Irinotecan (CPT-11) had a significantly worse median overall survival in cohorts treated without VP-16. In contrast, patient cohorts treated with VP-16 and a survival advantage remained, whereas Irinotecan should be used cautiously.

HG-01. ETOPOSIDE IMPROVES SURVIVAL IN HIGH GRADE GLIOMA: A META-ANALYSIS
Alexis Leonard and Johannes Wolff; Tufts University School of Medicine, Boston, MA, USA

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 575 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. 13.66 months, p = 0.031, 48 vs. 739 cohorts) and a significant survival gain advantage (p = 0.035) over cohorts treated without VP-16. In contrast, patient cohorts treated with Irinotecan (CPT-11) had a significantly worse median overall survival (9.89 months vs. 13.35 months, p = 0.007) and a survival disadvantage (p = 0.163) over cohorts treated without CPT-11. Subgroup analyses were performed such as newly diagnosed vs. recurrent, pediatric vs. adult, and glioblastoma multiforme vs. anaplastic astrocytoma. The trend toward a survival advantage for VP-16 and a survival disadvantage for CPT-11 remained, though significance was lost when subgroups became small. CONCLUSION: Results from this study suggest that Etoposide improves overall survival and should be included in chemotherapy regimens for patients being treated for HGGs, whereas Irinotecan should be used cautiously.

HG-02. GBM STEM CELL NICHE DISRUPTING AGENTS IDENTIFIED THROUGH NOVEL HIGH THROUGHPUT COMPOUND LIBRARY SCREEN
Rajeshi Sengupta, Jayne Marassa, David Pwnica-Worms, and Joshua Kumb; Washington University in St Louis, School of Medicine, St Louis, MO, USA

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 (PVN). 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 PVN that incorporated extra-cellular matrix (Matrigel), primary human brain microvascular endothelial cells (HBMECs) and primary GBM specimens or established GBM cell line cultures. Co-culture of GBM cells with HBMECs resulted in their co-localization and enhanced GBM cell growth. Genetic and pharmacological manipulation of the CXCL12 pathway revealed that the localizing and trophic effects of endothelial cells on GBM cells were dependent upon CXCL12 and CXCR4 but not CXCR7. To identify additional molecules and pathways that mediate a significant survival advantage, we used this co-culture system to screen the Spectrum Collection compound library. While most compounds in this 2000 component library were without effect, we identified a small but diverse group of drugs that blocked the trophic effects of the HBMECs 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. These data further validate the trophic and sanctuary effects of the GBM PVN and identify new candidate therapeutics for GBM therapy.

HG-03. PEPTIDE VACCINE THERAPY FOR CHILDHOOD GLIOMAS: INTERIM RESULTS OF A PILOT STUDY
Ian Pollack1, Regina Jakacki1, Lisa Butterfield2, and Hideho Okada2; 1Children’s Hospital of Pittsburgh, University of Pittsburgh, Pittsburgh, PA, USA; 2University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA

INTRODUCTION: Malignant astrocytomas of the brainstem and cerebrum have multipotent 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 15 Montane 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, IL13RA2, and survivin. Endpoints were safety, T-cell responses, and survival. PRELIMINARY RESULTS: 23 patients were vaccinated, 42 vs. 739 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 (16.5 months vs. 11.3 months, p = 0.035, 48 vs. 739 cohorts) and a significant survival gain advantage (p = 0.035) over cohorts treated without VP-16. In contrast, patient cohorts treated with Irinotecan (CPT-11) had a significantly worse median overall survival (10.9 months vs. 14.2 months, p = 0.007) and a survival disadvantage (p = 0.163) over cohorts treated without CPT-11. Subgroup analyses were performed such as newly diagnosed vs. recurrent, pediatric vs. adult, and glioblastoma multiforme vs. anaplastic astrocytoma. The trend toward a survival advantage for VP-16 and a survival disadvantage for CPT-11 remained, though significance was lost when subgroups became small. CONCLUSION: Results from this study suggest that Etoposide improves overall survival and should be included in chemotherapy regimens for patients being treated for HGGs, whereas Irinotecan should be used cautiously.

HG-04. AN INTERNET-BASED SURVEY EVALUATING THE STANDARD OF CARE IN TREATING CHILDREN WITH NEWLY DIAGNOSED HIGH GRADE GLIOMA
Jason Fanguayo1 and Katherine E. Warren2; 1Children’s Memorial Hospital and Northwestern Feinberg School of Medicine, Chicago, IL, USA; 2National Cancer Institute, Bethesda, MD, USA

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 to adults with 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 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 neuro-oncologists. The remaining were pediatric neurosurgeons, radiation oncologists, and pediatricians. 65% had >10 years’ experience and 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 with focal radiation plus temozolomide followed by maintenance temozolomide (30%). CONCLUSIONS: The response rate to our survey was excellent, and the
demographic data indicates a group of experienced physicians who work at large academic and cancer centers. Despite this, there was no clear agreement 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-07. PHASE 1 IMMUNOTHERAPY TRIAL USING Glioblastoma Apoptotic BODY-PULED DENDRITIC CELLS**

