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BACKGROUND/OBJECTIVES. We present the results of multicenter retrospective study in children with diffuse intrinsic pontine glioma (DIPG). DESIGN/METHODS. From January 2010 to October 2018 142 patients with DIPG were observed/treated in Rogachev's center from 16 regional hospitals of Russia. All details of follow-up were available in 57 pts (40%). MRI was the main confirmation of diagnosis, 1 pt was biopsied, 2 – received partial resection. Histological types: 1 - DIPG, 1 - HGG, in 1 patient the biopsy wasn't informative. The distribution on the age in 57 pts (median 60 mths, 17–180 m): 9 (15,8%) patients was younger than 3 years, 48 (84,2%) patients were older than 3 years. There were 29 (50.8%) girls and 28 (49.1%) boys. 48 patients (84%) who received RT/CHT, and 9 (15,7%) patients were not treated. RT was performed in 47 patients, among them 20 (42%) – with parallel Temozolomid (TMZ), in 1 - with Valproic acid. 27 patients (47%) showed positive response to the therapy. 16 patients (28%) received TMZ after radiation therapy, 8 (50%) of them had positive answer. 19 pts (33%) are alive. The median to the first progression after RT was 9 m (1–57 m). The median of OS 12 m (1–63 m). Among 20 pts, who received TMZ during RT 17 pts died (85%). Among 26 pts, who did not receive TMZ 15 pts died (57,7%). Salvage (second) RT was performed in 17 children (29%) among them only 2 pts (11,8%) are alive. CONCLUSIONS: It is well know that not any therapy of DIPG can provide long-term survival. Adding TMZ to RT and salvage RT does not improve the effect of primary and relapse treatment also in our cohort. Further research is needed in this area for the development of new treatment strategies.

DIPG-21. ELECTRICAL INTEGRATION OF GLIOMA INTO NEURAL CIRCUITRY

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Pediatric high-grade gliomas (pHGGs) are a lethal group of cancers whose progression is robustly regulated by neuronal activity. Activity-regulated release of growth factors into the tumor microenvironment represents part of the mechanism by which neuronal activity influences pHGG growth, but this alone is insufficient to explain the magnitude of the effect that activity exerts on glioma progression. Here, we report that neuron-glioma interactions include bona fide synaptic communication. Single cell transcriptomic analyses of primary pediatric and adult glioma samples reveal unambiguous expression of synaptic genes by malignant glioma cells. Whole cell patch clamp recordings from xenografted, pediatric patient-derived glioma cells revealed the existence of AMPAR-mediated excitatory neurotransmission between pre-synaptic glutamatergic neurons and post-synaptic glioma cells. Millisecond timescale excitatory post-synaptic currents (EPSCs) that are depolarizing were observed in a subpopulation of pHGG cells and are associated with activity-induced glioma cell calcium transients. These excitatory axon-glioma synapses are reminiscent of the axon-glial synapses formed between neurons and oligodendrocyte precursor cells. A second electrophysiological response characterized by a prolonged (>1 sec) depolarization in response to neuronal activity was also observed. These longer duration currents are blocked by gap junction inhibitors, supporting the concept that gap junction-mediated tumor interconnections, such as observed with tumor microtubes, can function as an electrically coupled network. As neurotransmitter-mediated depolarization of normal neural precursor cells can profoundly affect precursor cell proliferation, differentiation and survival, we tested the hypothesis that depolarizing currents in pediatric glioma cells promote tumor growth. Using *in vivo*optogenetic techniques to depolarize xenografted pHGG cells expressing channelrhodopsin-2 (ChR2), we found that glioma depolarization robustly promoted proliferation, while expression of a dominant-negative AMPAR subunit (GluA2) that blocks neuron-glioma signaling inhibited pHGG xenograft growth and extended mouse survival. These findings suggest that integration of pediatric glioma into neural circuits promotes tumor progression.

