HIGH GRADE GLIOMAS

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 in order to improve 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 calculated 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 treated without CPT-11). Subset analyses 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, whereas Irinotecan should be used cautiously.

INTRODUCTION: Malignant astrocytomas of the brainstem and cerebral 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, T-cell responses against vaccine-targeted GAs, and EBV+ perivascular CD45+ CD133+ population. RESULTS: In 21 patients evaluable for response, 3 had rapidly progressive disease, 13 had stable disease, 1 had an MR, and 1 had prolonged disease-free status after surgery. ELISPOT analysis, completed in seven children, showed response to IL13Rα2 in 5, and EPHA2 in 3, and survivin in 3. CONCLUSION: Peptide vaccination in children with gliomas is generally well tolerated, and has preliminary evidence of both immunological and clinical activity. Pseudoprogession 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
Rajashri Segupta, Jayne Marassa, David Pwntca-Worms, and Joshua Ruben; 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 affect GBM cell interactions, 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 analyses 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
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: An internet-based survey evaluating the standard of care in treating children with newly diagnosed high grade glioma
Jason Fanguasy1 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 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 > 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/neo-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 (26.7%). 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 (30.7%). CONCLUSIONS: The response rate to our survey was excellent, and the

These data further validate the trophic and sanctuary effects of the GBM PVN and identify new candidate therapeutic approaches for treatment in GBM.
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-07. PHASE 1 IMMUNOTHERAPY TRIAL USING GLOBLIOBLASTOMA APOPTOTIC BODY-PULSED DENDRITIC CELLS
Christopher Moertel1, Michael Olin1, Tambra Dahlheimer1, Michael Gustafsson2, Darin Sumstad1, David McKenna1, Walter Low1, David Nasceni1, Allen Dietz2, and John Oliffst3; 1University of Minnesota, Minneapolis, MN, USA; 2Mayo Clinic, Rochester, MN, USA

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 both in vitro and shown to express tumor-associated antigens (IL13Rα2, 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 (5x10^6, 1x10^7, and 1.5x10^7), the remainder received 15 x 10^6 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 toxicities have been reported. Time to progression varies from 6.5 weeks for the patient treated at the first dose level to 35 weeks for one patient receiving 15 x 10^6 DC. The latter patient experienced a partial response at 20 weeks. Two patients have stable disease at 18.5 and 28 weeks, respectively. The dose is tolerable and feasible. Flow cytometry and demonstrated expansion of central memory T-cells amid declining effector memory cells following vaccination in three patients at a dose of 15x10^6 DC. CONCLUSION: Apoptotic body-pulsed DC vaccination was well tolerated and preliminarily demonstrated clinical activity but a minimum of 15x10^6 DC was required for a modulation of central memory T-cells.

HG-08. DISTINCT DRIVER MUTATIONS DEFINE EPIGENOMIC AND BIOLOGICAL SUBGROUPS OF CHILDHOOD AND YOUNG ADULT GLOMABLASTOMA
Dominik Sturm1, Hendrik Witt1, Volker Hovestadt2, Dong Anh Khuong Quan1, David T.W. Jones1, Carolin Konermann3, Elke Pfaffli4, Andrey Korshunov5, Marina Rizhova6, Till Milde7, Olaf Witt1, Marc Zapatka8, V. Peter Collins8, Marcel Kool1, Guido Reifenberger9, Dominik Sturm1, Hendrik Witt1, Volker Hovestadt2, Dong Anh Khuong Quan1, David T.W. Jones1, Carolin Konermann3, Elke Pfaffli4, Andrey Korshunov5, Marina Rizhova6, Till Milde7, Olaf Witt1, Marc Zapatka8, V. Peter Collins8, Marcel Kool1, Guido Reifenberger9

Glioblastoma (GBM, WHO grade IV), the most common primary brain tumor, carries a universally dismal prognosis. While there are molecular and genetic data indicating age-specific differences, it remains unclear whether pediatric GBM is biologically distinct from adult GBM. We have recently identified two recurrent mutations in H3F3A, substituting amino acid ranges in the replication-independent histone 3 variant H3.3 (K27, G34), in one-third of pediatric GBM. In the present study we shed further light on the role of H3F3A mutations in GBM and their impact on the epigenome. To comprehensively assess genome-wide DNA methylation patterns, we investigated an unbiased subset of childhood GBM. We previously discovered 35 recurrent mutations in the expression-associated CpG island set of H3F3A mutations spanning the GBM sample set. By target sequencing of these regions, we identified two H3F3A mutations strongly associated with a distinct global DNA methylation pattern, clearly discernible from those of four additional GBM subgroups, including an IDHI mutation-specific Cpg-Island Methylator Phenotype (CIMP) subgroup, two seemingly receptor tyrosine kinase-negative GBM subgroups, and one subgroup harboring mitogen-activated protein kinases. Imputation of two H3F3A mutations gave rise to GBMs in

HG-05. CHARACTERISATION OF PRIMARY GLOBLIOBLASTOMA CELL LINES WITH PARTICULAR REGARD TO CILENGITIDE SENSITIVITY
Christina Mullins1, Piek Jungreis2, Schubert Julia3, and Classen Carl Friedrich4; 1University Childrens Hospital, Rostock, Germany; 2Department of Neurosurgery, University of Rostock, Rostock, Germany

Glioblastoma multiforma (GBM) still has a very poor prognosis even with today’s aggressive combination therapy of resection followed by radiochemotherapy with Temozolomide. This underlines the necessity of new therapeutic strategies. Recent clinical data support a possible role for Cilengitide, a new integrin inhibitor, in GBM treatment. Since established GBM cell lines may contain numerous selection artefacts, they do not represent the large heterogeneity of molecular characteristics in patients and may be inappropriate to predict the therapeutic efficacy of a new compound. 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 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 characteristics, and response to Cilengitide, 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 individual patient cell lines, we established a multi-parametric, multi-centre study using a multivariate, in vitro-studies analysing or predicting the efficacy of immunological, small molecular, biological-based or other therapeutic strategies. The project was supported by the Förderkreis für 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 GLOBLIOBLASTOMA
Stephen Kerr1, Julia Saling2, Martin Roskoski1, Henry Friedman1, and Darell Bigner2; 1The Preston Robert Tisch Brain Tumor Center at Duke, Durham, NC, USA; 2West Liberty University, West Liberty, WV, USA