**HG-08. DISTINCT DRIVER MUTATIONS DEFINE EPIGENOMIC AND BIOLOGICAL SUBGROUPS OF CHILDHOOD AND YOUNG ADULT Glioblastoma**
Dorinuj Sturm, Hendrik Witt, Volker Hovestad, Dong Anh Khanh Quan, David T.W. Jones, Carolin Konermann, Elke Paffi, Andrey Korshunov, Marina Rushova, Til Millde, Olaf Witt, Marc Zapata, V. Peter Collins, Marcel Kool, Guido Reifenberger, Peter Lichter, Anders M. Lindroth, Christoph Plass, Nada Jabbado, and Stefan M. Pfister.

**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 inhibitor that is believed to have broad potential applications in cancer. An elegant yet simple solution is the establishment of permanent individual patient tumor cell lines. These represent an invaluable source for detailed molecular, biochemical or functional analyses. Based on a close cooperation and logistics between surgery, pathology, laboratory researchers and animal care facilities, we cultured cell lines of about 30 primary brain tumors, most of them GBM. Further, tumors were established in a subcutaneous mouse model. Tumors and cell lines were characterised by morphology, growth in vitro, and genetic and molecular features, revealing a heterogeneous pattern in each case. Further, the sensitivity of individual cell lines towards Temozolomide, Cilengitide, and the combination of both was analysed. In the field of individualized patient care, preclinical studies need to permit a multitude of in-vitro-studies analysing or predicting the efficacy of immunological, small molecular, biological-based or other therapeutic strategies. The project was supported by the Forderkreis fur krebskranke Kinder Rostock e.V., a Mecklenburg-Vorpommern graduate scholarship, and a cooperation with Merck-Serono Research and Development (Dr. Goodman).

**HG-06. Efficacy of Combination Therapy with Ponatinib (AP24534) +/- Bevacizumab Against Pediatric Glioblastoma**
Stephen Kerr, Julia Salie, Martin Roskoski, Henry Friedman, and Darel Rigter.

**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 established the efficacy of ponatinib in combination with bevacizumab using both a subcutaneous and intracranial model. METHODS: The pediatric brain tumor xenograft D-2159MG was grown in athymic BALB/c mice in both a subcutaneous and intracranial model. After tumor size reached 200-500 mm³ subcutaneously or 3 days after intracranial implantation, groups of 10 mice were randomly assigned to groups treated with either drug vehicle/control, ponatinib (30 mg/kg/day), PO for 21 days, bevacizumab (5 mg/kg twice weekly) IP for 5 weeks or the combination of ponatinib and bevacizumab. Tumor responses for subcutaneous xenografts were assessed by tumor growth delay and regression and survival. In the intracranial model, there were significant (p < 0.001) growth delays of 16.48 and 19.53 days were achieved for single agent effects of ponatinib and bevacizumab respectively. The combination of agents resulted in a 38% increase in survival over the additive effect of single agents. CONCLUSION: In our results, we demonstrate the activity of ponatinib as a single agent against D-2159 MG in both models. When combined with bevacizumab, ponatinib produced significantly beneficial results in D-2159 MG. Additional studies need to be conducted to further validate these findings in other pediatric brain tumor xenografts.
different anatomic compartments, which likely originate from distinct precursor-cell populations, one of which completely lacks expression of OLG1/2 - early indicators of neural lineage commitment. Our findings show that the cellular origin of cell lines leading to GBM 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.

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 installation of GlialEd wafer. 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 (World grade III). Since surgery, treatment of the radiotherapy (54 Gy) with concomitant temozolomide (200 mg/m²/day) followed by six four-weekly cycles of oral 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 right pterional craniotomy and image-guided macroscopic resection of the tumour and implantation of seven carmustine impregnated wafers (GlialEd®). 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 few 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 GlialEd® in children and young people with relapsed HGG particularly in the setting of a second complete resection.

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Case Report: A 15-year-old female presented with temporal lobe epilepsy. MRI showed a heterogeneous 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). Since surgery, treatment with radiotherapy (54 Gy) followed by six four-weekly cycles of oral temozolomide (200 mg/m²/day) and concomitant 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 right pterional craniotomy and image-guided macroscopic resection of the tumour and implantation of seven carmustine impregnated wafers (GlialEd®). The middle cerebral artery was enucleated in tumour and skeletonized to enable a macroscopic clearance. Histology confirmed relapse grade III astrocytoma. The patient then received six cycles of procarbazine and lomustine. Three years later she is well and disease free.

Discussion: Relapsed HGG carries a dismal prognosis in both adults and children and there are few 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 GlialEd® in children and young people with relapsed HGG particularly in the setting of a second complete resection.

