NEURO-ONCOLOGY

Abstracts

HIGH GRADE GIOMAS

HG-01. ETOPOSIDE IMPROVES SURVIVAL IN HIGH GRADE GIOMA: A META-ANALYSIS
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BACKGROUND: Despite standard therapy, high grade gliomas (HGGs) have a very poor prognosis. There are numerous phase I and phase II trials investigating novel agents and multimodal approaches that seek to improve upon survival in these patients. The purpose of this study was to evaluate the therapeutic efficacy of topoisomerase inhibitors in the treatment of HGGs. METHODS: We compared the efficacy of chemotherapy drugs in a meta-analysis of 775 HGG studies, including 82,332 patients. Survival gain was defined as an increase in median overall survival compared to the predicted value as computed by multivariate analysis. RESULTS: Patient cohorts treated with Etoposide (VP-16) had a significant improvement in median overall survival (15.45 months vs. 13.06 months, p = 0.031, 48 vs. 739 cohorts) and a survival advantage (p = 0.035) over cohorts treated without CPT-11. Subgroup analyses revealed that newly diagnosed patients treated with Etoposide had a median overall survival of 9.89 months vs. 13.35 months, p = 0.007) and a survival disadvantage (p = 0.163) over cohorts treated without CPT-11. Additionally, a subgroup of patients with BSG had symptomatic pseudoprogression, with transient neurologic deterioration and tumor enlargement followed by stabilization. Among 22 patients evaluable for response, 4 had rapidly progressive disease, 14 had stable disease for 3 months, 2 had PRs, 1 had an MR, and 1 had PR with BSG had symptomatic pseudoprogression, with transient neurologic deterioration and tumor enlargement followed by stabilization. Among 22 patients evaluable for response, 4 had rapidly progressive disease, 14 had stable disease for 3 months, 2 had PRs, 1 had an MR, and 1 had prolonged disease-free status after surgery. ELISPOT analysis, completed in 24 patients, showed response to IL13Rα2 in 5, EphA2 in 3, and survivin in 3. CONCLUSION: Peptide vaccination in children with gliomas is generally well tolerated, and has preliminary evidence of both immunologic and clinical activity. Pseudoprogression can initially be difficult to distinguish from true progression and aggressive management may be warranted.

HG-02. GBM STEM CELL NICHE DISRUPTING AGENTS IDENTIFIED THROUGH NOVEL HIGH THROUGHPUT COMPOUND LIBRARY SCREEN
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A diagnosis of Glioblastoma multiforme (GBM) carries a dismal prognosis and new approaches to treatment are needed. Among the potential high impact targets in GBM is the GBM stem-like cell within peri-vascular niche (PNV). In this domain, GBM stem-like cells exhibit enhanced growth and relative resistance to the effects of chemotherapy and radiation therapy. To identify the molecular pathways that mediate the intercellular cross-talk between endothelial cells and brain tumor cells, and to identify novel agents for disrupting these pro-tumor interactions, we performed an in vitro high throughput compound library screen for drugs that disrupted the niche effect of the peri-endothelial domain. In order to perform this screen we developed a co-culture model of the PNV that incorporated extra-embryonic teleost tissues. To identify additional molecules and pathways that mediate endothelial and GBM cell interactions we used this co-culture system to screen the Spectrum Collection compound library. While most compounds in this 2000 compound library were without effect, we identified a small but diverse group of drugs that blocked the trophic effects of the HHBECs on GBM cells. In addition, we identified a second set of compounds, which were highly toxic to GBM cells in monoculture but had no effect when administered to co-cultures.

HG-03. PEPTIDE VACCINE THERAPY FOR CHILDHOOD GLIOMAS: INTERIM RESULTS OF A PILOT STUDY
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INTRODUCTION: Malignant astrocytomas of the brainstem and cerebralm hemispheres and multiply recurrent low-grade gliomas carry a poor prognosis despite current treatments, and new therapeutic approaches are needed. Having gained significant experience with immunotherapy for adult gliomas, we extended these insights to childhood gliomas, based on our observations regarding their profiles of glioma-associated antigen (GAA) expression. METHODOLOGY: We initiated a pilot trial of subcutaneous vaccinations with peptides for GAA epitopes in Montanide every 3 weeks for 3 courses with intramuscular injections of poly-ICLC in HLA-A2+ children with newly diagnosed brainstem gliomas (BSG), cerebral high-grade gliomas (HGG), or recurrent gliomas. GAs were EphA2, IL13Rα2, and survivin. Endpoints were safety and T-cell responses against vaccine-targeted GAs, assessed by ELISPOT and tetramer analysis. Treatment response was evaluated clinically and by MR imaging. RESULTS: To date, 24 children have been enrolled, 13 with newly diagnosed BSG, 3 with newly diagnosed HGG, and 6 with recurrent gliomas. No dose-limiting non-CNS toxicity has been encountered. One child with a BSG had transient tumor enlargement in association with acute neurological deterioration 4 months after beginning vaccination that later regressed and culminated in a sustained partial response (PR), consistent with pseudoprogression. Two other children with BSG had symptomatic pseudoprogression, with transient neurologic deterioration and tumor enlargement followed by stabilization. Among 22 patients evaluable for response, 4 had rapidly progressive disease, 14 had stable disease for > 3 months, 2 had PRs, 1 had an MR, and 1 had prolonged disease-free status after surgery. ELISPOT analysis, completed in 24 children, showed response to IL13Rα2 in 5, EphA2 in 3, and survivin in 3. CONCLUSION: Peptide vaccination in children with gliomas is generally well tolerated, and has preliminary evidence of both immunologic and clinical activity. Pseudoprogression can initially be difficult to distinguish from true progression and aggressive management may be warranted.

HG-04. AN INTERNET-BASED SURVEY EVALUATING THE STANDARD OF CARE IN TREATING CHILDREN WITH NEWLY DIAGNOSED HIGH GRADE GLIOMA
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INTRODUCTION: High grade gliomas (HGG) represent approximately 10% of all pediatric central nervous system (CNS) tumors. Despite a variety of therapies, outcomes remain dismal. In contrast, adult high grade gliomas (HGG) there is no apparent standard of care (SOC) for the treatment of children with HGG after surgery. We undertook an internet-based survey to better understand what the perceived SOC is for children > 3 years with newly diagnosed HGG. METHODS: An 8 question internet-based survey was e-mailed to 120 physicians who treat children with CNS tumors. Demographic data, including medical specialty, experience and institutional affiliations were collected. Respondents were asked what they consider as SOC for children with newly diagnosed HGG after a maximal surgical resection. RESULTS: The entire survey was completed by 62.5% (75/120) of respondents. 83% (62/75) identified themselves as pediatric oncologists/neuro-oncologists. The remaining were pediatric neurosurgeons, radiation oncologists and neurologists. 65% had >10 years experience and approximately 84% worked in a large academic or cancer center. More than 70% answered that their affiliated institution sees more than 5 pediatric HGG patients each year. The most commonly answered SOC was to treat patients on any available Phase I or II clinical trial (267%). In the absence of a clinical trial, physicians most commonly answered that they personally would treat a newly diagnosed patient with focal radiation plus temozolomide followed by maintenance temozolomide (30%). CONCLUSIONS: The response rate to our survey was excellent, and the

Published by Oxford University Press on behalf of the Society for Neuro-Oncology 2012.
demographic data indicates a group of experienced physicians who work at large academic and cancer centers. Despite this, there was no clear accepted SOC for children with newly diagnosed HGG. Even the most common responses were given by less than 1/3 of participants. This survey highlights that the SOC for children with newly diagnosed HGG remains controversial and unclear.

HG-06. EFFICACY OF COMBINATION THERAPY WITH PONATINIB (AP24534) +/- BEVACIZUMAB AGAINST PEDIATRIC GLIOBLASTOMA

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INTRODUCTION: Ponatinib (AP24534) is an oral multi-targeted kinase inhibitor that is believed to have broad potential applications in cancer. Ponatinib was designed using ARIAD’s computational and structure-based drug design platform to inhibit the enzymatic activity of BCR-ABL with very high potency and broad specificity. Ponatinib also exhibits potent activity for other key kinases involved in cancer including FGFR1, VEGFR2, TIE2, PDGFR, KIT and SRC. This study evaluated the efficacy of ponatinib in combination with bevacizumab using both a subcutaneous and intracranial human GBM model. METHODS: The pediatric brain tumor xenografts D-2159 MG were grown in athymic BALB/c mice in both a subcutaneous and intracranial model. After tumor size reached 200-500 mm³ subcutaneously or 3 mm diameter at 3 days after intracranial implantation, groups of 10 mice were randomly assigned to treatment groups. Imiquimod cream was applied at the injection site just prior to vaccination and 24 hours later. The vaccine schedule dictated administrations every 2 weeks for 8 weeks then monthly to progression or a total of 52 weeks. Patients were imaged monthly and blood was drawn to evaluate toxicity and immune response. RESULTS: 7 patients have been enrolled to date. No evidence of related toxicity was reported. Time to progression ranged from 6.5 weeks for the patient treated at the first dose level to 35 weeks for one patient receiving 15 x 10⁶ DC. The latter patient experienced a partial response at 20 weeks. Two patients have stable disease at 18.5 and 28 weeks, respectively. One patient was evaluated. Follow-up analyses demonstrated expansion of central memory T-cells amidst declining effector memory cells following vaccination in three patients at a dose of 15x10⁶ DC. CONCLUSION: Apoptotic body-pulsed DC vaccination was well tolerated and preliminarily demonstrated clinical activity but a minimum of 15x10⁶ DC was required for a modulation of central memory T-cells.