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 model. METHODS: The pediatric brain tumor xenografts-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 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 intracranial responses in the replication-independent histone 3 variant H3.3 (K27, G34), in one-third of pediatric GBM. In the present study we shed further light on the role of H3F3A mutations in GBM and their impact on the epigenome. To comprehensively assess genome-wide DNA methylation patterns, we investigated an unbiased subset of childhood GBMs (n = 50) and a preselected cohort of young adult cases (n = 25) using the Illumina 540k methylation array. We also analyzed the profiles of 74 adult GBMs previously investigated by The Cancer Genome Atlas Consortium, and complemented our analyses by targeted sequencing of H3F3A and IDHI in 450 GBMs of all ages. H3F3A mutations are mutually exclusive with IDHI mutations and other hallmark genetic events in GBM, e.g. EGFR amplification, and CDKN2A deletion. Each H3F3A mutation is highly correlated with a distinct global DNA methylation pattern, clearly discernible from those of four additional GBM subgroups, including an IDHI mutation-specific Cpg-Island Methylator Phenotype (CIMP) subgroup, two seemingly receptor tyrosine kinase-negative GBM subgroups, and one subgroup harboring mitogen-activated protein kinases. In accordance, two H3F3A mutations gave rise to GBMs in...
different anatomic compartments, which likely originate from distinctly precancer-cell populations, one of which completely lacks expression of OLIG1/2 - early indicators of neural lineage commitment. Our findings suggest that the close coupling of cell origin and oncogenic pathway 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.

---

**HG-09. PROLONGED SURVIVAL ASSOCIATED WITH THE USE OF INTRAOPERATIVE CARMUSTINE WAFERS (GLIADEL®) IN A PAEDIATRIC PATIENT WITH RECURRENT HIGH GRADE GLIOMA.**

Barry Pizer1, Alireza Salehzadeh1, Andrew Brodbelt2, and Conor Mallucci2; 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 Glialdel® wafers.

**CASE REPORT:** A 13 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). Significant involvement of the surgical margins and the inability to deliver concomitant chemoradiotherapy (54 Gy [72 Gy/32 weeks]) with concomitant temozolomide (75 mg/m²/day) followed by six-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 (Glialdel®). The middle cerebral artery was encased 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 therefore any 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 Glialdel® 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.**

Maria Braskas1, Julita Perzyk1, Andrea Morales1, Jaqueline de Oliveira2, Gabriela Roberto2, Kazuo Umezawa2, Elisa Valeza1, Eduardo Rigo1, Carlos Scridel1, and Luiz Tondel1; 1Faculty of Medicine of Ribeirao Preto - USP, Ribeirao Preto/Sao Paulo, Brazil; 2Faculty of Science and Technology, Keio University, Yokohama, Japan

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, Deydroxymethylpsycosynominic (DHMEQ) a novel NF-kb 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-kb constitutes the point of convergence of many oncogenic pathways. In the present study the effects of NF-kb inhibition by DHMEQ (alone or combined with TMZ or ionizing radiation) were evaluated on pediatric glioblastoma (SF188) and pilocytic astrocytoma (R286) cell lines by means of proliferation (XTT assay), viability (Trypan blue exclusion), cell cycle dynamics, clonogenic capacity, apoptosis (Nucview 488 caspase-3 kit®), necrosis (propidium iodate staining), migration (wound healing scratch) and invasion on matrigel® assays. Cells were treated with concentrations (2.5, 5, 10 and 20 ug/ml) drastically sensitized cells to ionizing radiation at 2, 4 and 6Gy doses. These results show the anti-tumor effect of DHMEQ suggesting being a good candidate for a new chemotherapeutic agent against pediatric gliomas, to be further explored on animal models.

---

**HG-11. SENSITIVITY OF PEDIATRIC HIGH GRADE GLIOMA TO CHEMOTHERAPY.**

Susanna J.E. Veringa1, Dannus G. Van Vuurden1, Pieter Wesseling1, W. Peter Vanderzouwen2, David P. Noskel3, Tom Wurdinger1, Gertjan L.J. Kaspers1, and Esther Hulleman1; 1Department of Pediatric Oncology - Hematology, VU University Medical Center, Amsterdam, The Netherlands; 2Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands; 3Department of Neurosurgery, VU University Medical Center, Amsterdam, The Netherlands

**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-responsivity 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. The cDNA of 21 compounds, including classical chemotherapeutics and novel agents, were exposed to 21 compounds. Expression of ABC transporters P-glycoprotein (P-gp), breast-cancer resistance protein (BCRP1) and multidrug-resistance associated protein (MRP1) in the cultures and tumor sections were assessed by Western blotting and immunohistochemistry, respectively.

**RESULTS:** Expression of the chemotherapeutics doxorubicin, vincristine, and melphalan 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-12. PRIMARY POSTOPERATIVE CHEMOTHERAPY WITHOUT IRRADIATION FOR TREATMENT OF HIGH GRADE GLIOMA IN CHILDREN UNDER 3 YEARS OF AGE: PRELIMINARY RESULTS FROM THE ST. JUDE YOUNG CHILDREN 2007 (SJC07) TRIAL.**

Karen Wright1, Alberto Bromsuer2, Anne Bendel3, Daniel Bowers2, John Crawford4, Paul Fisher1, Tim Hassall1, Gregory Armstrong1, Justin Baker1, Ibrahim Qaddoumi5, Giles Robinson1, Cynthia Wtemore1, Paul Klimo6, Frederick Boop7, Arza Omar-Thomas1, David Ellison1, and Amar Gajjar8; 1St. Jude Children’s Research Hospital, Memphis, TN, USA; 2UT Southwestern, Dallas TX, USA; 3Stanford, Palo Alto, CA, USA; 4Children’s Hospital Minneapolis, Minneapolis, MN, USA; 5Royal Children’s Hospital, Brisbane, QLD, Australia; 6Rady Children’s Hospital, San Diego, CA, USA; 7Le Bonheur Children’s Hospital, Memphis, TN, USA