HG-10. NF-kB INHIBITION BY DHMEQ EFFICIENTLY IMPAIRS IN VITRO GROWTH AND INVASION IN PEDIATRIC ASTROCYTIC TUMORS, SENSITIZING CELLS TO CONVENTIONAL TREATMENTS

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1Alder Hey Children’s Hospital, Liverpool, UK; 2The Walton Centre, Liverpool, UK

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 installation of GlialEd® 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 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 few 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 GlialEd® in children and young people with relapsed HGG particularly in the setting of a second complete resection.

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, Dehydroxymethylepoxyquinomide (DHMEQ) a novel NF-κB inhibitor has shown potent anti-tumor and chemo-sensitizing properties. This nuclear factor is constitutively activated in most tumors, including pediatric gliomas. Because of its ability to control the expression of critical genes involved in apoptosis, differentiation, chemo-resistance and invasion, NF-κB constitutes the point of convergence in most tumors, including pediatric gliomas. Because of its ability to control the expression of critical genes involved in apoptosis, differentiation, chemo-resistance and invasion, NF-κB constitutes the point of convergence in most tumors, including pediatric gliomas. Because of its ability to control the expression of critical genes involved in apoptosis, differentiation, chemo-resistance and invasion, NF-κB constitutes the point of convergence in most tumors, including pediatric gliomas. Because of its ability to control the expression of critical genes involved in apoptosis, differentiation, chemo-resistance and invasion, NF-κB constitutes the point of convergence in most tumors, including pediatric gliomas. Because of its ability to control the expression of critical genes involved in apoptosis, differentiation, chemo-resistance and invasion, NF-κB constitutes the point of convergence in most tumors, including pediatric gliomas.

In an attempt to elucidate this discrepancy the expression of drug efflux transporters BCRP1 and MRP1 on the blood-tumor barrier was assessed by Western blotting and immunohistochemistry, respectively. RESULTS: Exposure to the chemotherapeutics doxorubicin, mitoxantrone, melphalan, and vinblastine 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
Olela Alonso1, Carmen de Torres1, Mariona Sunol2, Eva Rodriguez1, Laura Álamo1, Andrea Parareda1, Teresa Cardesa1, Hector Salvador1, Vermónica Celis1, Antonio Guillén2, Gemma Marquith2, Jordi Muchard1, Carlos Trampal1, Maria Luisa Martín1, Monica Rebollo1, and Jaume Morà1
1Hospital St Joan de Deu, University of Barcelona, Pediatric Oncology, Esplugues, Barcelona, Spain; 2Hospital St Joan de Deu, Pediatric Neurosurgery, Esplugues de Llobregat, Barcelona, Spain; 3PET Unit, CRC-Molecular Image Center, IAT, Barcelona, Spain; 4Hospital St Joan de Deu, Pediatric Neuroradiology, Esplugues de Llobregat, Barcelona, Spain

INTRODUCTION: After a pilot study suggesting that irinotecan/cisplatin (I/C) may be effective for pediatric gliomas, we conducted a phase II controlled trial (EndeC2009/010742-59). METHODS: patients diagnosed with high-risk (HR) gliomas (HGG, DIPG, or HR-LGG) received sixteen weekly outpatient iv. cycles of C (30mg/kg of 11C-Methionine 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 7-17y (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, Pilomyxoid A = 1, Ganglioglioma = 1, NFI = 2), were included. Four patients died of disease (2HGG, 2DIPG). Clinically significant responses (PR + SD) were found in 14 (51%) patients (3PR/9LGG/1AAX), progressive disease in 8 (3DIPG/3AA/2GGLG) and 6 patients remain on treatment. PET-Methionine described 6 cases of metabolic stable disease, 4 of partial response and three of progression. SUV increasing significantly differentially expressed between normal brain and HGG samples. 106 microRNAs were upregulated and 501 downregulated (mostly to a minor degree) between normal brain and HGG samples. 106 microRNAs had a fold change greater than 3, with miR-34a, miR-21 known to have oncogenic effect in adult GBM, and miR-21 was significantly differentially expressed (downregulated in HGG). Biostatistical analysis was accomplished using the Genespring analysis platform (Benjamini-Hochberg multiple test correction) and microRNA target prediction using the miRanda algorithm. RESULTS: Validated selected microRNAs were undertaken using real-time-PCR. CONCLUSION: Analyzing frozen samples only, 551 microRNAs were significantly differentially expressed between HGG, LGG and normal brain. 50 microRNAs were upregulated and 501 downregulated (mostly to a minor degree) between normal brain and HGG samples. 106 microRNAs had a fold change greater than 3, with miR-34a, miR-21 known to have oncogenic effect in adult GBM, and miR-21 was significantly differentially expressed. Comparing HGG directly with LGG, 10 microRNAs were significantly altered, with miR-34a (a repressor of MYCN) the most differentially expressed (downregulated in HGG). Multiple microRNAs were significantly differentially expressed between FFPE and frozen samples. Microarray results were validated on real-time-PCR. CONCLUSIONS: The expression levels of several microRNAs are significantly altered between pediatric HGG and LGG, including microRNAs known to have substantial involvement in cancer related pathways. miR-21 in particular is a key player of therapeutic interest and potentially targetable by anti-sense genetic techniques.

