201 in DIPG. Given recent literature implicating the activation of ClpP by ONC201 and dysregulation of the metabolome in other tumors, we are currently examining these downstream effects in DIPG. Ultimately we hope to elucidate whether ClpP targeting can be used to better diagnose and improve therapeutic options in DIPG

#### HGG-37. UPFRONT TARGETED THERAPY FOR THE TREATMENT OF BRAFV600E-MUTANT PEDIATRIC HIGH-GRADE GLIOMA – A MULTI-INSTITUTIONAL EXPERIENCE

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**Introduction:** Sustained responses to molecular targeted therapy with BRAF with or without MEK inhibitors have been reported in patients with recurrent BRAFV600E-mutant pediatric high-grade gliomas (pHGG). The role of upfront targeted therapy in this population, however, has not yet been established. **Methods:** We performed a retrospective, multi-institutional record review of patients with BRAFV600E-mutant pHGGs, treated with off-label BRAF and/or MEK inhibitors as part of their initial adjuvant therapy. **Results:** Seventeen patients were identified (median age at diagnosis, 8.8 years, range 1.8–20.2). Histologic diagnoses included HGG/glioblastoma (n=10), anaplastic ganglioglioma (n=3), high-grade neuroepithelial tumor (n=2), diffuse midline glioma (n=1) and anaplastic astroblastoma (n=1). Ten patients underwent biopsy (n=8) or subtotal resection (n=2), while near-total or complete resection was accomplished in seven. Concomitant genetic alterations including CDKN2A/B loss, H3K27M and TERT promoter mutations were found in eight tumors. Thirteen patients received focal radiation therapy (RT) and one received craniospinal irradiation prior to targeted therapy. Adjuvant targeted therapy was initiated shortly after diagnosis or completion of RT. Five patients received BRAF-inhibitor monotherapy (dabrafenib or vemurafenib). Twelve patients received combination therapy with the addition of a MEK inhibitor (trametinib). For patients with measurable disease, best responses per COG criteria were CR (n=3), PR (n=7), SD (n=3) and PD (n=1). With median follow-up of 29 months (range 8–78), two-year PFS and OS for the cohort were 74.7% and 81.1%, respectively. Ten (59%) patients remain free of disease recurrence or progression. Grade 3 or higher toxicities were reported in four patients (neutropenia, skin toxicity/photosensitivity, fatigue and weight loss), leading to therapy discontinuation in two. **Conclusions:** Upfront targeted therapy for patients with BRAFV600E mutant pHGG appears tolerable and effective, with a durable disease control rate that is superior to historical data. This promising paradigm is currently being evaluated prospectively in the COG ACNS1723 clinical trial.

#### HGG-38. DE NOVO PYRIMIDINE SYNTHESIS INHIBITION INDUCES REPLICATION CATASTROPHE MEDIATED CELL DEATH IN DIFFUSE MIDLINE GLIOMA

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Diffuse midline gliomas (DMG) are aggressive and lethal pediatric brain tumors that cannot be cured by conventional therapeutic modalities. Using a genome wide CRISPR screen we identified the *de novo* pyrimidine biosynthesis pathway as a metabolic vulnerability in DMGs. BAY2402234 is a small molecule inhibitor of DHODH -a rate limiting enzyme in the *de novo* pyrimidine biosynthesis pathway. BAY2402234 induces cell death in DMG cells at low nanomolar concentrations while sparing adult glioblastoma cells and normal astrocytes. Further investigations revealed dramatic reduction in cellular UMP pools, the precursor for all pyrimidine nucleotides, after DHODH inhibition, specifically in DMG cells. Cytotoxicity of DHODH inhibition in DMG cells is rescued by exogenous uridine, supporting UMP depletion as the mechanism underlying DMG cell death and also showing that cell death is an “on target” response to BAY2402234. Cell death induced by BAY2402234 is a consequence of replication fork stalling as evident by accumulation of chromatin-bound RPA foci and g-H2AX. Stalled replication forks eventually collapse, resulting in replication catastrophe and apoptosis.