**BACKGROUND:** High grade gliomas (HGG) are rare in children under 3 years of age, yet young children may have a better prognosis than older naturally affected tumors. Few prospective studies have described outcomes without primary radiotherapy, which carries a high risk for poor neurocognitive and endocrinologic outcomes. We aim to assess the role of primary chemotherapy in an attempt to avoid irradiation in children <3 years of age with HGG. **METHODS:** Twenty-three patients <3 years of age with HGG have been enrolled on SJC07 between December 2007 and January 2012. Treatment includes 4 identical cycles of induction chemotherapy (CT), followed by 2 months of conventional and autologous CT and 6 months of oral CT. We anticipate 3 additional years of follow up. **FININDINGS:** Neuroimaging response for 6 anaplastic
astrocytomas (AA), 5 HGGs not otherwise specified, 4 malignant glioneu- ronal tumors, 4 glioblastoma multiformes, 3 high grade neuroepithelial tumors and 1 astroblastoma. Patients included 11 females and 12 males with median age 12 months (8 days-35 months) at enrollment. There were 14 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 those patients enrolled at the end of therapy; the other patient has stable resid- ual 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 (1 HGG, 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-13. PHASE 2, SINGLE ARM, CONTROLLED TRIAL OF IRINOTECAN AND CISPLATIN IN CHILDREN WITH HIGH-RISK GLIOMAS

Oxela Cruz1, Carmen de Torres1, Mariona Sunol1, Eva Rodriguez1, Laura Alföno1, Andreu Parareda2, Teresa Cardesa2, Hector Salvador2, Vincenzo Celis3, Antonio Guillén2, Gemma García2, Jordi Muchart4, Carlos Trampal5, Maria Luisa Martín1, Monica Rebelo1, and Jaume Mora1;
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; 4PET Unit, CRC-Molecular Image Center, IAT, Barcelona, Spain; 5PET Unit, CRC-Molecular Image Center, IAT, Barcelona, Spain

INTRODUCTION: After a pilot study suggesting that irinotecan/cis- platin (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 25, Ep = 100/25, 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-14. Glioblastoma Multiforme with Drop Metastases

Anna Pietrowski, Agnieszka Wolska, and Patricia Coyle; University Hospital at Stony Brook, Stony Brook, NY, USA

INTRODUCTION: Glioblastoma multiforme (GBM) with drop metastasis is rare, occurs late in the course of the disease and indicates a poor prognos- is. 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 gen- eralized anxiety, headache and blury vision. Neuroimaging revealed a mass at the splenium of the corpus callousum, 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 radio- therapy. He was concomitant treated with bevacizumab and show some benef- ocy. The patient was found to have progression of disease then started bevacizumab therapy. Ten months later, the patient developed low back pain and bilateral gel weakness and neuroimaging revealed drop metastases. A PET-enhancing lesion at the T3-T4-L1, L2-3 which was not visible on CT. Cranial MRI and brain MR angiography showed extramedullary, intradural. He received approximately 3,000 cGy radiation therapy to the spine and continued bevacizumab therapy for three months until he transitioned to hospice care. DISCUSSION: This case underscores the importance of having a high suspicion for leptomeningeal metastasis in patients with intracranial GBM and radiculopathy. Spinal MRI with gad- olinium enhancement is the best diagnostic test. GBM is an aggressive tumor that spreads via direct extension along white matter tracts or via cerebrospinal fluid. Proximity of tumor to the third and lateral ventricles seems to be a crucial component. In a study of 600 patients with supratentorial gli- oblastoma, the incidence of symptomatic metastasis was 2%. In a series of 267 patients, investigators found that 3 of them, 1.1% developed spinal drop me- tastasis. 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-15. Differential Expression of MicroRNAs between High and Low Grade Astrocytoma

Stuart Smith1, Hazel Rogers1, Russell Kerr1, and Jude Grundy1;
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 particu- lar the low grade (LGG) and HGG. METHODS: We examined whether there are differences in microRNA expression profiles between pediatric astrocytomas of different grades and normal brain. METHODS: The cohort consisted of 116 samples, 101 from tumors with confirmed histological diagnosis as astrocytomas (WHO grades I-V: 77 HGG and 24 LGG), 2 from normal brain and 13 cell lines. 43 samples of RNA were extracted from FFPE material and 60 from fresh frozen material. The Nanostring nCounter microRNA array system was used to assess expression levels of 747 microRNAs. Biostatistical analysis was accomplished using the Genespring analysis platform (Benjamini-Hochberg multiple test correction) and microRNA target prediction using the miRanda algorithm. Validation of selected microRNAs was undertaken using realtime-PCR.

RESULTS: Analysing 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-140, miR-21 identified to have oncogenic effects in adult GBM and most 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 signifi- cantly differentially expressed between FFPE and frozen samples. Microarray results were validated on realtime-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 therapeutic target and potentially targetable by anti-sense genetic techniques.

HG-16. Gliomatosis Cerebri in a Nine Year Old Boy: Case Review and Review of the Literature

Diane Puccetti, Shahriar Salamati, Tabassum Kennedy2, Jason Fanguzaro1, Neha Patel1, Kristin Bradley3, Kristin Casey1, and Bermans Iskandar1;
1University of Wisconsin, American Family Children's Hospital, Madison, WI, USA; 2University of Wisconsin; 3T-DT level Care and Ethics, Madison, WI, USA; 4Children's Memorial Hospital, Chicago, IL, USA

Gliomatosis Cerebri is a rare tumor defined as a diffuse glial tumor infiltr- ating more than two brain, involving eloquent areas, and may extend to infratentorial structures. This entity occurs much less often in chil- dren 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 36 months after his diagnosis. His treatment consisted of a combination of radiotherapy 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 Osugi1, Kao Yamasaki1, Hiroki Fujisaki1, Hiroko Fukushima2, Takeshi Inoue2, Yasuhiro Matsusaka3, Hiroaki Sakamoto3, and Junichi Harada4; 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 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 leukoencephalopathy 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 Simonetumab 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 Vlueschouwer, Hilko Ardon, Frank Van Calenbergh, Raf Sciot, Guido Wilms, Johan Van Loon, Jan Goffin, and Stefaan Van Gool; University Hospital Leuven, Leuven, Belgium

Adult patients with relapsed high grade glioma are a very heterogenous 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%, 11.5% 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 selecting patients for patients participating in 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

Dianne Puccetti1, Shahrar Salama2, David Rusinak3, Neha Patel3, Kristin Bradley2, Kristin Casey1, Peter Knight1, Kenan Onel1, David Wargowsky1, Amy Stettner2, and Bermans Iskandar2; 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, leukemias 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

Azyat Al-Ghazawi1, Wiyada Punjaruk1, Beth Coyle1, and Ian Kerr2; 1University of Nottingham-School of Biomedical Sciences, Nottingham, UK; 2University of Nottingham-School of Clinical Sciences, Nottingham, UK