HG-07. PHASE I IMMUNOTHERAPY TRIAL USING GLIOBLASTOMA APOPTOTIC BODY-PULSED DENDRITIC CELLS

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BACKGROUND: We recently reported that tumor cell vaccines cultured in 5% oxygen (O₂) had enhanced the immunogenicity relative to those grown in standard atmospheric O₂. A Phase I clinical trial was initiated to evaluate the safety of a dendritic cell (DC) vaccine pulsed with apoptotic bodies from our cell line, GBM6, grown in 5% O₂. GBM6 was extensively characterized and shown to express tumor-associated antigens (IL13RA2, Sox2, Epha2, etc.). METHODS: Patients ranging from 3 to 71 years with recurrent GBM (n = 6) or ependymoma (n = 1) were enrolled. Monocytes were collected via apheresis, matured into DC and pulsed with apoptotic bodies derived from GBM6. The first three patients received escalating doses of DC (5x10⁶, 1x10⁷, and 1x10⁸), the remainder received 15 x 10⁶ DC. Pulsed dendritic cells were injected subcutaneously. Imiquimod cream was applied at the injection site just prior to vaccination and 24 hours later. The vaccine schedule dictated administration every 2 weeks for 8 weeks then monthly to progression or a total of 52 weeks. Patients were imaged monthly and blood was drawn to evaluate toxicity and immune response. RESULTS: 7 patients have been enrolled to date. No evidence of related toxicity was reported. Time to progression ranged from 6.5 weeks for the patient treated at the first dose level to 35 weeks for one patient receiving 15 x 10⁶ DC. The latter patient experienced a partial response at 20 weeks. Two patients have stable disease at 18.5 and 28 weeks, respectively. One patient was evaluated. Follow-up analyses demonstrated expansion of central memory T-cells amidst declining effector memory cells following vaccination in three patients at a dose of 15x10⁶ DC. CONCLUSION: Apoptotic body-pulsed DC vaccination was well tolerated and preliminarily demonstrated clinical activity but a minimum of 15x10⁶ DC was required for a modulation of central memory T-cells.
different anatomical compartments, which likely originate from distinct precursor-cell populations, one of which completely lacks expression of OLIG1/2 - early indicators of neural lineage commitment. Our findings shed light on the cellular origin of and on the pathogenesis of leading to GMB tumorigenesis, and provide several new targets which may be further exploited both for molecular diagnostic purposes and for the development of therapeutic strategies targeting DNA methylation or downstream effectors.

HG-09. PROLONGED SURVIVAL ASSOCIATED WITH THE USE OF INTRAOPERATIVE CARMUSTINE WAFERS (GLIADEL®) IN A PAEDIATRIC PATIENT WITH RECURRENT HIGH GRADE GLIOMA.
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INTRODUCTION: The prognosis for patients with high-grade glioma (HGG) that relapse after standard treatment including radiotherapy remains extremely poor. We report an adolescent who relapsed shortly after completing therapy and who has shown prolonged survival following further treatment with surgery and the instillation of GliaEl® wafers.

CASE REPORT: A 15 year old female presented with temporal lobe epilepsy. MRI showed a heterogenous tumour in the right middle cranial fossa. Two image-guided resections were performed to achieve macroscopic clearance. Histology gave a diagnosis of anaplastic astrocytoma (WHO grade III). Six cycles of chemotherapy and surgical resection were followed by two weekly cycles of carboplatin and temozolomide (200 mg/m²/day for 5 days in accordance with the Children’s Cancer and Leukaemia Group Guidelines. Five months after the end of therapy, a surveillance MRI scan showed evidence of local relapse. The patient underwent a wide resection and image-guided macroscopic resection of the tumour and implantation of three Carmustine impregnated wafers (GliaEl®). The middle cerebral artery was enucleated in tumour and skeletonised to enable a macroscopic clearance. Histology confirmed relapse grade III astrocytoma. The patient then received six cycles of procarbazine and lomustine. 3 years later she is well and disease free. DISCUSSION: Relapsed HGG carries a dismal prognosis in both adults and children and there are limited strategies that give promise for a reasonable chance of cure. Carmustine impregnated wafers have been extensively investigated in adults in the up-front treatment of HGG with evidence from randomised trials that they are associated with a survival benefit in selected patients. The evidence base in relapsed disease is less strong but this case gives support to further investigation of the use of for GliaEl® in children and young people with relapsed HGG particularly in the setting of a second complete resection.

HG-10. NF-κb INHIBITION BY DHMEQ EFFICIENTLY IMPAIRS IN VITRO GROWTH AND INVASION IN PEDIATRIC ASTROCYTIC TUMORS, SENSITIZING CELLS TO CONVENTIONAL TREATMENTS
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Despite the improvements in neurosurgery, radiation treatment, and the advent of Temozolomide (TMZ), the outcome of pediatric patients with astrocytic tumors is still poor. Recently, Dehydroxymethylpoxquinomycin (DHMEQ) a novel NF-κb inhibitor has shown potent anti-tumor and chemo-sensitizing properties. This nuclear factor is constitutively activated in chemo-resistant and invasion, NF-κb constitutes the point of convergence for resistance to drugs that are toxic to pHGG cells. However, the role of chemotherapy is still limited. This clinical non-response is in part thought to be caused by drug resistance. Therefore, we aim to identify novel compounds that effectively inhibit HGG growth, and to determine the expression of three major drug efflux transporters of the ATP-binding cassette (ABC)-containing family in pHGG cultures and corresponding tumor tissue. MATERIALS & METHODS: Nine primary pHGG cell cultures were derived from tumor samples obtained via biopsy/resection or autopsy, including three pontine gliomas. All were exposed to 21 compounds including classical chemotherapeutics and novel agents. Cell survival was assessed after four days by use of the Acumen X3 laser scanning cytometer. Expression of ABC transporters P-glycoprotein (P-gp), breast cancer resistance protein (BCRP1) and multidrug-resistant associated protein (MRP1) in the cultures and tumor sections was assessed by Western blotting and immunohistochemistry, respectively. RESULTS: Exposure to the chemotherapeutics doxorubicin, topotecan, melphalan, and the novel agents vandetanib and bortezomib induced significant cell death in vitro in the pHGG cell cultures. However, these chemotherapeutics have never proven to be effective in clinical trials. In an attempt to elucidate this discrepancy the expression of drug efflux transporters was explored. In most cultures MRP1 expression was observed, while P-gp and BCRP1 were absent. Accordingly, P-gp, MRP1 and BCRP1 were present in the tumor vasculature, and MRP1 was also found in the tumor cell membrane. CONCLUSIONS: Drug screening revealed several effective chemotherapeutics and novel agents against pHGG cells in vitro. Further, our data suggest that the drug efflux transporters P-gp, MRP1 and BCRP1 on the blood-tumor barrier constitute an important mechanism for resistance to drugs that are toxic to pHGG cells.

HG-11. SENSITIVITY OF PEDIATRIC HIGH GRADE GLIOMA TO CHEMOTHERAPY
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BACKGROUND: Pediatric high grade gliomas (pHGG) are difficult to treat and associated with an extremely poor prognosis. Current treatment consists of a combination of surgery, radiotherapy, and/or chemotherapy, however, the role of chemotherapy is still limited. This clinical non-response is in part thought to be caused by drug resistance. Therefore, we aim to identify novel compounds that effectively inhibit HGG growth, and to determine the expression of three major drug efflux transporters of the ATP-binding cassette (ABC)-containing family in pHGG cultures and corresponding tumor tissue. MATERIALS & METHODS: Nine primary pHGG cell cultures were derived from tumor samples obtained via biopsy/resection or autopsy, including three pontine gliomas. All were exposed to 21 compounds including classical chemotherapeutics and novel agents. Cell survival was assessed after four days by use of the Acumen X3 laser scanning cytometer. Expression of ABC transporters P-glycoprotein (P-gp), breast cancer resistance protein (BCRP1) and multidrug-resistant associated protein (MRP1) in the cultures and tumor sections was assessed by Western blotting and immunohistochemistry, respectively. RESULTS: Exposure to the chemotherapeutics doxorubicin, topotecan, melphalan, and the novel agents vandetanib and bortezomib induced significant cell death in vitro in the pHGG cell cultures. However, these chemotherapeutics have never proven to be effective in clinical trials. In an attempt to elucidate this discrepancy the expression of drug efflux transporters was explored. In most cultures MRP1 expression was observed, while P-gp and BCRP1 were absent. Accordingly, P-gp, MRP1 and BCRP1 were present in the tumor vasculature, and MRP1 was also found in the tumor cell membrane. CONCLUSIONS: Drug screening revealed several effective chemotherapeutics and novel agents against pHGG cells in vitro. Further, our data suggest that the drug efflux transporters P-gp, MRP1 and BCRP1 on the blood-tumor barrier constitute an important mechanism for resistance to drugs that are toxic to pHGG cells.
HG-13. PHASE 2, SINGLE ARM, CONTROLLED TRIAL OF IRINOTECAN AND CISPLATIN IN CHILDREN WITH HIGH-RISK GLIOMAS

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INTRODUCTION: After a pilot study suggesting that irinotecan/cisplatin (I/C) may be effective for pediatric gliomas, we conducted a phase II controlled trial (EudraCT:2009-010742-59). METHODS: patients diagnosed with high-risk (HR) gliomas (HGG, DIPG, or HR-LGG) received sixteen weekly outpatient iv. cycles of C (30mg/m²) and 5FU (1AA; both GTR) died prior to completion of treatment. CONCLUSIONS: These preliminary results support similar studies in which a subgroup of patients with HGG who present in early childhood can avoid irradiation with the use of postoperative chemotherapy alone.

HG-14. GLOBLASTOMA MULTIFORME WITH DROP METASTASES

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INTRODUCTION: Glioblastoma multiforme (GBM) with drop metastasis is rare, occurs late in the course of the disease and indicates a poor prognosis. Recent advances in cancer treatment prolong survival and provide adequate time for these metastases to display clinical symptoms. CASE REPORT: A 47 years old right-handed Caucasian male presented with generalized anxiety, headache and blurry vision. Neuroimaging revealed a mass at the splenium of the corpus callosum, a butterfly glioma per MRI of the brain. He underwent stereotactic biopsy and pathology was consistent with GBM. The lesion was deemed unresectable. The patient started radiotherapy and chemotherapy. He died of sepsis 3 months after hospital admission.