Cytotoxic effects of DHODH inhibition are further exacerbated by inhibition of the intra-S checkpoint protein, ATR. Combined treatment of DMG cells with DHODH and ATR inhibitors resulted in enhanced accumulation of chromatin-bound RPA, g-H2AX, replication fork collapse and apoptosis. Importantly, *in vivo* studies verify that both BAY2402234 (DHODHi), and BAY1895344 (ATRi), cross the blood-brain barrier, accumulate in the brain at therapeutically relevant concentrations, and induce DNA damage in intracranial DMG xenografts in mice. Taken together, our studies have identified DHODH inhibition as a DMG-specific vulnerability resulting in cell death; the mechanism of DHODH-induced cell death led us to identify combined inhibition of DHODH and ATR as a synergistic therapy against DMG tumors.

#### HGG-39. ALTERNATIVE SPLICING OF NEUROFIBROMIN 1 IS ASSOCIATED WITH ELEVATED MAPK ACTIVITY AND POOR PROGNOSIS IN HIGH-GRADE GLIOMA

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Despite a good understanding of the coding mutations underlying high-grade gliomas (HGG), their prognosis remains poor. We sought to characterize their transcriptional alterations and how this contributes to pathogenesis. We analyzed a large cohort of pediatric HGG (pHGG) by DNA sequencing (n=79) and RNA-Seq (n=63 plus normal brain, n=20), finding spliceosome mutations that are associated with increased splicing burden. High levels of alternative splicing were found in known cancer driver genes, with enrichment for chromatin regulators (including the SWI/SNF and NuRD complexes) and the RAS/MAPK pathway, in particular *neurofibromin 1* (*NF1*). Both pediatric and adult HGG preferentially expressed *NF1-II*, a less active RAS GTPase, resulting in increased RAS/MAPK activity resulting from inclusion of exon23a into the GAP-related domain of *NF1*. In IDH wild-type, adult HGG, *NF1-II* was associated with reduced survival independently from RAS/MAPK pathway mutations. *NF1* exon23a splicing was regulated by REST-mediated suppression of splicing factors controlling its inclusion. Together, our results identify a novel mechanism by which HGG can activate RAS/MAPK signaling and other oncogenic pathways to promote tumorigenesis independently from direct mutations.

#### HGG-40. FOCUSED ULTRASOUND ENHANCES ETOPOSIDE DELIVERY IN A MURINE PONTINE GLIOMA MODEL

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**Background:** Diffuse intrinsic pontine glioma (DIPG) is a devastating pediatric brain cancer with limited treatment options and poor survival. The delivery of systemic therapies in this disease is severely limited by the blood-brain barrier (BBB). Focused ultrasound combined with intravenous microbubbles (FUS+MB) can effectively open the BBB permitting the entry of drugs across the cerebrovasculature. Etoposide is a chemotherapy frequently used in pediatric oncology with well-established anti-tumor effects but limited efficacy when administered systemically in DIPG. Given that FUS+MB in DIPG is not well studied, our goal was to determine the feasibility of ultrasound-mediated BBB opening and etoposide delivery in a preclinical murine pontine glioma model. **Methods:** A syngeneic, orthotopic model was established by stereotactic injection of PDGF-B+PTEN<sup>-/-</sup>p53<sup>-/-</sup> murine glioma cells into the pons of B6 albino mice. Mice were randomly divided into control (n=6) or FUS+MB groups (n=6). A single-element, spherical-segment FUS transducer (center frequency=1.5MHz) driven by a function generator through a power amplifier was used with concurrent microbubble injection to sonicate the tumor and its margins on post-injection day 14. Immediately after treatment, 5 mg/kg of intraperitoneal etoposide was administered to all 12 mice. All animals underwent cardiac puncture and blood sampling, followed by transcardiac perfusion and brain harvesting. Liquid chromatography-mass spectrometry was performed on both serum and tumor tissue to measure etoposide levels. **Results:** Contrast-enhanced MRI demonstrated successful BBB opening in all FUS+MB mice. Compared to control (mean=20.98ng/g), etoposide concentration in the sonicated tumor tissue (mean=164.77ng/g) was nearly eight times greater. Lastly, the mean brain tumor-to-serum ratio was more than fivefold higher in the treated mice (1.50%) compared with the control mice (0.28%) (P<0.005). **Conclusions:** FUS+MB is hereby shown successful in BBB opening and enhanced delivery of etoposide in a preclinical pontine glioma model.