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-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-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 < 0.001). C6-etoposide 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.
HG-21. DELTA-24-RGD IN COMBINATION WITH SALINOMYCIN FOR THE TREATMENT OF HIGH-GRADE PEDIATRIC GLIOMAS AND DIPGs

Evelyn Xieppl,1 Claudia Rodriguez,1 Maria Gonzalez-Huarte,1 Maria Teresa Tuion,1 Idoya Zapet,1 Sonia Tejada-Solis,1 Ricardo Diaz Valle,1 Juan Fueyo,1 Candelaria Gomez-Manzano,1 and Marta M. Alonso1

1University Hospital of Navarra, Pamplona, Navarra, Spain; 2Complejo Hospitalario de Navarra, Pamplona, Navarra, Spain; 3UT MD Anderson Cancer Center, Houston, TX, USA

Brain tumor stem cells (BTSCs) are a preferred therapeutic target since these cells have been proposed to be a possible source of cancer resilience 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 (HGGs) 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 HGGs 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 antiangioma effect. Interestingly, salinomycin alone or in combination with the virus resulted in 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 decreased in the expression levels of genes involved in angiogenesis, invasion and tumorigenesis. 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 HGGs and DIPGs.

HG-22. EVALUATING DIFFERENTIAL CNS TISSUE PENETRATION OF ANTITUMOR AGENTS IN A NOVEL ANIMAL MODEL

Devang Pastakia1, Cynthia McCully1, Robert Murphy1, John Bacher1, Marvin Thomas1, Emilie Steffen-Smith1, Kadharbatcha Saleem2, Stuart Waldbridge3, Brigitte Widemann1, and Katherine Warren1; 1National Cancer Institute, Bethesda, MD, USA; 2National Institute of Mental Health, Bethesda, MD, USA; 3National Institute of Neurological Disease and Stroke, Bethesda, MD, USA

BACKGROUND: The overall outcome for children with diffuse intrinsic pontine gliomas remains poor. We hypothesize that the lack of efficacy of any chemotherapy agents for this disease is related to differences in the blood-brain barrier (BBB) throughout the central nervous system, with certain areas crucial to basic functioning (such as the pons) having a more restrictive BBB. We developed a novel animal model in rhesus macaques (Macaca mulatta) using microdialysis (MD), a continuous sampling technique based on diffusion, to simultaneously measure drug penetration into the cerebral cortex, pons, and CSF. METHODS: Custom made MD probes (CMA, Solna, Sweden) were stereotactically placed into the frontal cortex and pons of 4 adult male rhesus macaques based on coordinates determined by pre-surgical MRI. After completion of in vivo retrodialysis, an intravenous infusion of temozolomide was administered. Single continuous MD samples from the pons and cortex were collected over 4 hours with concurrent serial plasma and CSF samples. Post-surgical MRI was obtained for 2 animals and post-mortem pathology was performed on all 4 animals to confirm probe placement. Temozolomide concentrations for all samples were quantified using HPLC/tandem mass spectroscopy. RESULTS: Microdialysis probe placement was tolerated in all four animals with confirmation of proper placement into the pons and cortex in 3 of 4 animals. Measurable drug quantities were present in both the pons and cerebral cortex for samples analyzed to date. CONCLUSIONS: We have developed a new animal model using MD to assess differences in drug penetration within the CNS. We were able to confirm in 3 of 4 animals proper placement of both MD probes using either MRI or gross pathology. Temozolomide drug concentrations can be quantitated with the pons and cortex using microdialysis; further pharmacokinetic assessment, assay development and survival studies will be done to validate this animal model.

HG-23. DIFFERENTIAL PROFILES OF MicroRNAs IN PEDIATRIC AND ADULT HIGH GRADE GLIOMAS

Evelina Międle,1 Francesca Buttarelli,1 Antonella Arcella1, Federica Bagli2, Aigli Papatsiera3,4, Ted Baldi5,6,9, G. Carusso2,7, M. Antonelli1,8, V. Donofrio4, I. Mora2,8, Paolo Nozza4, Alberto Galullo1, Felice Giaconsaper,1 and Elisabetta Ferretti4; 1Departments of Experimental Medicine Sapienza University, Rome, Italy; 2Department of Radiological, Oncological and Pathological Science Sapienza University, Rome, Italy; 3Neurological Institute, Pozzilli, Italy; 4Ospedale Paussilp, Naples, Italy; 5Ospedale Regina Margherita, Turin, Italy; 6Ospedale Gaslini, Genoa, Italy

MicroRNAs (miRNAs) have been identified as key molecules in gene expression regulation both in development and in cancer. Aim of the study was to provide new insight in the molecular regulatory circuits involved in Pediatric and Adult HGG. To this end high-throughput miRNA profiles have been carried out in two series of HGG samples. Total RNA was isolated from 13 Pediatric HGG (9 fresh frozen + 4 FFPE samples) and 23 Adult HGG (6 fresh frozen + 17 FFPE samples). miRNA profiling was performed using quantitative RT-PCR utilizing Taqman low density array. Statistical analysis was carried out using StatMiner (Integromics) software. The significantly modulated microRNAs were defined as a P value ≤ 0.05. Moreover the samples have been analyzed for their gene expression features focalizing particularly on pathways with a known role in the regulation of tumorigenesis (e.g. Sonic Hedgehog, Notch). Comparison of miRNA expression patterns with those obtained after temozolomide (TMZ) treatment as found in the literature. Median PFS and OS were 4.6 and 20.5, 3.4 and 18.8, 7.8 and 13.3 months in recurrent grade III astrocytoma, oligodendroglioma, and oligoastrocytoma, respectively. Compared with TMZ, no grade III/IV toxicity was reported and median OS tended to be higher although there was no difference in median PFS. The perceived benefit of immunotherapy was more pronounced in astrocytic tumors. We provide the first description of immunotherapy in recurrent grade III glioma as safe, promising, and feasible.