HG-15. DIFFERENTIAL EXPRESSION OF MICRORNAS BETWEEN HIGH AND LOW GRADE ASTROCYTOMAS

Priscila de De´ u, Pediatric NeuroRadiology, Esplugues de Llobregat, Barcelona, Spain;1Hospital St Joan de Deu, University of Barcelona, Pediatric Oncology, Esplugues, Barcelona, Spain;2LGG, 5 pts HR-LGG; 33% of LGG-tumors studied. No BRAFV600e mutations were found. 1, NF1 methylation analysis of MGMT promoter pattern in DNA-repair assesseed with MRI plus volumetric analysis. Biological studies included malignant gliomas received radiation at the end. Objective response was assessed with MRI plus volumetric analysis. Biological studies included metabolic studies was monitored with PET examination injecting 4MBq/Kg of 11C-Methionin in 25 patients. PET data were analysed semiquantitatively obtaining the standardized uptake value (SUV) in the tumor and normal cerebral cortex (SUV ratio) at diagnosis and at the end of chemotherapy. RESULTS: Since November/2009, 27 patients aged 7m-17y (mean = 66% of LGG-tumors studied. No BRAFV600e mutations were found. 1, NF1 methylation analysis of MGMT promoter pattern in DNA-repair/MSI and in LGG BRAF fusion transripts or mutation analysis in the tumor specimen. Analysis of metabolic changes was monitored with PET examination injektion of 4MBq/Kg of 11C-Methionin in 25 patients. PET data were analysed semiquantitatively obtaining the standardized uptake value (SUV) in the tumor and normal cerebral cortex (SUV ratio) at diagnosis and at the end of chemotherapy. RESULTS: Since November/2009, 27 patients aged 7m-17y (mean = 84-months), diagnosed with DIPG (n = 3), HGG (n = 4), anaplastic-ependymoma (n = 4), atypical neurocytoma (n = 1), LGG n = 15 (PA = 4), Astrocytoma NOS = 7, Proliferim = A = 1, Ganglioglioma = 1, NFI = 2), were included. Four patients died of disease (2HGG, 0DIPG). Clinically significant responses (PR + SD) were found in 14 (51%) patients (3F/3LGG/10HGG). progressive disease in 8 (3DIPG/3AA/2LGG). Pet remain on treatment. PET-Methionine described 6 cases of metabolic stable disease, of 4 of partial response and three of progression. SUV increasing in [11C]MET-PET correlated with progressive HGG. All tumors showed a stable pattern in DNA-repair/MSI analysis and unmethylated MGMT-promoter. KIAA1549-BRAF fusion transcripts were found in 66% of LGG-tumors studied. No BRAFV600e mutations were found. After a median follow-up of 11 months (26-2m), OS (%) and PFS (%) are: HGGs = 50/23, Ep = 100/23, DIPG = 33/0, HR-LGG = 100/40%. No grade 3-4 toxicities were observed. All but DIPG or relapsed HGG patients completed the protocol. Radiation was avoided in all HR-LGG patients. CONCLUSION: The I/C regimen is well tolerated and shows activity for the treatment of HR-gliomas in children.

HG-16. GLIOMATOSES CEREBRI IN A NINE YEAR OLD BOY: CASE REVIEW AND REVIEW OF THE LITERATURE

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Glioneomatos Cerebi is a rare tumor defined as a diffuse gial tumor infiltrating the brain without obvious mass effect and may extend to infratentorial structures. This entity occurs much less often in children than in adults. Our nine year old patient presented with seizures and Magnetic Resonance Imaging of his brain showed non-enhancing
abnormalities involving white and gray matter with cortical thickening involving multiple lobes of the brain. A biopsy was performed revealing a pathologic diagnosis of Grade 3 anaplastic astrocytoma. Our patient survived three months after his diagnosis. His treatment consisted of a combination of radiation therapy and chemotherapy including VEGF inhibitors. The MRI scan obtained at the time of his progressive disease showed marked enhancement with further areas of the brain involved along with a midline shift. We discuss his clinical course and a review of the pediatric literature for this rare and poor prognostic disorder.

**HG-17. RADIOTHERAPY PLUS CONCOMITANT TEMOZOLOMIDE FOR GLIOMATOSIS CEREBRI: A REPORT OF THREE CASES**

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**INTRODUCTION:** Gliomatosis cerebri (GC) is an uncommon glial neoplasm. A standard therapy has not been identified, and few reports are available about the therapeutic courses of pediatric GC. We report three patients with GC who were treated with radiotherapy and concomitant temozolomide (TMZ) (75 mg/m2/day, daily), followed by adjuvant chemotherapy including TMZ (150-200 mg/m2 x 5 days, every 28 days). CASE 1: An 8-year-old girl was initially treated as encephalopathy with repeated pulsed steroids. Three months after her initial presentation, a biopsy provided the definitive diagnosis of GC. After one cycle of cisplatin/etoposide, which resulted in progressive disease, RT (whole brain irradiation, 36 Gy) concomitant with TMZ was initiated. She improved clinically and the tumor showed regression on MRI. After the first cycle of adjuvant TMZ and etoposide, however, the tumor again progressed. Despite treatment with hydroxyurea (HU), TMZ/HU, interferon-beta and bevacizumab/irinotecan, the tumor never regressed and she died 29 months after the diagnosis. CASE 2: A 9-year-old girl was diagnosed with left thalamic glioblastoma and GC. She was treated with RT (36 Gy to the craniospinal axis with an 18 Gy boost to tumor) and TMZ. Grade 4 leukopenia was observed, which recovered after 10 days cessation of TMZ. The tumor showed a good response. However, after the second cycle of adjuvant TMZ, the tumor progressed. Despite treatment with nimustine or interferon-beta, she died 7 months after the diagnosis. CASE 3: A 12-year-old boy with GC received RT (whole brain irradiation, 36 Gy) and TMZ, followed by adjuvant TMZ combined with interferon-beta. His neurological symptoms improved. Seven months after diagnosis, his symptoms rapidly progressed and continue to deteriorate even with administration of nimustine and a combination of hydroxyurea and imatinib. CONCLUSION: RT was effective against GC, however, the usefulness of concomitant administration of TMZ was unclear. The PFS was 3, 4.5, and 7 months, respectively in these three patients.

**HG-18. STRATIFICATION ACCORDING TO HGG-IMMUNO RPA MODEL PREDICTS OUTCOME IN PATIENTS WITH RELAPSED MALIGNANT GLIOMA TREATED BY ADJUVANT POSTOPERATIVE DC VACCINATION**

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Adult patients with relapsed high grade glioma are a very heterogeneous group with however an invariably dismal prognosis. We stratified patients with relapsed HGG treated with re-operation and postoperative dendritic cell (DC) vaccination according to a simple recursive partitioning analysis (RPA) model to predict outcome. Based on age, pathology (grade II or III), Karnofsky performance score and mental status, 117 adult patients with relapsed HGG, undergoing re-operation and postoperative adjuvant DC vaccination were stratified into 4 classes. Kaplan-Meier survival estimates were generated for each class of this HGG-IMMUNO RPA model. Extent of resection (requirement for immunotherapy) was documented but not included in the prognostic model. Kaplan-Meier overall survival estimates revealed significant ($p < 0.0001$) differences amongst the 4 HGG-IMMUNO RPA classes. Long-term survivors, surviving more than 24 months after the re-operation and vaccination are seen in 54.5%, 26.7%, 11.3% and 0% for the classes I, II, III and IV respectively. The HGG-IMMUNO RPA classification is able to predict overall survival in a large group of adult patients with a relapsed HGG, treated with re-operation and postoperative adjuvant DC vaccination in the HGG-IMMUNO-2003 cohort comparison trial. The model appears useful for prognostic patient counseling after postoperative DC vaccination trials. A substantial number of long-term survivors after relapse are seen in class I to III, but no in class IV patients. The data can be of use to develop similar models for children with relapsed HGG, especially as currently several new treatment approaches are studied in children with relapsed HGG.

**HG-19. GLIOBLASTOMA MULTIFORME IN A CHILD SUBSEQUENTLY DIAGNOSED WITH LI-FRAUMENI SYNDROME**

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Glioblastoma multiforme (GBM) is a relatively uncommon tumor in the pediatric age group accounting for less than 10% of the histological subtypes in contrast to the larger percentage found in the adult population. Li-Fraumeni syndrome is an even rarer autosomal dominant disorder associated with the development of soft tissue and bone sarcomas, premenopausal breast cancer, brain tumors, leukemia and adrenocortical carcinoma. We describe the case of a twelve year old girl who, after presentation of symptoms including headaches and vomiting, was found on magnetic resonance imaging to have a large mass in the frontal lobe. She underwent a gross total resection. Pathology revealed a Grade IV astrocytoma (GBM). Careful review of her family history was quite concerning for the possibility of an inherited familial cancer syndrome specifically Li-Fraumeni syndrome. Genetic testing was performed and confirmed a germline mutation in the TP53 tumor suppressor gene. Her treatment has included radiation therapy, along with chemotherapy and a vascular endothelial growth factor (VEGF) inhibitor. She has no evidence of disease 32 months from her initial resection. We will review her case and present the published literature regarding Li-Fraumeni and pediatric brain tumors.

**HG-20. CHEMOTHERAPY DECREASES MIGRATION BUT INCREASES RESPONSE TO OXIDATIVE STRESS IN A HIGH GRADE GLIOMA CELL LINE**

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High grade glioma (HGG) is an aggressive brain tumour with a survival rate <1 year due to their ability to resist chemotherapy. In this study, the rat C6 glioma cell line and two drug selected derivatives (C6-irinotecan and C6-irinotecan) were used to investigate the different mechanisms of HGG chemoresistance. Two-dimensional gel electrophoresis, combined with mass spectrometry sequencing and gene expression microarrays were used to identify changes in protein and gene expression between the cell lines. Ingenuity Pathway Analysis was then used to propose target cellular pathways that may be differentially active. Expression of candidate proteins and genes was confirmed by western blotting and QRT-PCR. The response of the cell lines towards oxidative stress (OS) and their ability to migrate were determined by reactive oxygen species (ROS) production and wound migration assays respectively. Several signalling pathways were suggested to be differentially regulated in the C6 versus the drug treated cell lines including migration (BMP7 and EGR1) and drug resistance via ROS (catalase, PRP19, and lamin A). C6-irinotecan responded better to OS since it is resistant to ROS produced after the addition of a higher concentration of tert-butyl hydroperoxide (TBHP) (1mM, P < 0.0001). C6-irinotecan cells migrated at a slower rate than C6 and C6-Etoposide cell lines. Hence, HGG cells undergo changes in migration and their response to OS after prolonged treatment with irinotecan and etoposide respectively. Ongoing work includes analysis of the correlation between the expression of these proteins and outcome on a paediatric HGG TMA.
Brain tumor stem cells (BTSCs) are a preferred therapeutic target since they have been proposed to be a possible source of cancer resistance to conventional anti-cancer therapies. Oncolytic adenoviruses designed to replicate in and destroy tumor cells selectively represent a promising therapeutic strategy that could improve the outcome of children with high-grade gliomas (pHGGs) and DIPGs. Delta-24-RGD is an adenovirus that is currently being tested in adults with malignant gliomas in a Phase I clinical trial with promising results. Recently, salinomycin has proven to be highly effective against a model of breast cancer stem cells. We hypothesize that Delta-24-RGD in combination with salinomycin could be successfully implemented for the treatment of pHGGs and DIPGs, and specifically for the eradication of BTSCs. We performed MTT assays to evaluate in vitro the antitumor effect of Delta-24-RGD and/or salinomycin and TMZ in a panel of BTSCs lines (n = 4), and established pediatric glioma cell lines (n = 4). Our results showed that salinomycin displayed an IC50 ranging from 100 to 10,000 folds less than TMZ in the same cell lines. Combination of Delta-24-RGD resulted in a synergistic antiangioma effect. Interestingly, salinomycin alone or in combination with the virus reduced the number of self-renewal cells in treated-BTSCs. Cell cycle analysis showed that salinomycin or combination-treated cells did not arrest and progressed through the cell cycle to finally die by autophagic cell death. Global gene expression analysis revealed a significant decrease in the expression levels of genes involved in angiogenesis, proliferation, and stemness. At the moment we are testing in vivo the efficacy of this combination treatment using an intracranial model and a DIPG model in nude mice. Altogether our data show that Delta-24-RGD in combination with salinomycin is able to overcome BTSCs chemoresistance and could constitute a promising agent against pHGGs and DIPGs.
minor effects on survival in vitro. However, local TMZ treatment increased the survival of glioma-bearing mice. Systemic TMZ induced a transient decline in leukocyte number, which was not seen following local administration. In vivo, CD8+ cells were specifically sensitive. TMZ treatment to naïve mice increased relative numbers of FoxP3+ CD25+ CD4+ T regulatory cells / CD4+ in the blood. Preliminary results showed that local but not systemic TMZ improved long-term survival of GL-GM immunized glioma-bearing mice. Proliferating T cells were reduced by the immunotherapy when TMZ treated but not in systemical TMZ treated mice, implying that the immuno-suppressive effects of TMZ was avoided by local administration.