HG-14. GLIOBLASTOMA 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, which was concomitant to everolimus therapy. There were no gross total resections. One patient presented with metastatic disease. Twelve of 14 patients (86%) who completed therapy remain alive with no evidence of disease (NED) at a median of 33 months (12-45 months) from diagnosis. Of six patients who were alive at the end of therapy; the other patient has stable residual disease. Of the remaining nine patients, two elected to come off study. Three patients are currently on active treatment- 2 with NED and 1 with stable disease. Four patients developed progressive disease on therapy, 2 of whom had a HR-LGG (1 AA; 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-15. DIFFERENTIAL EXPRESSION OF MICRORNAS BETWEEN HIGH AND LOW GRADE ASTROCYTOMAS
Stuart Smith1, Han Miao Wu2, and Bermans Iskandar1
1Children’s Brain Tumour Research Centre, University of Nottingham, Nottingham, UK; 2Department of Neurosurgery, NUH NHS Trust, Nottingham, UK

INTRODUCTION: MicroRNAs play key roles in cell differentiation, proliferation and cell death and are key regulators in adult high grade glioma (HGG) though little work has examined their role in pediatric tumors, in particular low grade gliomas (LGG). METHODS: METHODS: 116 samples of pediatric HGG and LGG, including microRNAs known to have substantial therapeutic interest and potentially targetable by anti-sense genetic techniques. In a study of 600 patients with intracranial glioblastoma, the incidence of symptomatic metastasis was 2%. In a series of 267 patients, investigators found that 3 of them, 1.1% developed spinal drop metastasis. One study found an average of 8-14 months between the diagnosis of GBM and spinal metastasis. The average time interval between diagnosis of drop metastasis and death is 2-6 months.

HG-16. GLIOMATOSIS CEREBRI IN A NINE YEAR OLD BOY: CASE REVIEW AND REVIEW OF THE LITERATURE
Diane Puccetti1, Shahriar Salam2, Tabassum Kennedy2, Jason Fangusaro3, Neha Patel1, Kristin Bradley4, Kristin Casey1, and Bermans Iskandar1
1Children’s Memorial Hospital, Chicago, IL, USA; 2University of Wisconsin Hospital and Clinics, Madison, WI, USA; 3University of Wisconsin, Madison, WI, USA; 4Children’s Memorial Hospital, Chicago, IL, USA

Gliomatosis Cerebi is a rare tumor defined as a diffuse glial tumor infiltrating brain, involving multiple lobes and possibly extending 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
Yoshiko Nakano1, Keiko Okada1, Yuko Usugi1, Kai Yamasaki1, Hiroyuki Fujisaki1, Hiroko Fukushima2, Takeshi Inoue2, Yasuhiro Matusaka3, Hiroaki Sakamoto1, and Junichi Harai1; 1Department of Pediatric Hematology and Oncology, Children's Medical Center, Osaka City General Hospital, Osaka, Japan; 2Department of Pathology, Osaka City General Hospital, Osaka, Japan; 3Department of Pediatric Neurosurgery, Children's Medical Center, Osaka City General Hospital, Osaka, Japan

INTRODUCTION: Gliomatosis cerebi (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 bevacinumab/trirotinuc, 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 craniopinal 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 continued 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
Steven De Vleeschouwer, Hilko Ardon, Frank Van Calenbergh, Raf Sciot, Guido Wilms, Johan Van Loon, Jan Goefin, and Stefaan Van Goor; University Hospital Leuven, Leuven, Belgium

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 III or IV), 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. Further 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 HGGs, 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
Diane Puccetti1, Shahrizat Salama2, David Rusnak2, Neha Patel1, Kristin Bradley1, Kristin Casey1, Peter Knight1, Kenan Onel3, David Wargowski1, Amy Stettner1, and Bermans Iskandar1; 1University of Wisconsin, American Family Children's Hospital, Madison, WI, USA; 2University of Wisconsin Hospital and Clinics, Madison, WI, USA; 3University of Chicago, Chicago, IL, USA