HG-24. RESECTION AND IMMUNOTHERAPY FOR RECURRENT GRADE III GliOMA

Iris Elens, Steven De Vleeschouwer, Femke Pauwels, and Stefana Van Goed; University hospital Leuven, Leuven, Belgium

Despite surgery, radiotherapy, and chemotherapy, the prognosis of relapsed grade III gliomas remains poor. After promising results of immunotherapy in grade IV gliomas, we investigated its safety and efficacy in recurrent grade III gliomas. Thirty-nine patients received vaccines containing dendritic cells loaded with autologous tumor lysate after tumor resection. Progression-free survival (PFS) and overall survival (OS) were compared with those obtained after temozolomide (TMZ) treatment as found in the literature. Median PFS and OS were 4.6 and 20.5, 3.4 and 18.8, 7.8 and 13.3 months in recurrent grade III astrocytoma, oligodendroglioma, and oligoastrocytoma, respectively. Compared with TMZ, no grade III/IV toxicity was reported and median OS tended to be higher although there was no difference in median PFS. The perceived benefit of immunotherapy was more pronounced in astrocytic tumors. We provide the first description of immunotherapy in recurrent grade III glioma as safe, promising, and feasible.

HG-25. LOCAL BUT NOT SYSTEMIC TEMOZOLOMIDE COMBINED WITH IMMUNOTHERAPY IMPROVES THE SURVIVAL IN AN EXPERIMENTAL GLIOMA MODEL

Sara Fritze1, Sofia Eberstal, Emma Sandén, Edward Visse, Anna Darabi, and Peter Siesjö; Lund University, Lund, Sweden

BACKGROUND: Glioblastoma multiforme (GBM), the most common primary malignant brain tumor has despite standard treatment with temozolomide (TMZ) still a poor prognosis. An up-and-coming treatment approach is immunotherapy combined with conventional treatment. We have previously cultured glioma-bearing mice (GL261/ C57Bl6) by peripheral immunization using GM-CSF-producing tumor cells (GL-GM). However the effect of combining immunotherapy with TMZ has not been investigated sufficiently. Chemotherapy may have a dual effect on immunity. Despite the fact that chemotherapeutic agents induce lymphopenia, some chemotherapeutic agents have been shown to act immunostimulatory by targeting specific immunosuppressive subpopulations (T regulatory cells and MDSCs) or by inducing immunogenic cell death of tumor cells, thus increasing the amount of tumor antigens available for T cell antigen-processing. AIMS AND METHODOLOGY: The study was undertaken to determine whether TMZ administered systemically (i.p. 50 mg/kg/day for 3 days) or locally (i.c. using an 72h-osmotic pump filled with 2.5 mg/ml TMZ / brain infusion kit) would increase survival of GL261 tumor-bearing mice either as monotherapy or in combination with GL-GM immunization. Survival was monitored and blood samples were collected and analyzed for leukocytes using flow cytometry. RESULTS: Despite that TMZ was toxic to the GL261 cell line in vitro, systemic TMZ had only
HG-26. MANAGEMENT OF PROGRESSIVE GLIOMATOSIS CEREBRI (GC) IN A CHILD WITH COMBINATION ORAL CHEMOTHERAPY
Patrick McDonald1, Jens Wroegemann2, Sherry Krawitz3, Marc Del Bigio4, and David Eisenstat5
1University of Alberta, Edmonton, AB, Canada; 2University of Manitoba, Winnipeg, MB, Canada; 3University of Alberta, Edmonton, AB, Canada

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. In adults, whole brain radiation therapy is considered. 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 neuropathologists 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/day 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 3 weeks off therapy. Three remain on treatment with remains consistent with acute ITP. CRA was continued for another 6 months. 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
Johannes Wolff1, Robert Kwiciecin2, Torsten Pietsch3, Andreas Faldum4, Rolf-D. Kortmann5, Monica Warmuth-Metz6, Stefan Rütkowski7, Irene Slavé8, and Christof and Maria Kramm9; 1Tufts Medical Center, Boston, MA, USA; 2Institute of Biostatistics and Clinical Research, UK Muenster, Münster, Germany; 3Institute for Neuropathology, U. Bonn, Bonn, Germany; 4Radiation Oncology, University Hospital Leipzig, Leipzig, Germany; 5Diagnostic Imaging, University Hospital, Würzburg, Germany; 6UK, Hamburg, Germany; 7University Hospital Wiesbaden, Wiesbaden, Germany; 8University Hospital, Halle, Germany

We tested the beneficial effect of High Dose Methotrexate (MTX) in a phase III study. The patients were to receive two cycles of MTX 5g/m² over 24 hours prior to standard treatment with induction treatment of radiation and simultaneous Cisplatin Etoposide Vincristine and Fosfamide and maintenance with Lomustine predmison and vincristine. The control group was treated with MTX. This report describes the data at the time 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, 8 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 presenta- tion. Grade IV, III, and enrollment based on radiology findings of DIPG were 50, 35, 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 MTX group and 57 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, IRINOTECAN AND BEVACIZUMAB AT SEATTLE CHILDREN’S HOSPITAL
Urmila Uparkar, Russell Geyer, Ralph Ermoian, Richard Ellenbogen, and Sarah Leary; Seattle Childrens Hospital, Seattle, WA, USA

Children with high-grade glioma (HGG) and diffuse intrinsic pontine glioma (DIPG) have historically had a dismal outcome. Anti-angiogenic 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 (1 patient), recurrent glioblastoma (1 patient) 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 diagnosis of cerebritis. Kaplan-Meier estration was 90% and 66% with extension to the right parietal and occipital lobes another biopsy was performed. Local and external neuropathologists 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/day 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 3 weeks off therapy. Three remain on treatment with remains consistent with acute ITP. CRA was continued for another 6 months. 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-29. ALDEHYDE DEHYDRGENASE INHIBITION WITH DISULFIRAM SUPPRESSES Glioblastoma Multiforme CELL GROWTH AND AUGMENTS TEMOZOLOMIDE CYTOTOXICITY IN VITRO
Jennifer Truong1, Kasi Hu1, Abbas Fotovati1, Stephen Yip2, Richard Kast3, Brian Toyotara1, and Sandra Dhan1; 1Department of Pediatrics, University of British Columbia, Vancouver, BC, Canada; 2Department of Pathology & Laboratory Medicine, Centre for Translational and Applied Genomics, BC Cancer Agency, Vancouver, BC, Canada; 3Medical Oncology Division, University of Vermont, Burlington, VT, USA; 4Division of Neurosurgery, BC Cancer Agency, Vancouver, BC, Canada

Evidence suggests a drug resistant brain tumor initiating cell (BTC) subpopulation 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). GBM cell lines SF188 and U251 were tested with DSF or in combination with currently used therapy, Temozolomide (TMZ). Normal human astrocytes were tested with DSF as a non-cancerous or in combination with currently used therapy, Temozolomide (TMZ). Normal human astrocytes were tested with DSF as a non-cancerous DSF was effective against U251 (adult GBM), aBT001 (adult GBM) and high grade glioma (HGG) and diffuse intrinsic pontine glioma (DIPG) and confirmed this effect with a second ALDH inhibitor, DEAB. The drug DSF was effective against U251 (adult GBM), aBT001 (adult GBM) and
Dazo (pediatric medulloblastoma) cell lines, but demonstrated no harmful effects on normal human astrocytes. In addition, the efficacy of TMZ on tumor growth inhibition was enhanced when ALDH1a3 isoform was knocked down in GBM cells. In conclusion, the inhibition of ALDH activity using DSF is a safe and effective approach to eliminate GBM cells, improve efficacy of current treatments, and prevent BTC driven recurrence.