HG-26. MANAGEMENT OF PROGRESSIVE GLIOMATOsis Cerebri (GC) in a Child with Combination Oral CHemoTherAPy

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INTRODUCTION: Gliomatosis cerebri (GC) is diagnosed radiologically as glioma involving more than one lobe of cerebral cortex and histologically by invading neoplastic astrocytes. Management of this rare glioma variant is not standardized. This case highlights the role of radiation therapy in the management of this disease. There are no clinical trials in adults or children to guide management.

CASE REPORT: A Caucasian male child presented at age 2 years with status epilepticus requiring intravenous antiepileptic drugs (AED). Imaging revealed an enhancing lesion limited to a gyrus of right posterior parietal cortex. CSF studies were negative. A provisional diagnosis of cerebritis was made. Biopsy after asymptomatic progression was inconclusive but with extension to the right parietal and occipital lobes another biopsy was performed. Local and external neurosurgeons diagnosed an infiltrating Grade III astrocytoma consistent with GC. Unfortunately, significant progression necessitated subtotal resection; the diagnosis remained unchanged. We commenced Temozolomide (TMZ) using a dose dense schedule of 75-85 mg/m2/dose days 1-21/28 day cycle with concurrent cis-retinoic acid (CRA) given 50 mg/m2/dose bid, D1-21/28. After 2 cycles there was a partial response (PR) and therapy was continued for 12 total cycles with stable disease (SD). Thrombocytopenia occurred with the 12th cycle necessitating a change in treatment. Moreover after 12 months off therapy, there was further asymptomatic progression and we elected to treat a second time with TMZ/CRA using the same dosing schedule; SD followed a minor PR. The child completed a second 12 month course of TMZ and 18 months of CRA. He remains clinically stable off AED since initial chemotherapy without progression 18 months off therapy.

CONCLUSION: Management of GC must be individualized, factoring age, extent of disease and QOL. Treatment with concurrent dose dense TMZ/CRA may be considered in children with GC and retreatment is feasible.

HG-27. HIGH-DOSE METHOTREXATE IMPROVED SURVIVAL IN PEDIATRIC HIGH GRADE GLIOMA: RESULTS OF HIT-GBM-D

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We tested the the benefit of High Dose Methotrexate (MTX) in a phase III study. The patients were to receive two cycles of MTX 5g/m²q over 24 hours prior to standard treatment with induction treatment of radiation and simultaneous Cisplatin Etoposide Vincristine and Ifosfamide and maintenance with Lomustine prednisone and vincristine. The control group was treated omentum MTX. This report describes the first look at the data with further follow up data coming in. 117 patients from 52 institutions in Germany, Switzerland, Austria, Spain, USA and Slovenia. 66 patients were male, the mean age was 10.5 years (range 3.4 to 17.8), 39 tumors were located in the pons, 5 in other brain stem locations, 20 in basal ganglia or third ventricle, 8 in cerebellum, 40 in cerebrum, 2 in the spinal cord, and 3 in overlapping locations. 7 tumors were metastatic at presentation. Grade IV, III, and enrollment based on radiology findings of DIPG were 50, 33, and 32 respectively. Surgeries were gross total resection, subtotal resection, partial resection, biopsy and no surgery 19,20,24,25, and 29 respectively. The median overall survival was 1.202 years. Previously known prognostic factors were confirmed: Survival was superior after gross total resection and inferior for DIPG. 60 patients were randomized in the 77% received the prescribed dose of MTX, while three only received one. 57 were randomized in the control arm, and received no MTX. The survival of patients receiving two cycles of methotrexate was superior 1.51 versus 0.86, and 1.09 for 2 cycles, 1 cycle, and no MTX, respectively (P = 0.035). Progression free survival was similar with 0.84, 0.37, and 0.62 years respectively (P = 0.195). We conclude that these data suggest a benefit of high dose methotrexate when given prior to standard radiochemotherapy for some pediatric patients with high grade glioma.

HG-28. SURVIVAL OF PEDIATRIC PATIENTS WITH NEWLY DIAGNOSED HIGH-GRADE GLIOMA AND DIFFUSE INTRINSIC PONTINE GLIOMA TREATED WITH TEMOZOLOMIDE, IRINOotecAN AND BEvacizUMAB AT SEATTLE Children’S Hospital

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Children with high-grade glioma (HGG) and diffuse intrinsic pontine glioma (DIPG) have historically had a dismal outcome. Antiangiogenic therapy including bevacizumab has not been evaluated as a component of initial therapy in this patient population. Standard therapy for patients with HGG or DIPG treated at Seattle Children’s Hospital since April, 2009 has consisted of focal radiation therapy with concurrent temozolomide followed by 12 cycles of maintenance therapy with temozolomide, irinotecan and bevacizumab. The records of patients treated on this regimen were retrospectively reviewed. Survival was analyzed according to the method of Kaplan and Meier. Factors associated with survival were evaluated using the log-rank test. Ten patients have been treated on this regimen, seven with HGG and three with DIPG. Histologic diagnosis included glioblastoma multiforme (4 patients), anaplastic astrocytoma (GBM) cell and high-grade glioma not otherwise specified (2 patients with brainstem biopsy). The median patient age was 8.8 years (range 1.5 - 17.5 years). Three patients completed therapy, three progressed on therapy and one stopped because of nausea (1 patient), and one was lost to follow up (1 patient). The median follow up of 13 months, the 6, 12 and 24 month event free survival (EFS) was 90%, 66%, and 44% respectively. The 6, 12 and 24 month overall survival (OS) was 89%, 76% and 61%. Survival was not significantly associated with age, gender (6/10 male), extent of resection (4/10 near or gross total resection), or type of tumor (HGG vs. DIPG). This well-tolerated regimen appears to result in superior survival compared to historical treatment.

HG-29. ALDEHyDe DEHYDROGENASE INHIBITION INhIBits GBM TUMOR InitiATING cells (BTIC) and AUGMENTS TEMOZoLOMIDE Cytotoxicity in viTO

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Evidence suggests a drug resistant brain tumor initiating cell (BTIC) sub-population may be responsible for tumor recurrence in Glioblastoma multiforme (GBM). High aldehyde dehydrogenase (ALDH) activity is used as an indicator of tumor initiating capacity. We aim to target ALDH activity and investigate the therapeutic potential of ALDH inhibitor, Disulfiram (DSF). We previously shown that SF188 cells are resistant to TMZ. Here we report that ALDH enzyme activity was measured following ALDH1a1 sRNA transfection. This report describes the effect of DSF and TMZ in a neurosphere assay to examine BTIC self-renewal. We have previously shown that SF188 cells are resistant to TMZ. Here we report that TMZ has no effect on ALDH enzyme activity. In contrast, DSF blocks ALDH activity in SF188 cells treated with DSF at concentrations >100nM. At similar concentrations, BTIC neurosphere growth can be blocked in SF188 and BT74 cells treated with DSF. We also found that ALDH inhibition with DSF enhanced cytotoxicity to TMZ (10μM) and confirmed this effect with a second ALDH inhibitor, DeAB. The drug DSF was effective against U251 (adult GBM), A172 (adult GBM) and
HIGH-DOSE CHEMOTHERAPY MALIGNANT BRAIN TUMOR WITH NON-MYELOABLATIVE HG-31. SELECTIVE AND TARGETING TREATMENT OF T-cells exhibit significantly enhanced effector functions. CONCLUSION: The pattern of heterogeneity in HGG favors near complete confer survival advantage to treated animals over controls. In cytotoxicity assays, bi-specific T-cells exhibited a significantly higher T-cells induced simultaneous depletion of HER2

Interestingly, bi-specific T-cells and pool of HER2.CAR and IL13R

survival and expansion of HER2-ve

cells expressed either or both antigens. Transduction efficiency for bi-specific

in-vivo experiments. RESULTS: 40-70% of cells in primary HGG tissues and established cell lines expressed HER2 or IL13Ra2, whereas >80% of cells expressed either or both antigens. Transduction efficiency for bi-specific

CAR T-cells was ~70%. In co-culture experiments, bi-specific T-cells showed increased proliferation and enhanced cytokine release. Furthermore, HGG cells treated with HER2.CAR T-cells showed selective survival and apoptosis of HER2+

IL13Ra2 HGG cells. In cytotoxicity assays, bi-specific T-cells exhibited a significantly higher degree of tumor cell killing over control. Bi-specific T-cells were found to confer survival advantage to treated animals over controls. CONCLUSION: The pattern of heterogeneity in HGG favors near complete targeting of tumor subpopulations. Pooled CAR T-cells as well as bi-specific CAR T-cells effectively target antigen escape variant tumor cells, yet bispecific T-cells exhibit significantly enhanced effector functions.