DIPG-22. GENETIC MODELING IMPLICATES RAS AND MYC AS KEY EPIGENETICALLY ACTIVATED TRANSCRIPTIONAL TARGETS OF H3K27M-DRIVEN CANCER

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Histone 3 mutations at lysine 27 (H3K27M) are frequent drivers of midline gliomagenesis, occurring in ~80% of diffuse intrinsic pontine gliomas (DIPG) and leading to widespread H3K27me3 changes through PRC2 inhibition. Although H3K27M mutations appear to cooperate with additional mutations including *TP53* and *PDGFRA*, the exact oncogenic function of H3K27M is unknown, and *in vivo* models have not yielded tumors driven by H3K27M alone. Here we created a genetic mouse model by microinjection, expressing H3.3K27M under control of the *Fabp7* promoter, which is active in the brain from approximately E14.5, primarily in radial glia and astrocyte precursors, but also allows expression in non-brain developing and adult tissues. H3.3K27M expression in the developing brainstem led to SOX10 upregulation via loss of H3K27me3 at the *Sox10* promoter, resulting in an expression profile consistent with K27M-mutant DIPG, including an oligodendrocyte precursor cell (OPC) signature along with RAS and EMT activation. H3.3K27M induced tumors in multiple organs that were driven by K27M alone and, when combined with *Trp53* loss, led to primary high-grade gliomas. The tumors had a cell-type independent expression signature featuring RAS and MYC activation, which overlapped with human DIPG and pointed to a core K27M transcriptome. Furthermore, as in human DIPG, mouse tumors spontaneously mutated the RAS pathway and MYC to lock in pathway activation. Our data suggest that RAS and MYC are core pathways that will need to be targeted in order to effectively treat this devastating disease.

DIPG-23. EFFICACY OF THE SOLUBLE PANOBINOSTAT (MTX110) IN PRECLINICAL DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG) MODELS

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INTRODUCTION: Large drug screening and preclinical studies identified the pan-histone deacetylate inhibitor panobinostat as a promising treatment for DIPG. However, blood brain barrier functional integrity in DIPG limits drug penetration and remains a key cause of resistance to therapies. Therefore, we explored the therapeutic potential of water-soluble formulation of panobinostat MTX110 in our preclinical models and tested the efficacy in DIPG in vivo models using convection-enhanced delivery (CED). METHODS: CellTiter-Glo assay was used assess antiproliferative effects in DIPG derived cell lines (SF8628, NEM157, SF10423, SF10693). Effect on cell cycle was assessed using FACS analysis. To test safety, concentrations from 30 uM to 3000 uM were administered via CED into the striatum of the rat brain. In vivo activity of MTX110 via CED was assessed using a DIPG patient derived model (SF8628). RESULTS: MTX100 showed a strong antiproliferative and cytostatic effect on halting progression through G0/G1 in all tested DIPG cell lines with similar efficacy than the original formulation at submicromolar concentrations. Immunohistochemistry analysis showed no significant changes in doses up to 1000uM. CED of 100uM MTX110 significantly prolonged survival (control: median survival 52 days; MTX CED: median survival 64 days; p=0.0372). CONCLUSION: Our study confirmed that CED administration of MTX110 provides a potent antitumor effect. An ongoing trial is testing the safety and efficacy of CED of MTX110 in newly diagnosed patients.

DIPG-24. DIFFUSE INTRINSIC PONTINE GLIOMAS EXHIBIT HIGH BASAL DNA DAMAGE AND ARE VULNERABLE TO INHIBITION OF DNA DAMAGE REPAIR PATHWAYS