HG-30. TARGETING TUMOR HETEROGENEITY IN CHILDHOOD HIGH GRADE GLIOMA (HGG): BISPICIFIC T-CELLS EXHIBIT ENHANCED EFECTOR FUNCTIONS AND OFFSET ANTIGEN LOSS ESCAPE VARIANTS

Meenakshi Hegde, Amanda Corder, Kevin Chow, Malini Mukherjee, Aidin Ashford, Vita Brawley, Helen Heslop, 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-13Rα2 specific CARs. METHODS: We used characterizations for tumor and cell lines from HGG patients to develop a bi-specific effector T-cells expressing HER2.CAR and IL13Rα2.CAR were generated by sequential retroviral transduction. Their functionality was tested against HER2/IL13Rα2 expressing tumors as well as HGG cells. Uni-specific HER2.CAR and IL13Rα2.CAR T-cells, their pooled product, and GBM401 cells T-cells served as controls. Orthotopic mouse 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 IL13Rα2, 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 in vivo. Blunting of HER2+ve/IL13Rα2+ve tumor cells and vice-versa. Interestingly, bi-specific T-cells and pool of HER2.CAR and IL13Rα2.CAR T-cells induced simultaneous depletion of HER2+ve/*IL13Rα2 +ve 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 bi-specific T-cells exhibit significantly enhanced effector functions.

HG-31. SELECTIVE AND TREATING TREATMENT OF MALIGNANT BRAIN TUMOR WITH NON-MYELOABLATIVE HIGH-DOSE CHEMOTHERAPY

Tai-Tong Wong,1 Fei-Yi Yang,2 Maggie Liu,1 Hsiang-Fa Liang,3 Meenakshi Hegde,4,5 and Nabil Ahmed2; 1Hospital for Sick Children, UofT, Toronto; 2Toronto Western Hospital, UofT, Toronto, ON, Canada; 3Institute of Cancer Research, London, UK; 4Division of Pediatric Neurosurgery, Neurological Institute, Taipei Veterans General Hospital, National Yang Ming University School of Medicine, Taipei, Taiwan; 5Division of Hematology and Oncology, Department of Medicine, University of Toronto, Toronto, ON, Canada

Cancer cells acquire resistance to several modalities of treatment including radiotherapy. This resistance is often attributed to abberant 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 chemotheraphy and radiotherapy resistance. Here we demonstrated that the DNA repair protein alkylpurine-DNA-N-glycosylase (APN) involved in short patch base excision repair (BER) contributes to resistance to temozolomide (TMZ) an oral alkylating agent in PHGG. Silencing APN expression in TMZ-resistant PHGG cells enhanced 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 APN as a novel ATM substrate that directly phosphorylates APN, thus linking the ATM DNA damage response pathway with short patch BER. Loss of APN-APN reduced its ability to protect cancer cells against temozolomide and other alkylating agents. Clinically, binary expression of activated ATM and high APN 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 thus this effect was synergistic increased ATM inhibition. Collectively, our study demonstrates a novel ATM-APN TMZ resistance axis in glioma and that selective targeting of BER and ATM signaling may be of therapeutic relevance.

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

Sameer Agnihotri1, Christian Ternaman1, Chris Jones1, Gelareh Zadeh2, James Rukia2, and Cynthia Hawkins1; 1Hospital for Sick Children, UofT, Toronto, Ontario, Canada; 2Toronto Western Hospital, UofT, Toronto, ON, Canada

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 chemotheraphy and radiotherapy resistance. Here we demonstrated that the DNA repair protein alkylpurine-DNA-N-glycosylase (APN) involved in short patch base excision repair (BER) contributes to resistance to temozolomide (TMZ) an oral alkylating agent in PHGG. Silencing APN expression in TMZ-resistant PHGG cells enhanced 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 APN as a novel ATM substrate that directly phosphorylates APN, thus linking the ATM DNA damage response pathway with short patch BER. Loss of APN-APN reduced its ability to protect cancer cells against temozolomide and other alkylating agents. Clinically, binary expression of activated ATM and high APN 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 thus this effect was synergistic increased ATM inhibition. Collectively, our study demonstrates a novel ATM-APN 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 Filipček1, Monika Drogosiewicz2, Marta Perek-Poźniak1, Ewa Świeszewska1, Bozenka Dembowska-Bąginska1, Elżbieta Jurkiewicz2, and Danuta Perek1; 1The Children’s Memorial Health Institute, Department of Oncology, Warsaw, Poland; 2The Children’s Memorial Health Institute, Department of Radiology, 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 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 oligodendroglioma, 1 anaplastic oligodendroglioma and 13- glioblastoma. 7 pts received this regimen as a salvage treatment in patients with disease relapse/progression then as pre-irradiation chemotherapy in patients with measurable residual tumors. 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 oligodendroglioma, 1 anaplastic oligodendroglioma and 13- glioblastoma. 7 pts received this regimen as a salvage treatment in patients with disease relapse/progression then as pre-irradiation chemotherapy in patients with measurable residual tumors.
HG-34. CLINICAL AND EPIDEMIOLOGICAL CHARACTERISTICS OF MALIGNANT TRANSFORMATION OF PAEDIATRIC LOW GRADE GLIOMA INTO HIGH GRADE GLIOMA: THE HIT EXPERIENCE

Alexander Krumm1, Fabian Falkenstein1, Johannes Wolff2, Robert Kweiecin3, Torsten Petri1, Astrid Gnekow2, and Christof Kramm3
1University Childrens Hospital, Halle, Saxony-Anhalt, Germany; 2Childrens Hospital, Augsburg, Bavaria, Germany; 3The Floating Hospital for Children, Boston, MA, USA; 4IBKF, Muenster, Northrhine-Westfalia, Germany; 5Neuropathology, Bonn, Northrhine-Westfalia, Germany

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 Society of 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 diagno- sis 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/fibril-lary astrocytoma WHO II (n = 11), pilocytic astrocytoma WHO I (n = 8), ganglioglioma WHO I (n = 2), and pleomorphic xanthoastrocytoma (n = 1). In 13/22 (60%) of anaplastic astrocytoma, 8/22 (36%) of ganglioglioma and 3/22 (14%) of juvenil astrocytoma WHO IV the histological diagnosis of the LGG was confirmed. Malignant transformation was observed in 1 pt and 1 had PD. 1 pt who showed PR on irinotecan carboplatin regimen.