HG-31. SELECTIVE AND TARGETING TREATMENT OF MALIGNANT BRAIN TUMOR WITH NON-MYELOABLATIVE HIGH-DOSE CHEMOTHERAPY Tai-Tong Wong1, Pei-Yi Yang2, Maggie Lu2, Hsiang-Fa Liang3, HSun-El Wang4, Ren-Shyan Liu5, Ming-Chie Teng6, and Chueh-Chuan Yen7; 1Division of Pediatric Neurosurgery, Neurological Institute, Taipei Veterans General Hospital, National Yang Ming University School of Medicine, Taipei, Taiwan; 2Department of Biomedical Imaging and Radiological Science, National Yang Ming University, Taipei, Taiwan; 3Drug Delivery Laboratory, Biomedical Technology and Device Research Laboratory, Industrial Technology Research Institute, Hsinchu, Taiwan; 4Molecular-Genetic Imaging Core, National Research Program for Genomic Medicine, National Yang Ming University School of Medicine, Taipei, Taiwan; 5Division of Hematology and Oncology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan

PURPOSE: To develop non-myeloablative high dose tumor tissue chemotheraphy for the treatment of highly malignant or recurrent brain tumors in children. METHOD: We established an human GBM mouse models by injecting GBM cells into the right striatum of CB-17 scid/scid mice. We transplanted human GBM8401-Luc tumor cells into the right striatum of CB-17 scid/scid mice. The mice were then treated with TMZ and/or Doxil. The results were analyzed using in vivo imaging.

HG-30. TARGETING TUMOR HETEROGENEITY IN CHILDHOOD HIGH GRADE GLIOMA (HGG): BISPECIFIC T-CELLS EXHIBIT ENHANCED EFFECTOR FUNCTION AND OFFSET ANTIGEN LOSS ESCAPE VARIANTS Meenakshi Hegde, Amanda Corder, Kevin Chow, Malini Mukherjee, Aidin Ashour, Vita Brawley, Helen Helen, Stephen Gottschalk, Eric Yvon, and Nabil Ahmed; Baylor College of Medicine, Houston, TX, USA

BACKGROUND: Preclinical and early clinical studies have shown Chimeric Antigen Receptor (CAR)-redirected T-cell therapy to be a potentially effective approach against cancer. Downregulation/mutation of targeted antigens is a common tactic used by cancer cells to create antigen-loss escape variants; culminating in relapse. Targeting multiple antigens on tumor cells, simultaneously, could offset this escape mechanism.

OBJECTIVES: The intent of this project was to develop an effective adoptive cell therapy for childhood HGG by simultaneously targeting two glioma restricted and validated antigens, using T-cells genetically modified to express HER2 and IL-13Ra2 specific CARs. METHODS: We used character templating for chemotherapy and cell line generation. Bi-specific effector T-cells expressing HER2.CAR and IL13Ra2.CAR were generated by sequential retroviral transduction. Their functionality was tested against HER2+/IL13Ra2+ proteins as well as HGG cells. Uni-specific HER2.CAR and IL13Ra2.CAR T-cells from pooled human GBM8401-Luc glioma cells T-cells served as controls. Orthotopic murine model of HGG was used for in-vivo experiments. RESULTS: 40-70% of cells in primary HGG tissues and established cell lines expressed HER2 or IL13Ra2, whereas >80% of cells expressed either or both antigens. Transduction efficiency for bi-specific CAR T-cells was ~70%. In co-culture experiments, bi-specific T-cells showed increased proliferation and enhanced cytokine release. Furthermore, HGG cells treated with HER2.CAR T-cells showed selective survival and apoptosis of HER2+/IL13Ra2+ tumor cells and vice-versa. Interestingly, bi-specific T-cells and pool of HER2.CAR and IL13Ra2.CAR T-cells induced simultaneous depletion of HER2+ / IL13Ra2+ HGG cells. In cytotoxicity assays, bi-specific T-cells exhibited a significantly higher degree of tumor cell killing over control. Bi-specific T-cells were found to confer survival advantage to treated animals over controls. CONCLUSION: The pattern of heterogeneity in HGG favors near complete targeting of tumor subpopulations. Pooled CAR T-cells as well as bi-specific CAR T-cells effectively target antigen escape variant tumor cells, yet bispecific T-cells exhibit significantly enhanced effector functions.

HG-32. AN ATM-APNG DNA REPAIR AXIS CONFERS AN ALKYLATING AGENT RESISTANCE PHENOTYPE IN ADULT AND PEDIATRIC HIGH-GRADE GLIOMA Sameer Agnihotri1, Christian Ternamian1, Chris Jones1, Gelaehr Zach2, James Kurtz3, and Cynthia Hawkins1; 1Hospital for Sick Children, UofT, Toronto, ON, Canada; 2Toronto Western Hospital, UofT, Toronto, ON, Canada; 3Institute of Cancer Research, London, UK

Cancer cells acquire resistance to several modalities of treatment including radiation therapy. This resistance is often attributed to aberrant signaling in DNA damage response pathways resulting from genetic mutations, epigenetic alterations or genome instability. Glioblastoma multiforme (GBM) is the most common and lethal primary brain tumor in adults and pediatric high-grade gliomas (PHGG) represent approximately 10-15% of all pediatric brain tumors, behave very aggressively and have an abysmal prognosis. Common to both is a poor understanding of the mechanisms for chemotherapy and radiotherapy resistance. Here we demonstrate that the DNA repair protein alkylpurine-DNA-N-glycosylase (APNG) involved in short patch base excision repair (BER) contributes to resistance to temozolomide (TMZ) an oral alkylating agent in PHGG. Silencing APNG expression in TMZ-resistant PHGG cell lines enhanced TMZ responsiveness, while exogenously expressing APNG in TMZ-sensitive PHGG lines conferred resistance to TMZ in vitro and in vivo. Surprisingly, we observed activated ataxia telangietasia mutated (ATM) kinase in steady state conditions in PHGG and adult GBM cells. We identified APNG as a novel ATM substrate that directly phosphorylates APNG, thus linking the ATM DNA damage response pathway with short patch BER. Loss of phospho-APNG reduced its ability to protect cancer cells against temozolomide and other alkylating agents. Clinically, binary expression of activated ATM and high APNG correlated with the worst overall survival in adult gliomas with current studies focusing on the survival benefit in pediatric gliomas. TMZ resistant PHGG and GBM cells were sensitized to TMZ as measured by cell viability and apoptosis with methoxyamine and that the effect was synergistic with ATM inhibition. Collectively, our study demonstrates a novel ATM-APNG TMZ resistance axis in glioma and that selective targeting of BER and ATM signaling may be of therapeutic relevance.

HG-33. IRINOTECAN WITH CARBOPLATIN FOR HIGH GRADE GLIOMAS (HGG) IN CHILDREN Iwona Filipiak1, Monika Drogosiewicz2, Marta Perek-Polnik3, Ewa Szwieszkowska1, Bozena Dembowska-Bagniska1, Elzbieta Jurkiewicz2, and Danuta Perek1; 1The Children’s Memorial Health Institute, Department of Oncology, Warsaw, Poland; 2The Children’s Memorial Health Institute, Department of Radiotherapy, Warsaw, Poland

Since responses to irinotecan in HGG have been reported we have introduced a combination of irinotecan and carboplatin at first as a second line salvage treatment in patients with disease relapse/progression then as pre-irradiation chemotherapy in patients with measurable residual tumors. AIM: To assess response to irinotecan and carboplatin regimen for HGG in children. PATIENTS AND METHODS: 16 pts were assessable for response. Two were diagnosed with anaplastic oligodendroglioma, 1 anaplastic oligodendroglioma and 13- glioblastoma. 7 pts received this regimen as a second/third line treatment and 9 after tumor resection, prior to radiotherapeutic chemotherapy. Consisted of 5-7 days course of carboplatin 50 mg/m² in 1 hour infusion and carboplatin 250 mg/m² on day 1st given every 3 weeks. Response to chemotherapy was evaluated using RANO criteria, toxicity was assessed according to CTC. RESULTS: 16 pts received from 1-14 courses (median 4) of chemotherapy. Out of 7 pts with disease progression /relapse, previously treated with chemotherapy and
HG-34. CLINICAL AND EPIDEMIOLOGICAL CHARACTERISTICS OF MALIGNANT TRANSFORMATION OF PAEDIATRIC LOW GRADE GLIOMA INTO HIGH GRADE GLIOMA: THE HIT EXPERIENCE

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Malignant transformation (MT) of low grade glioma (LGG) into high grade glioma (HGG) represents a tumor biological phenomenon, which accounts for the development of up to 5% of adult HGG. Relatively little is known about MT in pediatric patients. Thus, the HIT-LGG and HIT-HGG data bases of the HIT brain tumor network within the German Sarcoma Group, Paediatric Oncology and Haematology (GPOH) were screened for MT by the following parameters: Development of a HGG at the site of a previous LGG, confirmation of HGG by central neuropathological review, histopathological confirmation of previous LGG, interval between diagnosis of LGG and HGG of at least 6 months. Twenty-two patients (11 males, 11 females) with potential MT were identified. Median age at diagnosis of LGG was 11.6 years (range 0.7-16.9), at diagnosis of HGG 14.4 years (range 2.0-23.6). Median interval between onset of LGG and HGG was 2.5 years (range 0-8.7). Only three patients had undergone radiotherapy and/or chemotherapy for their previous LGG. Previous LGG included diffuse/fibrillary astrocytoma WHO II (n = 11), pilocytic astrocytoma WHO I (n = 8), ganglioglioma WHO I (n = 2), and pleomorphic xanthoastrocytoma (n = 1). Interval for malignant transformation from LGG to anaplastic astrocytoma or glioblastoma multiforme WHO IV (n = 6), anaplastic pilocytic astrocytoma WHO III (n = 3), anaplastic oligoastrocytoma WHO III (n = 2), and anaplastic ganglioglioma WHO III (n = 2). The cumulative incidence rate for development of HGG was 2 ± 0.5% at ten years after diagnosis of any LGG (n = 1876), 1 ± 0.5% after diagnosis of pilocytic astrocytoma I (n = 1347) and 11.8 ± 3.7% after diagnosis of diffuse/fibrillary astrocytoma II (n = 149). Children with HGG after MT seem to have a better overall survival than pediatric patients with primary HGG (n = 372; p = 0.018). The essential support by the Deutsche Kinderkrebsstiftung is gratefully acknowledged.