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 adenocortical 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-etoposide and C6-trirotinuc) 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-etoposide 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-trirotinuc 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 trirotinuc 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 these cells 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 antitumoral 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 with Delta-24-RGD resulted in a synergistic antitumor effect. Interestingly, salinomycin alone or in combination with the virus reduced the number of self-renewal cells in BTSC-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, invasion, 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.
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 child, a 7-year-old male, was treated with TMZ and radiotherapy, and then was retreated with a 3 month course of combination chemotherapy consisting of cisplatin (CDDP), cytarabine (CRA), and TS-1 followed by a second course of chemotherapy using high dose methotrexate (MTX) (cycle 1). At the age of 2 years, the child presented with a seizure and a new enhancing lesion was noted on MRI. Biopsy revealed a very low grade glioma. Immunohistochemistry showed ALDH2 expression, consistent with a glioma not otherwise specified (2 patients with brainstem biopsy). The child completed a second 12 month course of TMZ and 18 months of CRA. He remains clinically stable off AED since initial diagnosis. The overall survival rates were: 50, 35, and 32 respectively. Survival was not significantly associated with age, gender (6 male, 4 female), extent of resection (4 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-31. SELECTIVE AND TARGETING TREATMENT OF MALIGNANT BRAIN TUMORS WITH NON-MYOELABLATIVE HIGH-DOSE CHEMOTHERAPY

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PURPOSE: To develop non-myeloablative high dose tumor tissue chemotherapies for the treatment of highly malignant or recurrent brain tumors in children. METHOD: We established human GBM mouse models by intracranial injection of GFP-expressing GBM cells into the parenchyma of NOD-scid mice. 2. To develop the technologies of dual enhanced drug delivery system, we integrated both focused ultrasound also active liganded nanosphere drug delivery system, we integrated both focused ultrasound also active liganded nanosphere drug delivery system and magnetic resonance imaging (MRI) after tumor cell transplant and enhanced drug delivery. RESULTS: Compared with the control group, the 5 mg/kg injections of AP-1 Lipo-Dox followed by repeated pulsed HIFU showed significantly enhanced accumulation of drug in the sonicated tumor and demonstrated significantly elevated tumor-to-normal brain drug ratio (p < 0.001). Tumor treatment by AP-1 Lipo-Dox with repeated sonication significantly slowed the growth of the tumor by days 12 after tumor implantation. CONCLUSIONS: This study demonstrated that the targeted liposomal doxorubicin along with repeated selective HIFU to the tumor region will be a promising enhanced drug delivery strategy to achieve non-myeloablative selective high dose chemotherapy to tumor tissue.

HG-32. AN ATM-APNG DNA REPAIR AXIS CONFERS AN ALKYLATING AGENT RESISTANCE PHENOTYPE IN ADULT AND PEDIATRIC HIGH-GRADE GLIOMA

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Background: 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 telangiectasia 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 APPO-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 to this effect was synergistic with TMZ and 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. IBRITONECAN WITH CARBOPLATIN FOR HIGH GRADE GLIOMAS (HGG) IN CHILDREN

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Since responses to irinotecan in HGGs 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 and toxicity of irinotecan and carboplatin regimen for HGG in children. PATIENTS AND METHODS: 16 pts were assessable for response. Two were diagnosed with anaplastic oligoastrocytoma, 1 anaplastic oligodendroglioma and 13- glioblastoma. 7 pts received this regimen as a second /third line treatment and 9 after tumor resection, prior to radiotherapy. Chemotherapy consisted of 5 day courses of TMZ and 1 mg/kg chemotherapeutic agent at 3 week intervals. 12 pts had 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 Stroke of Paediatric Oncology and Hematology (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 0.5-8.7). Only three patients had undergone radio- and chemotherapy for their previous LGG. Previous LGG included diffuse/fibillary astrocytoma WHO II (n = 11), pilocytic astrocytoma WHO I (n = 3), ganglioglioma WHO II (n = 2), and pleomorphic xanthoastrocytoma (n = 2). The cumulative incidence rate for development of MT was 2 ± 0.5% per year at ten years after diagnosis of any LGG (n = 1887). 1 ± 0.5% after diagnosis of pilocytic astrocytoma (n = 1347) and 11.8 ± 3.7% after diagnosis of diffuse/fibillary astrocytoma II (n = 372). The essential support by the Deutsche Kinderkrebshilfstiftung is gratefully acknowledged.

HG-35. GENE EXPRESSION PROFILING REVEALS THAT Glioblastoma Multiforme (GBM) PROMOTE THE FORMATION OF THE VASCULARITY AND ASSOCIATED GERMAL NICHES THAT CONSTITUTE THE MICROENVIRONMENT OF PRIMARY GBM SPECIMENS

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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 vascularity and associated germinai 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 swap experiments showed that it is the glioblastoma cells which downregulate THBS1 and endothelial cells that upregulate CXCL1, reinforcing the hypothesis that the anti-angiogenic effort 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 CXCL1 is upregulated in the second line treatment of myelosuppression and gastrointestinal toxicities were the most common and manageable. CONCLUSION: Iromtican with carboplatin regimen shows activity against high grade glioma in children and has acceptable toxicity. Supported by The National Centre for Research and Development.