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

Zhifeng Dong1, Peter Siegel1, Andreas Von Diemling2, Damien Faury3, Uri Tabori4, Peter Lichter2, Christoph Plass1, Jacek Majewski1, Stefan M. Pfister1, and Nada Jabado2
1McGill University, Montreal, QC, Canada; 2German Cancer Research Center, DKFZ, Heidelberg, Germany; 3Montreal Children’s Hospital, McGill University Health Center, Montreal, QC, Canada; 4Hospital for Sick Children Research Institute, University of Toronto, Toronto, ON, Canada

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 critical positions within the histone tail (K27M, G34R/V) involved in key regulatory post-translational modifications. Mutations in ATRX (a-thalassaemia/mental retardation syndrome X-linked) and DAXX (death-domain associated protein), encoding two sub-units of the chromatin remodel complex required for H3.3 incorporation at pericentric heterochromatin and telomeres, 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

Rishi Lulla1, Maria Cheever2, Tord Alden3, Arthur DiPatri1, Shivakumar Tompala4, Stewart G. Farley2, and Jaswon Fangasaro1
1Children’s Memorial Hospital and Northwestern University Feinberg School of Medicine, Chicago, IL, USA; 2San Jorge Children’s Hospital, Santurce, Puerto Rico

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 curatively described in the literature. METHODS: 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) thalamic tumors. Histologic diagnosis of high grade glioma (HGG) 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 (35%), hemiparesis (41%) and visual disturbances (33%). Patients with unilateral tumors were more likely to present with hydrocephalus and require shunt placement as compared to unilateral...
worse outcome. To investigate the frequency and prognostic impact of genetic alterations in a homogenously treated patient cohort, we analysed 49 consecutive cases enrolled into the multicenter HIT-HGG trials; in 49 genetic alterations in a homogenously treated patient cohort, we analysed worse outcome. To investigate the frequency and prognostic impact of genetic alterations in a homogenously treated patient cohort, we analysed

HG-38. A PHASE II STUDY OF ERLOTINIB DURING AND AFTER RADIOThERAPY IN NEWLY DIAGNOSED PEDIATRIC HIGH-GRADE GLIOMAS

Ibrahim Qaddoumi, Yong Liu, Thomas E. Merchant, Mehmet Kokak, Aminar Pan Panandikker, Gregory F. Armstrong, Cynthia Wernore, Amar Gajjar, and Alberto Broniscer; St. Jude Children’s Research Hospital, Memphis, TN, USA

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-day TOY for (from diagnosis to initial post RT) treatment was 61.1% (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 (SE 8.0). 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 did not improve PFS in children with HGG.

HG-39. GENETIC ALTERATIONS AS POSSIBLE MARKERS FOR RISK STRATIFICATION OF PEDIATRIC HIGH GRADE GLIOMA PATIENTS: IDENTIFICATION OF ACCUMULATION AND PDGFRa GAIN/AMPLIFICATION AS ADVERSE PROGNOSTIC FACTORS IN THE GERMAN HIT-HGG TRIAL

Geirr H. Gielen1, Anja zur Muehlen1, Christoph Kramm2, and Torsten Pietsch1; 1Department of Neuropathology, University of Bonn, Germany; 2Department of Pediatric Armstrong, Cynthia Wernore, Amar Gajjar, and Alberto Broniscer; St. Jude Children’s Research Hospital, Memphis, TN, USA

HG-40. A FUNCTIONAL GENETIC APPROACH IN PATIENT-DERIVED GLIOBLASTOMA STEM CELLS REVEALS PRE-mRNA SPlicing COMPONENTS TO BE CANCER-LETHAL GENE TARGETS

Christopher Hubert, Yu Ding, Chad Toledo, Patrick Paddison, and James Olson; Fred Hutchinson Cancer Research Center, Seattle, WA, USA

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 ≥ 3 yet primary tumors. Importantly, unlike serum-cultured NSCs, 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 govern the 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 (AML) from these patients. 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 requirement for splicing of multiple transcripts using RNAi and 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 GLIOBLASTOMA REGULATE A SELF-RENEWAL GENE Signature

Meera Nandhabalan1, Lynn Bjerke1, Dorine Bax1, Diana Carvalho1, Illiriana Bajrami1, Alan Ashworth1, Christopher Lord1, Darren Hargrave2, Rui Reis3, Paul Workman1, and Chris Jones1; 1Institute of Cancer Research, Sutton, UK; 2Great Ormond Street Hospital, London, UK; 3Barretos Cancer Hospital, Barretos, Brazil

Mutations in genes encoding histone H3 proteins have recently been reported to underlie approximately 30% of paediatric glioblastomas (pGBM) and up to 80% diffuse intrinsic pontine glioma (DIPG), though they 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/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 (KNS42) 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 (Y379K). H3F3A G34V mutation associated 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 HOXA9/ HOXJ10 expression in pGBM is thought to be linked to the H3K36 methyltransferase activity of the nuclear receptor-binding SET domain protein 1 (NSD1) in UPN98:NSD1 fusion positive cases, whilst KNS42 itself harbours a homozygous R1618H mutation in the H3K36 methyltransferase SET domain-containing 2 (SETD2A) 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

Suzie Little1, Sergey Popov1, Alexia Jury1, Anna Burford2, Lawrence Doey2, Safa Al-Sarraj2, Juliane Jurgensmeier3, and Chris Jones1; 1Institute of Cancer Research, Sutton, UK; 2Kings College Hospital, London, UK; 3AstraZeneca, Alderly Park, UK

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 the tumour. DNA copy number profiling has to date 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 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 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 differential 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, or none of the above genes, could be found in 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 KDR, 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

Diana Carvalho1, Lynn Bjerke1, Donnie Box2, Linz Chen3, Iwanka Kozarewa1, Suzanne Baker1, Richard Grundy1, Alan Ashworth4, Christopher Lord1, Darren Hargrave4, Rui Reis2, and Chris Jones1; 1Institute of Cancer Research, Sutton, UK; 2St Jude Childrens Research Hospital, Memphis, TN, USA; 3University of Nottingham, Nottingham, UK; 4Great Ormond Street Hospital, London, UK; 5Barretos Cancer Hospital, Barretos, Brazil

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 this fusion in a single case of glioblastoma (age 1.2 years). Next, we applied the iCNA algorithm to identify copy number aberrations with intra-tumour cell death in this population. These data provide the first clues as to the biological consequences of H3F3A G34V mutations in pHGG, as well as identifying novel targets for drug development in mutant positive patients.