HG-35. GENE EXPRESSION PROFILING REVEALS THAT Glioblastoma Multiforme (GBM) FACILITATE INCREASED ANGIOGENESIS IN THE SURROUNDING MICROENVIRONMENT

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One of the hallmarks of glioblastoma is its abundant vasculature. These blood vessels support the tumor in a number of ways, including providing nutrients but also as a niche for glioblastoma cancer stem cells. Tumors like Glioblastoma multiforme (GBM) promote the formation of their vasculature and associated germinal niches through reciprocal inductive interactions between GBM cells and endothelial cells. Targeting the primary drivers of angiogenesis, VEGF and bFGF, in anti-angiogenic cancer treatments has value but is not curative. We hypothesize that identification of additional angiogenic molecules and pathways will provide new avenues for treatment. We used microarrays to perform global gene expression profiling to identify genes that are increased or decreased in expression as a consequence of functional interactions between GBM and brain microvascular endothelial cells. We identified several regulators of angiogenesis whose expression is modulated by this cell-cell interaction, including Thrombospondin-1 (THBS1) and CXCL1. CXCL1 is a pro-angiogenic chemokine and THBS1 is a powerful anti-angiogenic protein. These two proteins have been previously implicated in cancer, but their precise mechanism in glioblastoma angiogenesis is not yet known. Conditioned media experiments showed that it is the glioblastoma cells which downregulate THBS1 and endothelial cells that upregulate CXCL1. To reinforce the hypothesis that these intercellular efforts between both cell types to increase the microenvironmental angiogenesis, Immunohistochemical evaluation of primary GBM specimens revealed CXCL1 to be significantly upregulated around blood vessels in tumors and qRT-PCR analysis showed that across 23 different primary glioblastoma tumors, THBS1 was consistently and strongly downregulated. The impact of CXCL1 upregulation and THBS1 suppression on angiogenesis was validated in in-vitro and in-vivo angiogenesis assays. We believe these results reveal the important factors other than VEGF in glioblastoma angiogenesis and provide a rationale to evaluate combinatorial targeted anti-angiogenic therapy for GBM.

HG-36. DRIVER MUTATIONS IN HISTONE H3.3 AND CHROMATIN REMODELING GENES IN PAEDIATRIC GliOMA

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Glioblastoma multiforme (GBM) is a lethal brain tumour in adults and children. However, DNA copy number and gene expression signatures indicate differences between adult and paediatric cases. To explore the genetic events underlying this distinction, we sequenced the exomes of 48 paediatric GBM samples. Somatic mutations in the H3.3-ATRX-DAXX, chromatin remodelling pathway were identified in 44% of tumours (21/48). Recurrent mutations in H3F3A, which encodes the replication-independent histone 3 variant H3.3, were observed in 31% of tumours, and led to amino acid substitutions at two tumourigenic hotspots. For two tumourigenic H3F3A variants H3F3A G34R and G34V involved in key regulatory post-translational modifications. Mutations in ATRX (α-thalassaemia/mental retardation syndrome X-linked) and DAXX (death-domain associated protein), encoding two subunits of a chromatin remodelling complex required for H3.3 incorporation in pericentric heterochromatin and telomer, were identified in 31% of samples overall, and in 100% of tumours harbouring a G34R or G34V H3.3 mutation. Somatic TP53 mutations were identified in 54% of all cases, and in 86% of samples with H3F3A and/or ATRX mutations. Screening of a large cohort of gliomas of various grades and histologies (n = 784) showed H3F3A mutations to be specific to GBM and highly prevalent in children and young adults. Furthermore, the presence of H3F3A/ ATRX-DAXX/TP53 mutations was strongly associated with alternative lengthening of telomeres and specific gene expression profiles. This, to our knowledge, the first report to highlight recurrent mutations in a regulatory histone in humans, and our data suggest that defects of the chromatin architecture underlie paediatric and young adult GBM pathogenesis.

HG-37. PRIMARY THALAMIC TUMORS OF ASTROCYTIC ORIGIN: A 20-YEAR EXPERIENCE AT CHILDREN'S MEMORIAL HOSPITAL

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INTRODUCTION: Primary thalamic tumors (TT) represent a small group of pediatric central nervous system tumors (CNS) with distinct clinical behavior when compared to other pediatric CNS tumors. This subset of tumors is not curative. Described in the literature, METHOD: We retrospectively reviewed the records of patients with TT of astrocytic origin at the Falk Brain Tumor Center between 1990 and 2010 to determine: the incidence of TT at our center, common presenting signs and symptoms, prognostic factors and overall survival (OS) based upon histopathology and unilateral versus bilateral location. RESULTS: Primary TT of astrocytic origin represented 3% of all diagnoses over the study period. Follow-up was available for 39 patients with unilateral (n = 27) or bilateral (n = 12) tumors. Histologic diagnosis of high grade glioma (FGT2) in 17 (44%) and low grade glioma (LGG) in 22 (56%) were equally distributed between unilateral and bilateral tumors. Common presenting symptoms included: headache and vomiting (55%), hemiparesis (41%) and visual disturbances (33%). Patients with bilateral tumors were more likely to present with hydrocephalus and require shunt placement as compared to unilateral
tumors (58% vs. 33%, p = 0.17). Median symptom duration was longer for bilateral tumors (4.5 months) than unilateral tumors (1 month). All patients with bilateral HGG and 92% of patients with unilateral HGG died of tumor progression. In our series, we found an accuracy of 51 months and diagnostic sensitivity. There was no significant difference in 3-year OS between patients with unilateral tumors (60%) or bilateral tumors (50%), regardless of histology. Patient age, extent of resection and presenting symptoms were not identified as prognostic factors in our series. CONCLUSIONS: In our prospective series, histological analysis, unilateral and bilateral primary astrocytic TTs had similar 5-year OS, suggesting that bilateral involvement alone is not a poor prognostic feature. Histologic diagnosis was the most important predictor of survival in our series.

HG-38. A PHASE II TRIAL OF ERLOTINIB DURING AND AFTER RADIOTHERAPY IN NEWLY DIAGNOSED PEDIATRIC HIGH-GRADE GLIOMAS
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BACKGROUND: Epidermal growth factor receptor (EGFR) protein is overexpressed in most pediatric high-grade gliomas (HGG). Based on the activity of erlotinib in adult HGG, we conducted a Phase II study combining local RT and erlotinib in children with newly diagnosed HGG. METHODS: Eligible patients ≥ 3 years and ≤ 21 years with any type of HGG, received local RT (59.4 Gy) following maximum surgical resection. Erlotinib started on the first day of RT at 120 mg/m² per day. Treatment with erlotinib lasted for 2 years if there were no signs of tumor progression or intolerable toxicities. 1- and 2-year progression free survival (PFS) was estimated for patients with intracranial anaplastic astrocytoma (AA) and glioblastoma multiforme (GBM). The statistical design included a stopping rule for accrual if the 2-year PFS was at most 10% for GBM and 25% for AA. RESULTS: The protocol was closed on November 17, 2010. A total of 41 patients (24 females) were treated. There were 21 cases of Glioblastoma multiforme (GBM) and 20 patients with anaplastic astrocytoma (AA). The median age at treatment initiation was 10.9 years (range, 3.3-21 years) and 16% (SE 7.3) while for GBM it was 15% for both (SE 10.8) and 16% (SE 7.3) while for GBM it was 15% for both (SE 10.8). Erlotinib was well tolerated. Twenty-four patients developed one or more grade 3 or 4 drug related toxicities. The non-hematologic toxicities included gastrointestinal (n = 11), dermatologic (n = 5), metabolic (n = 4), constitutional (n = 3), pain (n = 2) and bleeding (n = 1). The hematologic toxicities (mostly leucopenia) occurred in 16 patients. There was one death due to pancreatitis toxicity. DISCUSSION: Erlotinib was well tolerated but it did not improve PFS in children with HGG.

HG-39. GENETIC ALTERATIONS AS POSSIBLE MARKERS FOR RISK STRATIFICATION OF PEDIATRIC HIGH-GRADE GLIOMAS
Ibrahim Qaddoumi, Tong Lin, Thomas E. Merchant, Mehmet Kocak, Atimaram Pan, Panandiker, Gregory F. Armstrong, Cynthia Wetmore, Amar Gajjar, and Alberto Broniscer; St. Jude Children's Research Hospital, Memphis, TN, USA

Mutations in genes encoding histone H3 proteins have recently been reported to underlie approximately 30% of paediatric glioblastoma (pGBM) and up to 80% diffuse intrinsic pontine glioma (DIPG), though are largely absent from adult GBM and other paediatric malignancies. In particular, somatic mutations in H3F3A occur at or close to critical residues at which methylation marks are associated with transcriptional repression (H3K27me – K27M) or activation (H3K36me – G34R). The functional implications of these different mutations, and the mechanism by which they may be targeted clinically are not yet known. We have identified a pGBM cell line harboring a heterozygous H3F3A G34V mutation associated with increased levels of H3K36 trimethylation. This cell line additionally has the recurrent homozygous TP53 mutation (R342X) found in pGBM patients, as well as a novel heterozygous DAXX mutation (Y579X). H3F3A G34V found to correlate with the pre-mRNA splicing machinery. Notably, loss of the U2 snRNP protein PHF5a or its interacting partners resulted in cell cycle arrest and subsequent cell death only in GSCs, identifying the spliceosome as a specific molecular vulnerability in GBM. New treatment strategies for this disease are urgently needed. The identification of spliceosomal proteins as essential for the growth and maintenance of GSCs both adds to our understanding of glioblastoma biology and suggests novel targets for therapeutic intervention.