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BACKGROUNDL Diffuse intrinsic pontine gliomas (DIPGs) are fatal childhood gliomas and radiotherapy is the only modality that prolongs survival; however, all patients progress with no long term survivors, indicating an urgent need to develop new treatment approaches. METHODS: Genome wide CRISPR screen was used to identify the cellular pathway(s) critical for DIPG cell survival and doxycycline inducible shRNA were used for temporal knockdown of gene expression. Colony formation assays were performed to monitor effects on cell proliferation, comet assay and immunoflourescence based gamma-H2AX foci detection were used for DNA damage assessment, and caspase3/7 activity measured apoptosis. RESULTS: Our preliminary studies to understand radio- and chemo-resistance mechanisms in DIPGs uncovered high levels of persistent basal DNA double strand breaks (DSBs) in the majority of analyzed DIPG cell lines compared to neural stem cells and astrocytes, a phenomenon that promotes oncogenic potential. The basal increase in DSBs was independent of H3.3K27M mutational status. To pinpoint the molecular mechanism(s) that support proliferation and survival of DIPG cells with highly damaged DNA, we performed unbiased CRISPR screens in unperturbed cells and preliminary results suggest an addiction of DIPG cells to specific DSB repair factors. Further analyses confirmed this intrinsic reliance of DIPG cells on specific DSB repair pathways and the key role of DNA repair mechanisms in suppressing DNA damage induced apoptotic cell death. CONCLUSION: DIPG cells exhibit persistent DNA damage. To evade catastrophic genomic instability and cell death, DIPG cells hijack DNA repair pathways. Therefore, a promising therapy against DIPG would include inhibition of specific DNA damage response pathways that allow DIPGs to survive persistent DNA damage.

DIPG-25. GENETIC ALTERATIONS TARGETING THE MAPK PATHWAY CONFERS PRECLINICAL SENSITIVITY TO TRAMETINIB IN A CO-CLINICAL TRIAL IN DIPG

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The survival of children with DIPG remains dismal, and new treatments are desperately needed. The development of patient-specific *in vitro* and *in vivo* models represents one such opportunity, however the time taken to establish such models and the rapid disease progression has been thought to limit the utility of such an approach. We sought to explore a co-clinical trial model for DIPG patients enrolled in an ongoing biopsy-stratified study in order to identify rational therapeutic options with individualised preclinical evidence as to their efficacy. To date we have established novel patient-derived *in vitro* cultures from biopsy specimens of 11 patients, in both 2D (laminin matrix) and 3D (neurosphere) conditions, as well as orthotopic xenografts *in vivo*, with a high concordance in their molecular profile compared to the original tumour specimen (methylation BeadArray, exome, RNA sequencing). Cells were screened against a series of common and bespoke FDA-approved drugs based upon previous evidence in DIPG and/or the specific molecular alterations found in the patient sample. We identified a high degree of *in vitro* sensitivity to the MEK inhibitor trametinib (GI50 23-312nM) in samples which harboured genetic alterations targeting the MAPK pathway, specifically the non-canonical *BRAF*_G469V mutation and those affecting *PIK3R1*(N564D, H450E_VF_ins), with assessment of tumour volume *in vivo* by MRI. Allelic imbalance of *PIK3R1*_N564D by stochastic selection was observed in two independent cultures from the same patient showing a greater response to trametinib (6.4-fold) as well as a decreased sensitivity to dasatinib in the mutant-enriched culture (38-fold). RNAseq of the cultures revealed differential gene expression associated with hypoxia and interferon signalling linked to drug sensitivity. These data show the feasibility in generating patient-specific, testable hypotheses that may be clinically translated in a subset of patients, and we are currently exploring parallel resistance modelling to further inform novel treatment strategies at tumour progression.