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

Lynn Bjerke1, Lara Perryman2, Dorine Box2, Alexia Jury1, Sergey Popov2, Gary Box3, Florence Raynaud2, Darren Hargrave4, 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 not infrequent and may contribute to drug resistance. IGF1R is a key regulator of the HGF/MET pathway 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 fluorescent and single agents are consistent with a mild anti-proliferative response at clinically relevant concentrations (≤1mM) and incomplete suppression of downstream signalling as measured by p-PI3K and AKT phosphorylation. Exploring these pathways further we were able to identify upstream inhibitors of the PI3K and AKT pathway that induce a potent anti-proliferative effect in pHGG. These data highlight the presence of hitherto unrecognised 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-45. MOLECULAR DETERMINANTS OF EFFICACY OF MET RECEPTOR INHIBITION IN PAEDIATRIC GLIOBLASTOMA

Marta Viana-Pereira1, Marco Pereira1, Anna Burford2, Alexia Jury4, Sergey Popov2, Lara Perryman2, Dorine Box2, Tim Forsyth2, Ruth Tatevossian1, Denise Sheer3, Jose Pimental4, Rui Reis2, and Chris Jones1; 1Institute of Cancer Research, Sutton, UK; 2Queen Mary and Westfield College, London, UK; 3Hospital S. António, Lisbon, Portugal; 4Hospital S. Maria, Porto, Portugal; 5Barretos Cancer Hospital, Barretos, Brazil

Recent molecular profiling data has suggested the hepatocyte growth factor receptor (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 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′–3′ 2′-deoxycytidine treatment identified SPINT2, encoding a putative regulator of 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 methylation in 35/62 (56%) pHGG, 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.

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

Chitra Sarkar1, Preerna Jha1, Irene Rose Pia Patrick1, Kumar Somasundaram1, Pankaj Pathak1, Mehar Chand Sharma1, Vaishali Suri1, and Ashish Suri1;
1Department of Pathology, All India Institute of Medical Sciences, New Delhi, India; 2Department of Neurosurgery, All India Institute of Medical Sciences, New Delhi, India; 3Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore, India

Paediatric glioblastomas (pGBM) 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 controls were calculated. For validation of epigenetic alterations, mRNA expression 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 in both paediatric and adult GBMs. Interestingly, two genes were hypomethylated in pediatric GBMs with respect to adult GBMs 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 therapy targets 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 GLOBLASTOMA MULTIFORME BRAIN TUMOUR CELLS IN PART THROUGH LOSS OF SOX2 AND DELAYS TUMOUR PROGRESSION IN MICE**

Cathy Lee1, Abbas Fotovati1, Joanna Triscott1, James Chen1, Chitra Venugopal1, Ash Singh1, Chris Dunham1, John Kerr1, Marie Verreau1, Stephen Yip1, Hiro Wakimoto1, Chris Jones1, Aarthi Jayanthan2, Aro Nareen2, Sheila Singh1, and Sandra Dunn1;
1University of British Columbia, Vancouver BC, Canada; 2McMaster’s University, Hamilton ON, Canada; 3BC Cancer Agency, Vancouver BC, Canada; 4Mass Gen Hospital, Boston MA, USA; 5Royal Marsden Hospital, Surrey, UK; 6University of Calgary, Calgary AB, Canada

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 chemotherapy regimens 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 BI2536 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 SF188 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, BI2336 suppressed cell growth, down-regulated SOX2 and induced cell death. Furthermore, BI2336 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-47. GENE EXPRESSION PROFILING OF PEDIATRIC HIGH GRADE ASTROCYTOMAS REVEALS MTOR PATHWAY DYSREGULATION**

Nisha Jorge1, Takrama Haque1, Andre Nantel2, Damien Faury1, and Nadia Jabado1; 1Departments of Pediatrics and Human Genetics, Montreal Children’s Hospital, McGill University Health Center, Montreal, QC, Canada; 2Biotechnology Research Institute, Montreal, QC, Canada

Brain tumours are the leading cause of cancer-related mortality in children. Pediatric high grade astrocytomas (pHGA), including grade IV (glioblastoma, pGBM) and grade III (anaplastic astrocytomas, pAA), are rare but devastating brain tumours 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 highly expressed cell molecular characteristics. 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 = 4.6x10^-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 therapy targets 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-49. SIX YEARS COMPLETE REMISSION AFTER A RELAPSE OF GLOBLASTOMA IN A CHILD, TREATED WITH ADJUVANT DENDRITIC CELL-BASED TUMOUR VACCINATION**

Geraldine Giraud1, Stefan Holm1, Bengt Gustavsson2, and Stellan Van Gool3; 1Department of Woman and Child Health, Pediatric Oncology Unit, Karolinska Institutet, Stockholm, Sweden; 2Department of Neurosurgery, Karolinska Institutet, Stockholm, Sweden; 3Laboratory of pediatric immunology, Catholic University of Leuven, Leuven, Belgium

A 12 years old girl presented 2005 with epilepsy and MRI showed a right-sided temporal tumour. She was operated and microscopy showed a high grade astrocytoma grade III-IV (WHO). Treatment followed with chemotherapy (ICG and IFN protocol), 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-Immuno-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. Glioblastoma have relapsing glioblastoma and this is an excellent opportunity. 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

Roman Kizyma1, Zoryana Kizyma1, Lesya Dvornyak1, and Bogdan Kotsay2; 1Ukrainian Specialized Children Medical Centre, Lviv, Ukraine; 2Lviv National Medical University, Lviv, Ukraine

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.

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 confirmed 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 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 (53-60Gy) 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.