HG-40. A FUNCTIONAL GENETIC APPROACH IN PATIENT-DERIVED GLOIOBLASTOMA STEM CELLS REVEALS PRE-mRNA SPlicing COMPONENTS TO BE CANCER-LETHAL GENE TARGETS
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Glioblastoma multiforme (GBM) is the most lethal form of brain cancer in both adults and children. It is among the deadliest cancers with a median survival period of 12-14 months despite aggressive therapy and toxic side effects, underscoring the need for novel therapeutic targets specifically required by GBM cells. Many GBM are thought to arise from a neural stem cell (NSC) origin and, consistent with this premise, tumor-initiating GBM stem cells (GSCs) isolated from patients retain the NSC-like phenotype and molecular markers of primary tumors. Importantly, unlike serum-cultured NSC cell lines, GSCs retain the developmental potential and specific genetic mutations acquired as each patient’s tumor progressed from its cell of origin. We hypothesized that these genetic alterations driving GBM growth might also target unique molecular vulnerabilities within the cancer cells. To identify such novel gene targets required for GBM cell growth, but which are dispensable to normal cells, we performed genome-scale RNAi screens in multiple patient-derived GSC isolates and simultaneously counter-screened against primary untransformed acute myeloid leukemia. From these screens, we identified and validated the existence of GBM-lethal genes that, when inhibited, render patient GSCs sensitive to cellular stresses arising within these transformed cells. From these targets, we show that GSCs have an increased representation of genes involved in pre-mRNA splicing (PUMA, KNS42) which harbours a heterozygous mutation (H3K27me – K27M) or activation (H3K36me – G34R). The functional implications of these different mutations, and the mechanism by which they may be targeted clinically are not yet known. We have identified a pGBM cell line harboring a heterozygous H3F3A G34V mutation associated with increased levels of H3K36 trimethylation. This cell line additionally has the recurrent homozygous TP53 mutation (R342X) found in pGBM patients, as well as a novel heterozygous DAXX mutation (Y579X). H3F3A G34V found to correlate with the pre-mRNA splicing machinery. Notably, loss of the U2 snRNP protein PHF5a or its interacting partners resulted in cell cycle arrest and subsequent cell death only in GSCs, identifying the spliceosome as a specific molecular vulnerability in GBM. New treatment strategies for this disease are urgently needed. The identification of spliceosomal proteins as essential for the growth and maintenance of GSCs both adds to our understanding of glioblastoma biology and suggests novel targets for therapeutic intervention.

HG-41. H3F3A MUTATIONS IN PAEDIATRIC GLOIOBLASTOMA REGULATE A SELF-RENEWAL GENE SIGNATURE
Meera Nandialahab1, Lynn Bjørke1, Dorine Bax1, Diana Carvalho1, Ilirjana Bajrami1, Alan Ashworth1, Christopher Lord1, Darren Hargrave2, Christopher Hubert, Yu Ding, Chad Toledo, Patrick Paddison, and James Olson; Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Mutations in genes encoding histone H3 proteins have recently been reported to underlie approximately 30% of paediatric glioblastoma (pGBM) and up to 80% diffuse intrinsic pontine glioma (DIPG), though are largely absent from adult GBM and other paediatric malignancies. In particular, somatic mutations in H3F3A occur at or close to critical residues at which methylation marks are associated with transcriptional repression (H3K27me – K27M) or activation (H3K36me – G34R). The functional implications of these different mutations, and the mechanism by which they may be targeted clinically are not yet known. We have identified a pGBM cell line model (KN42) which harbours a heterozygous H3F3A G34V mutation associated with increased levels of H3K36 trimethylation. This cell line additionally has the recurrent homozygous TP53 mutation (R342X) found in pGBM patients, as well as a novel heterozygous DAXX mutation (Y579X). H3F3A G34V found to correlate with the pre-mRNA splicing machinery. Notably, loss of the U2 snRNP protein PHF5a or its interacting partners resulted in cell cycle arrest and subsequent cell death only in GSCs, identifying the spliceosome as a specific molecular vulnerability in GBM. New treatment strategies for this disease are urgently needed. The identification of spliceosomal proteins as essential for the growth and maintenance of GSCs both adds to our understanding of glioblastoma biology and suggests novel targets for therapeutic intervention.

HG-42. MULTIPLE H3 MUTATIONS IN PAEDIATRIC GLOIOBLASTOMA REGULATE A SELF-RENEWAL GENE SIGNATURE
Meera Nandialahab1, Lynn Bjørke1, Dorine Bax1, Diana Carvalho1, Ilirjana Bajrami1, Alan Ashworth1, Christopher Lord1, Darren Hargrave2, Christopher Hubert, Yu Ding, Chad Toledo, Patrick Paddison, and James Olson; Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Mutations in genes encoding histone H3 proteins have recently been reported to underlie approximately 30% of paediatric glioblastoma (pGBM) and up to 80% diffuse intrinsic pontine glioma (DIPG), though are largely absent from adult GBM and other paediatric malignancies. In particular, somatic mutations in H3F3A occur at or close to critical residues at which methylation marks are associated with transcriptional repression (H3K27me – K27M) or activation (H3K36me – G34R). The functional implications of these different mutations, and the mechanism by which they may be targeted clinically are not yet known. We have identified a pGBM cell line model (KN42) which harbours a heterozygous H3F3A G34V mutation associated with increased levels of H3K36 trimethylation. This cell line additionally has the recurrent homozygous TP53 mutation (R342X) found in pGBM patients, as well as a novel heterozygous DAXX mutation (Y579X). H3F3A G34V found to correlate with the pre-mRNA splicing machinery. Notably, loss of the U2 snRNP protein PHF5a or its interacting partners resulted in cell cycle arrest and subsequent cell death only in GSCs, identifying the spliceosome as a specific molecular vulnerability in GBM. New treatment strategies for this disease are urgently needed. The identification of spliceosomal proteins as essential for the growth and maintenance of GSCs both adds to our understanding of glioblastoma biology and suggests novel targets for therapeutic intervention.
lethality screen for H3F3A mutant versus wild-type pGBM cells with siRNAs directed against 794 human kinases and tumour suppressor genes revealed a selective sensitivity of H3F3A G34V to multiple kinases associated with high-level phosphorylation, providing an individual approach to tumour cell death in this population. These data provide the first clues as to the biological consequences of H3F3A G34V mutations in pGBM, as well as identifying novel targets for drug development in mutant positive patients.

HG-42. INTRATUMORAL MUTUAL EXCLUSIVITY OF DUAL AMPLIFIED RECEPTOR TYROSINE KINASE GENES IN PAEDIATRIC GLIOBLASTOMA
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Glioblastoma is recognised for a pronounced intratumoral heterogeneity within its neoplastic cells. The generation of composite genomic profiles from bulk tumour samples has allowed for the mapping of putative genetic drivers of the disease, and the prioritisation of therapeutic targeting strategies designed to eradicate multiple tumour DNA copy number profiling has demonstrated that multiple RTK amplifications may frequently be found in the same glioblastoma specimens. Although tumour clonality would imply that these events would be present in all neoplastic cells, we previously noted a large proportion of these genes had distribution in situ hybridisation (FISH/CISH) experiments on pathological specimens that not all cells harbour individual amplification events. Further fine FISH-mapping of the two RTK genes that are most commonly amplified in adult and paediatric glioblastoma, the EGFR and PDGFRA receptor tyrosine kinases, revealed a greater than previously recognised concurrent amplification, with a remarkable degree of mutual exclusivity across entire tumour specimens. This was quantitated by assessing >40,000 cells from >200 distinct loci across 20 samples. Although some cases demonstrated a relatively uniform admixture of different DNA copy number across the sample, most showed significantly distinct frequency patterns in restricted topographical components of the tumour. Within an individual sample, cells harbouring one, both, or neither amplification could be found in parts of the tumour, presenting as a mosaic of genetically distinct cells, or forming foci where one event would strongly predominate. Specific to paediatric glioblastoma, we also identified a case with dual amplification of PDGFRα and PDGFRβ, in which every adjacent cell studied across the tumour specimen harboured either one gene amplification or the other in roughly equal proportions, and never both. These data have profound implications for designing efficacious therapeutic regimens, as the relative contributions of cell populations harbouring one or other genetic alteration to disease propagation, and the implications for targeted therapies, are not known.

HG-43. IDENTIFICATION OF NOVEL FUSION GENES IN PAEDIATRIC HIGH GRADE GLIOMA
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Chromosomal rearrangements resulting in novel fusion genes are among the most prevalent form of genetic alterations known in cancer, and numerous examples exist in both adult and childhood malignancies. To date however, none have been reported in paediatric high grade glioma (pHGG), so we have undertaken to search for novel structural rearrangements using three distinct approaches. Firstly, we took a candidate approach and screened a series of 83 pHGG for the fusion previously described in adult glioblastoma between PDGFRα and KDR (VEGFR2) at 4q12. Using RT-PCR and sequencing we identified the second reported instance of KDR/PDGFRα in a single case of glioblastoma (age 1.2 years). Next, we applied the (c)RNA algorithm to identify novel intrachromosomal rearrangements in an analogous mechanism to kdr/PDGFRα. We fine-mapped the fusions using custom Agilent oligonucleotide arrays and characterised the fusions DHX57:MAPK4 (2p22) and GGA(NACT2:RET (10q11) in cases of anaplastic astrocytoma (2 years) and recurrent glioblastoma (12.8 years), respectively. Finally, we sequenced the entire genomes of five paediatric glioma cell lines at ≥30x coverage using the Illumina HiSeq2000 platform, and screened for rearrangements using the BreakDancer (BD) package. We identified a median of 165 intragenic structural variants per genome, that were filtered based on BD confidence score, number and orientation of reads and by reverse mapping. Candidate fusions being systematically validated and screened in our pHGG cohorts include interchromosomal rearrangements resulting in TULIP:RPTOR (t6;17 - SF188), GORASP2:CDAC1 (t2:13 - KNS42) and PCSK3:MYO15A (p9;17 - UW799). These data highlight the presence of novel fusion genes in pHGG which may play important roles in the unique biology of the tumours as well as provide excellent candidates for novel therapeutic strategies.

HG-44. SELECTIVE TARGETING OF IGF1R BLOCKS MTOR INHIBITOR-INDUCED PI3K PATHWAY REACTIVATION IN PAEDIATIC GLIOBLASTOMA CELLS
Lynn Bjerke1, Lara Perrym1, Anna Burford2, Dorine Bax1, Alexa Jury1, Sergey Popov1, Gary Box3, Florence Raynaud2, Darren Hargrave1, Suzanne Eccles1, and Chris Jones1; 1Institute of Cancer Research, Sutton, UK; 2Great Ormond Street Hospital, London, UK

Amplification and overexpression of IGF1R in paediatric glioblastoma (pGBM) are known to be aberrantly expressed in both preclinical and clinical studies, and overexpression of IGF1R has been correlated with aggressive biological behaviour and decreased patient survival. Inhibitors of mitogen-activated protein kinase (MAPK) and mammalian target of Rapamycin (mTOR) are currently being developed as potential drugs to treat GBM, both in vitro and in vivo. We have previously shown that co-targeting IGF1R and mTOR could increase anti-proliferative effects and better suppress PI3K signalling in pGBM cells, and sought to explore this using a variety of clinically relevant IGF1R and mTOR inhibitors. We found that co-targeting IGF1R and mTOR blocked the mTOR inhibitor-induced inactivation of the PI3K pathway in SF188, one of our pGBM cell lines. Inhibition of phospho-IGF1R and phospho-mTOR was assessed by flow cytometry, western blotting and immunohistochemistry. SF188 cells were treated with a range of IGF1R and mTOR inhibitors for 48 hours, with IGF1R inhibition assessed by IGF1R and mTOR kinase inhibitor treatment identified by enrichment of downstream markers such as p-AKT, p-mTOR, p-4EBP1, and p-RPS6. Inhibition assays showed additive responses when IGF1R and mTOR inhibitors were combined, with the lack of stronger anti-proliferative effects possibly explained by a predominant inductions of apoptosis in pGBM cells. The small molecule IGF1R inhibitor OSI-906 and the ATP competitive mTOR kinase inhibitor AZD-6055 were both found to have sufficient CNS penetration to produce therapeutic levels of the drugs in our orthotopic pGBM xenograft models and represent a potentially clinically useful combination therapy in pGBM and DIPG patients.