DIPG-26. *ACVR1* R206H COOPERATES WITH H3.1K27M IN PROMOTING DIFFUSE INTRINSIC PONTINE GLIOMA PATHOGENESIS

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Diffuse intrinsic pontine glioma (DIPG) is an incurable pediatric brain tumor, resulting in the death of 200–300 children each year in the United States. Recently it was discovered that approximately 25% of all DIPG cases harbor activating *ACVR1*mutations, a gene that encodes Activin A receptor (ALK2), a receptor in the bone morphogenetic protein (BMP) pathway, and that DIPGs with ALK2 mutations commonly harbor an H3.1K27M mutation. Herein, we used the RCAS/TVA retroviral system to study the effects of *ACVR1* mutations and H3.1K27M on DIPG pathogenesis. *In vitro* expression of *ACVR1* R206H with and without H3.1K27M in nestin-expressing brainstem progenitors resulted in upregulation of mesenchymal markers and revealed Stat3 activation by gene set enrichment analysis (GSEA) analysis. Neonatal expression of *ACVR1* R206H or G328V in combination with H3.1K27M and p53 deletion in nestin-expressing brainstem progenitors induced glioma-like lesions expressing mesenchymal markers along with Stat3 activation but were not sufficient for full gliomagenesis *in vivo*. In combination with platelet-derived growth factor A (PDGFA) signaling, *ACVR1* R206H and H3.1K27M significantly decreased survival and increased tumor incidence. We demonstrate that targeting the BMP signaling may be an effective therapeutic strategy to treat *ACVR1* R206H mutant DIPGs as exogenous Noggin expression at tumor initiation significantly increased tumor latency and treatment of *ACVR1* R206H mutant murine DIPGs with LDN212854, an *ACVR1* inhibitor, significantly prolonged their survival. We confirm relevance of our model to the human disease as human DIPG models with *ACVR1* mutations were also sensitive to treatment with LDN212854 *in vitro*. Altogether, our studies demonstrate that *ACVR1* R206H and H3.1K27M promote tumor initiation, accelerate gliomagenesis, promote a mesenchymal profile in part due to Stat3 activation, and identify LDN212854 as a promising compound to treat children with DIPG.

DIPG-27. OPTIMIZING CLINICAL TRIAL DESIGN: PHARMACOKINETICS OF MARIZOMIB AND PANOBINOSTAT IN A NON-HUMAN PRIMATE MODEL

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BACKGROUND: Pre-clinical determination of disease-specific activity, effective dosing, safety, pharmacokinetics, and CNS delivery can optimize clinical trial designs. The proteasome inhibitor, marizomib, together with the HDAC inhibitor, panobinostat, is active and synergistic in pre-clinical DIPG studies, with target concentrations of 20 and 100 nM, respectively. The adult maximum tolerated dose (MTD) for marizomib is 0.8 mg/m^2. We evaluated the safety, tolerability and pharmacokinetics in a non-human primate model, predictive of pediatric patients. METHODS: Marizomib was administered (10-minute intravenous infusion) at three dose levels: 0.02 (n=4), 0.04 (n=5), and 0.06 mg/kg (n=1), equivalent to human doses (HED) of 0.4, 0.8, and 1.1 mg/m^2, respectively. Marizomib (dose 0.04 mg/kg) was subsequently administered (n=4) 1-hr post-panobinostat (dose 1 mg/kg, HED 20 mg/m^2, p.o.). Drug concentrations were determined by LC-MS/MS using validated assays and PK parameters calculated via noncompartmental methods. RESULTS: Marizomib +/- panobinostat was tolerable with the exception of one animal (single agent marizomib, dose 0.06 mg/kg, HED= 1.2 mg/m^2) that expired 12–20 hr post administration; no clear etiology was found at necropsy. Remaining adverse events were Gr 1, 2 with the exception of lymphocytopenia, Gr 3 (n=2). Marizomib demonstrated rapid plasma clearance (1.22–10.25 L/min), short plasma half-life (4.45–8.24 min), non-linear increase in AUC_{inf}, and no significant difference across dose levels. Conversely, in CSF, there was a trend toward increasing exposure with increasing dose. $T_{1/2}$ was longer in CSF than blood (18–25 min vs. 4–7 min, respectively). Comparing marizomib PK before and after panobinostat, $t_{1/2}$ and clearance were similar (mean 7.80 vs. 9.49 min, and 6.04 vs. 4.24 L/min, respectively); CSF AUC_{inf} increased, 65.69 vs. 121.68 min*nM, respectively. CONCLUSIONS: Marizomib penetrates into the CNS and CSF exposure was higher after panobinostat. This combination warrants clinical evaluation in DIPG; correlation of results with preclinical findings is planned.

DIPG-28. NTRK FUSIONS IN PEDIATRIC DIFFUSE INTRINSIC PONTINE GLIOMAS

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Diffuse intrinsic pontine gliomas (DIPGs) are effectively incurable brainstem tumors with extraordinarily limited treatment options. Decades of