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

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Glioblastoma multiforme (GBM) is a lethal brain tumor 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 tumors (21/48). Recurrent mutations in H3F3A, which encodes the replication-independent histone 3 variant H3.3, were observed in 31% of tumors, and led to amino acid substitutions in two out of 16 previously reported cases. Furthermore, 5% of tumors harboured the G34V mutation in H3F3A. Screening of a large cohort of gliomas of various grades and histologies showed H3F3A mutations to be specific to GBM and highly prevalent in children and young adults. Furthermore, the presence of H3F3A/ATRX-DAKX/TP5 mutations was strongly associated with alternative lengthening of telomeress and specific gene expression profiles. This is, 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 incompletely described in the literature. METHODOLOGY: 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. 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. The median time to first histologic diagnosis of high grade glioma (HGG) was 17 (4-45) and low grade glioma (LGG) in 22 (36%) 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.
FACTORS IN THE GERMAN HIT-HGG TRIAL

 dependent probe amplification (MLPA), we were able to analyse 49 HGG, 31 as glioblastoma (WHO grade IV). By using multiplex ligation-traction. The patients were treated with postoperative polychemotherapy
70 consecutive cases enrolled into the multicenter HIT-HGG trials; in 49 genetic alterations in a homogenously treated patient cohort, we analysed chromosomal material of chromosome arm 1q were identified to predict prospective series of pediatric HGG, p53 accumulation and gain of operative residual tumor was associated to EFS in both age groups. In a retro-
in adult high grade gliomas (HGG). Unfortunately, the significance of these markers is unclear in pediatric HGG which are believed to represent constitutional (n = 5), metabolic (n = 4), constitutional (n = 3), pain (n = 2) and bleeding (n = 1). The hematologic toxicities (mostly lymphopenia) 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-38. A PHASE II TRIAL OF ERLOTINIB DURING AND AFTER RADIOTHERAPY IN NEWLY DIAGNOSED PEDIATRIC HIGH-GRAD E GLIOMAS

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 43 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, 3.3-19). The median 30 month for (from diagnosis to death of (range, 0.2-30.3). As per off study date the 1- and 2-year PFS for AA was 42.7% (SE 10.8) and 16% (SE 7.3) while for GBM it was 15% for both (range, 0.2-30.3). Erlotinib was well tolerated. Twenty-four patients developed one or more grades 3 and 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 lymphopenia) 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 GLIOMA

Several genetic markers with prognostic/predictive impact including IDH1/2 mutation and MGMT promoter hypermethylation were identified in adult high grade gliomas (HGG). Unfortunately, the significance of these markers is unclear in pediatric HGG which are believed to represent biologically different tumors compared to adult HGG. In contrast, post- operative residual tumor was associated to EFS in both age groups. In a retrospective series of pediatric HGG, p53 accumulation and gain of chromosomal material of chromosome arm 1q were identified to predict worse outcome. To investigate the frequency and prognostic impact of genetic alterations in a homogenously treated patient cohort, we analysed 70 consecutive cases enrolled into the multicenter HIT-HGG trials; in 49 cases, formalin-fixed, paraffin embedded material was available for DNA extraction. The patients were treated with postoperative polychemotherapy (infants, n = 23) or radioschemotherapy (patients ≥ 3 years of age at diagno-
osis, HIT-HGG-2007, n = 45). 38 cases were diagnosed as WHO grade III HGG, 31 as glioblastoma (WHO grade IV). By using multiplex ligation-dependent probe amplification (MLPA), we were able to analyse 49/49 cases (100 %) for chromosome 1q gain, chromosome 22 loss, PTEN loss, PDGFRA gain/amplification, and CDKN2A loss. While we could not validate 1q gain (occurring in 21%) as a prognostic marker in this series, PDGFRA alterations (occurring in 21 %) were associated to shorter overall survival in HGG patients (p = 0.036). Using immunohistochemistry and MGMT promoter analysis, we found an accumulation of p53 gene product from diagnostic to postoperative samples (29%). In infant HGG patients, this p53 accumulation was found associated with shorter EFS (p = 0.029), but not to overall survival. In conclusion, we identified p53 gene accumulation and PDGFRA gene alterations as prognostic markers which may be useful for risk stratification of the pediatric HGG patients and can be evaluated by immunohistochemistry and MLPA representing robust, reliable and cost-efficient methods.

HG-40. A FUNCTIONAL GENETIC APPROACH IN PATIENT-DERIVED GLIOBlastoma stem cells REVEALS PRE-mRNA SPlicing COMPONENTS TO BE CANCER-LETHAL GENE TARGETS

Glialbloma multiforme (GBM) is the most lethal form of brain cancer in both adults and children. It is among the deadliest cancers with a median sur-