HG-45. MOLECULAR DETERMINANTS OF EFFICACY OF MET RECEPTOR INHIBITION IN PAEDIATRIC GLIOBLASTOMA
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Recent molecular profiling data has suggested the hepatocyte growth factor (HGF)/MET pathway is the least important in paediatric high grade glioma (pHGG) than for histologically similar adult lesions, although it may be more frequent in diffuse intrinsic pontine glioma (DIPG). We sought to explore the possibilities of targeting this pathway in the paediatric setting pHGG through a combination of molecular pathology of patient samples, genetic/epigenetic profiling of paediatric glioma cell lines as well as pharmacological/genetic inhibition in vitro. MET gene amplification by FISH was found in 3/123 (2.4%) pHGG, with overexpression of the receptor by immunohistochemistry in 20/136 (14.7%), significantly lower than that observed in adult HGG (27/284, 9.5%); amplification; 58/256, 22.6% overexpression, p < 0.001). No MET amplifications were observed in a panel of paediatric glioma cell lines, however in vitro treatment with the small molecule MET inhibitor PHA665752 revealed two lines (SF188 and Res259) to be sensitive to targeted inhibition, effects replicated by knockdown with siRNA. Similar results were observed with the dual ALK/MET inhibitor crizotinib for SF188, but not Res259. An epigenome-wide screen using 5'-aza-2'deoxycytidine treatment identified gene expression changes in the HGF/MET pathway, to be aberrantly silenced by promoter
Hypermethylation selectively in SF188 and Res259 cells, the latter of which also harbouring an additional novel heterozygous missense mutation in the SPINT2 gene. Methylation-specific PCR confirmed SPINT2 promoter hypermethylation in 35/62 (56%) pHBG, although no correlations with clinical outcome were observed. With concurrent SPINT2 hypermethylation / MET receptor expression identified as being predictive for PHA665752 efficacy in vitro, these data widen the possible subset of children with HGG who may benefit from anti-MET therapies in the clinic. Further work is aimed at extending these observations to the orthotopic in vivo setting, as well as understanding the mechanism of differential sensitivity to crizotinib in these cells.

Paediatric glioblastomas (GBM) are rare and currently there is insufficient information regarding their pathogenesis unlike their adult counterparts. Hence, this study was undertaken to gain insight into the genetic and epigenetic alterations in paediatric GBMs. The following mutations were studied by sequencing viz. TP53, IDH1 and H3F3A. Fluorescence in situ hybridization was done to assess EGFR amplification, PTEN deletion, and CDKN2A deletion. Also, MGMT methylation status was studied using methylation specific PCR. Further, 21 GBM cases along with 3 control normal brains from paediatric epilepsy surgery cases were studied using genome wide methylation profiling by Illumina Infinium HumanMethylation27 assay. Beta values were used to carry out Hierarchical Clustering Analysis (HCA) for all the genes and all samples. Differences between average beta values of GBM and control brains were determined on the basis of statistical significance. Methylation analysis was performed using quantitative real time PCR. Comparison with methylation data from adult GBM cases from the same institute was also done. Paediatric GBMs were characterized by TP53 mutation in 47% of cases, CDKN2A deletion in 31%, PTEN deletion in 40% and MGMT methylation in 50%. No case showed IDH1 mutation or EGFR amplification. HCA showed at least two clusters of paediatric GBM. In total, there were 162 hypermethylated and 1318 hypomethylated genes. There were 9 genes hypermethylated and 11 hypomethylated in both paediatric and adult GBMs. Interestingly, two genes were hypomethylated in pediatric while hypermethylated in adult GBM viz. FSD1 and GPRK2. However, there was no gene which was hypermethylated in pediatric and hypomethylated in adult. Expression analysis of these genes was done using real time PCR. This study highlights differences in the genetic and epigenetic alterations between pediatric and adult GBMs. Such a detailed understanding of the molecular pathogenesis is crucial for identification of relevant targets for designing of new therapeutic agents.

**HG-46.** **PAEDIATRIC GLIOBLASTOMAS: AN INTEGRATED GENETIC AND EPIGENETIC PROFILING STUDY**

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Brain tumours are the leading cause of cancer-related mortality in children. Pediatric high grade astrocytoma grade III-IV (WHO) are rare but devastating brain tumours accounting for 15% of all pediatric brain tumour cases. Grade III and IV pediatric astrocytomas are similarly treated and exhibit the poorest overall prognosis in pediatric oncology. To identify differences based on tumor grade and age, we investigated pHBG using transcriptome profiling. Our results show independent segregation of pHBG from pGBM patients highlighting distinct molecular characteristics between these subgroups. The 660 differentially expressed genes between grade III and IV pediatric astrocytomas were further investigated using the Ingenuity Pathway Analysis (IPA) software to achieve comprehensive analysis of biological functions. IPA identified significant downregulation of the mTOR pathway (p-value = 4.65x10^-4) that differentiated both subgroups. 13 genes involved in the mTOR pathway were found to be differentially regulated between both subgroups. PRKCB, a major member of the mTOR pathway involved in apoptosis and transcriptional regulation, was found to be upregulated in pAA compared to pGBM with a fold change of 2.723 (further validated using IHC staining on pAA and pGBM primary tumors). This is the first report of its kind showing differential regulation of pAA and pGBM within pHGA and identifying upregulation of mTOR pathway genes in pAA. We are further investigating the functional significance of this dysregulation in pAA. These results shed light on a pathway that may be amenable to therapy as drugs targeting it are already being used in clinical trials in children. They also further emphasize the need for better molecular classification of tumors for optimal therapeutic results in patients who have limited options for clinical trials and dismal outcome using current targeted therapies that exist without improved knowledge of the inherent biology of the tumor.

**HG-47.** **GENE EXPRESSION PROFILING OF PEDIATRIC HIGH GRADE ASTROCYTOMAS REVEALS mTOR PATHWAY DYSREGULATION**

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Grade III and IV pediatric astrocytomas are similarly treated and exhibit the poorest overall prognosis in pediatric oncology. To identify differences based on tumor grade and age, we investigated pHBG using transcriptome profiling. Our results show independent segregation of pHBG from pGBM patients highlighting distinct molecular characteristics between these subgroups. The 660 differentially expressed genes between grade III and IV pediatric astrocytomas were further investigated using the Ingenuity Pathway Analysis (IPA) software to achieve comprehensive analysis of biological functions. IPA identified significant downregulation of the mTOR pathway (p-value = 4.65x10^-4) that differentiated both subgroups. 13 genes involved in the mTOR pathway were found to be differentially regulated between both subgroups. PRKCB, a major member of the mTOR pathway involved in
HG-50. GIANT CONGENITAL ANAPLASTIC ASTROCYTOMA: A CASE REPORT
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BACKGROUND: intracranial congenital tumors are rare, especially the malignant ones. Several case reports were published so far about congenital anaplastic astrocytomas. REPORT: A huge total right hemisphere involving brain lesion was revealed in 7 days boy neonate by neurosonography and confirmed on MRI scan. Tumor resection was performed in the age of 5 weeks. Pathological and immunohistochemical examination revealed anaplastic astrocytoma with glioblastoma plots with Ki 67 of 18 %. As the control MRI showed no exact signs of residual mass and no involvement of the spinal cord and considering child’s poor overall condition, no adjuvant treatment was performed. Left hemiparesis and diffuse hypomyotony developed. Child received continuous rehabilitation treatment. At the age of 6 months ventriculoperitoneostomy was performed due to progressive ventriculomegaly. Periodic control MRI scans showed no tumor growth with ventricles and postoperative cyst size regression. Child is alive, 2 years of age with no symptoms of tumor progression. Still the quality of life is poor due to suffering from neurological disorders and blindness.

HG-51. PAEDIATRIC GLIOBLASTOMAS: AN TERTIARY INSTITUTIONAL EXPERIENCE OF CLINICOPATHOLOGICAL FEATURES IN A SERIES OF 24 CASES
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INTRODUCTION: Astrocytic tumours are the commonest paediatric primary brain tumours. Most of them are low grade. Glioblastomas (GBM) are most common in the adults and older population but they extremely uncommon in paediatric population. MATERIALS AND METHODS: Reviewed and histologically reconfirmed diagnosed cases of GBM, where paraaffin blocks and radiological details were available were retrieved from the departmental archival records during the period of 2004 to 2011 and were studied clinical, radiological, histological and molecular features. RESULTS: Of the total 33 GBM cases, 9 cases were excluded due to various reasons of borderline age of presentation of 18-20yrs, lack of paraaffin blocks and radiology. In total, 24 formed the study sample, which included 15 males and 9 female and age group at presentation as follows: 0-6yrs:1, 7-12yrs:9 and 13-18yrs:14. Supratentorial hemispheric location was predominant location (n=22; parietal, temporal & temporoparietal: 16, frontal & frontotemporal:5 & occipital:1) and 2 in posterior fossa. Most of cases were right sided (n=19), 3 were left sided and 2 were bilateral. All of them were heterogeneously enhancing and solid in nature. 5 cases additionally showed cystic change. Headache and vomiting were presenting features in all, additionally seizures, hemiparesis and visual symptoms were noted in 9, 3 & 2 respectively. Histologically, p53 immunopositivity was noted in 9. Evaluation for MGMT gene promoter methylation by gel based MS-PCR and EGFR gene by FISH are being done and the results of the same will elaborated in the main paper. Radiation (55-60Gy) was given in 22 and in 10 concomitant with adjuvant temozolomide has been given. Follow up data was available in 14 was variable between 3-19 months. 8 of them were alive with stable disease and 6 died (of which 3 had received temozolomide). CONCLUSIONS: Paediatric GBMs are uncommon and results of MGMT gene methylation and EGFR gene status are awaited for correlation with clinicoradiological features.