HG-41. H3F3A MUTATIONS IN PAEDIATRIC GLIOBLASTOMA REGULATE A SELF-RENEWAL GENE SIGNATURE

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 they are largely absent from adult GBM and other paediatric gliomas. In particular, somatic mutations in H3F3A occur at or close to critical residues at which methyltransfer marks are associated with transcriptional repression (H3K27me – K27m) or activation (H3K36me – G34R/V). 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 (KN542) which harbours a heterozygous H3F3A G34V mutation associated with increased levels of H3K36 trimethylation. This cell line additionally has the recurrent homogenous TP53 mutation (R342X) found in pGBM patients, as well as a novel heterozygous DAXX mutation (Y379X). H3F3A G34V was found to correlate with the epigenetically-regulated aberrant HOX gene expression associated with a self-renewal gene expression signature, MGMT-independent temozolomide resistance, and poor clinical outcome in pGBM. Aberrant HOX/A9/ HOX/A10 expression has been linked to the H3K36 methyltransferase activity of the nuclear receptor-binding SET domain protein 1 (NSD1) in NUP98:NSD1 fusion positive cases, whilst KN542 itself harbours a homozygous R1618H mutation in the H3K36 methyltransferase SET domain-containing 2 (SETD2) gene, suggesting two potential targetable mechanistic links. Carrying out a synthetic
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 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 through fluorescent and chromogenic in situ hybridisation (FISH/CISH) experiments on pathological samples 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 gene, 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 a mosaic presenting as a genetic mosaic of 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 harboring one or other genetic alteration to disease propagation, and the implications for targeted therapies, are not known.

HG-44. SELECTIVE TARGETING OF IGF1R BLOCKS MTOR INHIBITOR-INDUCED PI3K PATHWAY REACTIVATION IN PAEDIATRIC GLIOBLASTOMA CELLS

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Amplification and overexpression of IGF1R in paediatric glioblastoma (pGBM) and diffuse intrinsic pontine glioma (DIPG) suggests the receptor tyrosine kinase to be a rational target for anti-cancer treatment. Despite this, response to small molecule and antibody agents that target IGF1R have shown only modest effects in pGBM in vitro and in vivo. Evaluation of IGF1R fluorogenic inhibitors as single agents are consistent with a mild anti-proliferative response at clinically relevant concentrations (≤1μM) and incomplete suppression of downstream signalling as measured by PI3K and MAPK-related markers. Exploring these pathways further we were able to induce anti-proliferative effects of IGF1R inhibitors in the presence of wildtype AKT, to an extent surpassing metformin (mTOR) alone at just 0.1-0.2μM exposure in both H3F3A G34V mutant and wild-type pGBM cells, but with a co-commitant increase in phospho-IGF1R. We hypothesised that co-inhibition of IGF1R and mTOR could increase anti proliferative effects and better suppress PI3K pathway 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 increase in phospho-IGF1R and potentiated variable frequency of downstream signalling as assessed by p-AKT, p-mTOR, p-RPS6 and p-4EBP1 levels. Growth 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 induction of autophagy in pGBM cells. The small molecule IGF1R inhibitor OSI-906 and the ATP competitive mTOR kinase inhibitor AZD-8055 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-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 techniques. 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 PDGFRα:MAP4K3 in a single case of glioblastoma (age 1.2 years). Next, we applied the cDNA algorithm to identify neoplastic glioma cell lines at ≥30 copies of the relevant locus (H3F3A:DHX57 (10q11) in cases of anaplastic astrocytoma (2 years) and recurrent glioblastoma (12.8 years), respectively. Finally we sequenced the entire genomes of five neoplastic glioma cell lines at ≥30 copies of the relevant locus (H3F3A:DHX57/10q11, H3F3A:MAP4K3, H3F3A:TULP4/6q23, H3F3A:MAPK4K3 (2p22) and CGALNACT2:RET (10q11) in cases of anaplastic astrocytoma (2 years) and recurrent glioblastoma (12.8 years), respectively. Finally we sequenced the entire genomes of five neoplastic glioma cell lines at ≥30 copies of the relevant locus (H3F3A:DHX57/10q11, H3F3A:MAP4K3, H3F3A:TULP4/6q23, H3F3A:MAPK4K3 (2p22) and CGALNACT2:RET (10q11) in cases of anaplastic astrocytoma (2 years) and recurrent glioblastoma (12.8 years), respectively. Finally we sequenced the entire genomes of five neoplastic glioma cell lines at ≥30 copies of the relevant locus (H3F3A:DHX57/10q11, H3F3A:MAP4K3, H3F3A:TULP4/6q23, H3F3A:MAPK4K3 (2p22) and CGALNACT2:RET (10q11) in cases of anaplastic astrocytoma (2 years) and recurrent glioblastoma (12.8 years), respectively. Finally we sequenced the entire genomes of five neoplastic glioma cell lines at ≥30 copies of the relevant locus (H3F3A:DHX57/10q11, H3F3A:MAP4K3, H3F3A:TULP4/6q23, H3F3A:MAPK4K3 (2p22) and CGALNACT2:RET (10q11) in cases of anaplastic astrocytoma (2 years) and recurrent glioblastoma (12.8 years), respectively. Finally we sequenced the entire genomes of five neoplastic glioma cell lines at ≥30 copies of the relevant locus (H3F3A:DHX57/10q11, H3F3A:MAP4K3, H3F3A:TULP4/6q23, H3F3A:MAPK4K3 (2p22) and CGALNACT2:RET (10q11) in cases of anaplastic astrocytoma (2 years) and recurrent glioblastoma (12.8 years), respectively.

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 to be less 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 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 5/123 (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 PHA665732 revealed two active compounds (SF188 and Res259) to be sensitive to targeted inhibition, effects replicated by knockdown of siRNA. Similar results were observed with the dual ALK/MET inhibitor cizotinib for SF188, but not Res259. An epigenome-wide screen using 5′-azacytidine treatment identified SPINT2, encoding a regulatory of the HGF/MET pathway, to be aberrantly silenced by promoter
Abstracts

HG-46. PAEDIATRIC GLIOBLASTOMAS: AN INTEGRATED GENETIC AND EPIGENETIC PROFILING STUDY
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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 normal brains were compared using MANOVA, with p<0.05 considered significant. The study showed that 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 paediatric while hypermethylated in adult GBM viz. FSD1 and GPR62. However, there was no gene which was hypermethylated in paediatric 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 paediatric 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-47. GENE EXPRESSION PROFILING OF PEDIATRIC HIGH GRADE ASTROCYTOMAS REVEALS MTOR PATHWAY DYSREGULATION
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Brain tumors are the leading cause of cancer-related mortality in children. Pediatric high grade astrocytomas (pHGA), including grade IV (glioblastoma, pGBM) and grade III (anaplastic astrocytoma, pAA), are rare but devastating brain tumors accounting for 15% of all pediatric brain tumor 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 pHGA using transcriptome profiling. Our results show independent segregation of pAA 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 dysregulation 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-48. POLO-LIKE KINASE 1 (PLK1) INHIBITION KILLS GLIOBLASTOMA MULTIFORME BRAIN TUMOUR CELLS IN PART THROUGH LOSS OF SOX2 AND DELAYS TUMOUR PROGRESSION IN MICE
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Glioblastoma multiforme (GBM) ranks amongst the deadliest types of cancer and, given this, new therapies are urgently needed. To identify molecular targets, we queried a microarray profiling 467 human GBMs and discovered that polo-like kinase 1 (PLK1) was highly expressed in these tumours and that it clustered with the proliferative subtype. Patients with PLK1-high tumours were more likely to die from their disease suggesting that current therapies are inactive against such tumours. This prompted us to examine its expression in brain tumour initiating cells (BTICs) given their association with treatment failure. BTICs isolated from patients expressed 110-470 times more PLK1 than normal human astrocytes. Moreover, BTICs rely on PLK1 for survival because the PLK1 inhibitor B2536 inhibited their growth in tumoursphere cultures. PLK1 inhibition suppressed growth, caused G2/M arrest, induced apoptosis and reduced the expression of SOX2, a marker of neural stem cells, in SF18 cells. Consistent with SOX2 inhibition, the loss of PLK activity caused the cells to differentiate based on elevated levels of GFAP and changes in cellular morphology. We then knocked-down SOX2 with siRNA and showed that it too inhibited cell growth and induced cell death. Likewise in U251 cells, B2536 suppressed cell growth, down-regulated SOX2 and induced cell death. Furthermore, B2536 delayed tumour growth of U251 cells in an orthotopic brain tumour model, demonstrating that the drug is active against GBM. In conclusion, PLK1 level is elevated in GBM and its inhibition restricts the growth of brain cancer cells partly via SOX2 downregulation.

HG-49. SIX YEARS COMPLETE REMISSION AFTER A RELAPSE OF GLIOBLASTOMA IN A CHILD, TREATED WITH ADJUVANT DENDRITIC CELL-BASED TUMOUR VACCINATION
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A 12 years old girl presented 2005 with epilepsy and MRI showed a right-sided temporal tumour. She was operated and microsurgery showed a high grade astrocytoma grade III-IV (WHO). Treatment followed with chemotherapy (ICE protocol) and local radiotherapy 54 Gy together with temozolomide. Eight months after initial operation she was reoperated due to a local relapse and microscopy now showed a glioblastoma grade IV (WHO) and she was included in the Leuven HGG-Immu-2003 relapse protocol. She received in Leuven adjuvant dendritic cell-based immunotherapy in combination with oral temozolomide according to the schedule. Temozolomide was finished in September 2007 and the lyse vaccinations in May 2010. Today she is still, 6 years from the relapse, in complete remission. Children with relapsing glioblastoma have a grim prognosis. This treatment is under continuous development in Leuven. A pilot project is also running in Stockholm with similar methodology, to investigate the feasibility of such a complex treatment in another centre.
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 paraffin 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 paraffin 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 is 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 temozolamide 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 temozolamide). CONCLUSIONS: Paediatric GBMs are uncommon and results of MGMT gene methylation and EGFR gene status are awaited for correlation with clinicoradiological features.