HIGH GRADE GLIOMAS AND DIPG

HG-001. CULTURE CONDITION-DEPENDENT GROWTH OF EARLY PASSAGE PATIENT-DERIVED HUMAN GLOBLASTOMA CELL LINES

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In contrast to most other malignant diseases, especially in children, up to now glioblastoma multiforma (GBM) is a lethal diagnosis for most of the patients. Operation and radiotherapy are very effective to reduce the tumor burden, however, a strong adjuvant treatment is lacking. To target glioblastoma cells more effectively, it is crucial to understand the cellular signaling and regulatory pathways, particularly in the EGFR and VEGF-dependent pathways. Since it has been shown that glioblastomas are extremely heterogeneous regarding genetic, epigenetic or signaling regulation, receptor expression etc., analysis of individual patient derived tumor cells is particularly important. We established a collection of well-characterized heterogeneous early-passage brain tumor cell lines. August 2009, more than 26 clinical samples from patients with WHO grade IV GBM and Anaplastic Astrocytoma, WHO grade III, were collected. Cell lines were established that were in depth analysed both for genetic and epigenetic regulation, receptor expression, and sensitivity to cytostatic or targeted drugs. We found that cells in monolayer and spheroid cultures, and cells grown using standard and stem cell selective culture medium, respectively, or, further, tumors grown in xenograft models behave differently with regard to the receptor dependent signaling pathways. Establishment of such models is crucial to design targeted therapy approaches that allow direct transfer from the laboratory system to the clinical application.

HG-002. SURGICAL MANAGEMENT FOR PEDIATRIC INTRA-AXIAL BRAIN STEM TUMORS, SINGLE CENTER EXPERIENCE

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INTRODUCTION: Intra-axial brain stem tumors belong to the most challenging neurosurgical problems. For decades the brain stem remained a no-man’s land, but this concept changed recently due to advances in the diagnostic and surgical tools. MATERIAL AND METHODS: We review the surgical outcome of 21 pediatric cases surgically treated for brain stem glioma at the Neurosurgery Department, Alexandria University between 2008 and 2013. They underwent 29 surgical procedures and include 10 boys and 11 girls. Age ranged from 2-18 years (mean 9 years), and follow up period ranged from 6 to 63 months. RESULTS: Among 29 surgical procedures, 9 procedures were carried out at the midbrain, 13 at the pons, and 5 at the medulla oblongata. The remaining 2 surgical interventions were for CSF diversion (V-P shunt and 3rd ventriculostomy). Patients with high grade glioma or DIPG were treated by radiotherapy 54 Gy/30 fractions, while those with low grade glioma were treated by carboplatin and vincristine for 18 months. 8 patients were operated twice; 4 for tumor regrowth, 2 as a planned second stage surgery, and 2 for CSF diversion. Intra-operative neurophysiological monitoring was available for 19 procedures, and all cases were operated without neuronavigation or intra-operative MRI. Total resection was achieved in 8 out of 27 procedures (29.6%), the most common pathology was pilocytic astrocytoma (14 cases). There was no post-operative mortality in this series, worsening of the pre-operative neurological status occurred in 12 patients but was permanent in only 2 cases. Six cases died during the follow up period, 3 cases had anaplastic astrocytoma (WHO G 3) and 3 other children suffered from diffuse pontine glioma. CONCLUSION: Microsurgery is the mainstay in the management of focal pediatric brain tumors. Radiotherapy and/or chemotherapy are used in selected cases of focal tumors, however they represent the main treatment in diffuse glioma.

HG-003. IMMUNOTHERAPY FOR RELAPSED MALIGNANT GLIOMA IN CHILDREN

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The prognosis of children with relapsed malignant glioma remains poor. The results of new drugs, if available, on tumor control are disappointing. Active specific immunotherapy is a new promising treatment modality under development in several centres. We report an update of our experience. Children were treated with neurosurgery and vaccination with monocycle-derived mature dendritic cells loaded with tumor lysate. They had leukapheresis before weaning corticosteroids. Leukapheresis was performed via insert of a double-lumen inguinal venous access. Safety, feasibility and efficacy were endpoints of the HGG IMMUNO-2003 cohort study comparison. Forty five children (24 females) aged 2-17 years were included. Twelve patients had relapsed anaplastic astrocytoma, 2 anaplastic ependymoma, 2 Oligoastrocytoma grade III, 27 GBM, and 2 unspecified malignant glial tumors. They were treated with a median of 6 vaccines (4-17). The treatment was feasible. There were no treatment-related toxicities. Immunotherapy was given in ambulatory setting. Median PFS and OS of the total group were 3.5 and 12.1 months with 26.9% 2-year OS. Median PFS and OS of the subgroup with relapsed GBM were 2.7 and 10.4 months with 21.2% 2-year OS. The tail of the PFS curve remained stable at 17.7% after 16 months, with longest follow-up till 143.2 months. Similarly, the tail of the OS curve remained stable at 15.9% OS from 2.5 months. Because of low numbers, we could not detect any change in PFS or OS for the different cohorts in HGG-IMMUNO-2003. We could not detect any particular predictive parameter for long-term survival. Immunotherapy after neurosurgery provides a realistic second chance for children with relapsed malignant glioma, at least the tumor can be again resected up to a subtotal extent. Collaborative efforts are urgently needed to implement this technology in a network of centres, so that randomized clinical trials can be initiated.

HG-004. NEWCASTLE DISEASE VIROTHERAPY, A NOVEL IMMUNOTHERAPEUTIC APPROACH FOR HIGH GRADE GLIOMA

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In spite of current multimodal treatment the prognosis of Glioblastoma Multiforme patients remains poor. Here, we investigate oncolytic Newcastle disease virus (NDV), as a novel approach for glioma therapy in the murine orthotopic GL261 glioma model. Oncolytic viruses have the potential to lower tumor volume and viability, as well as to alter the immunosuppressive tumor microenvironment and stimulate antitumor immunity. This immune involvement has not been investigated in orthotopic glioma and the mechanisms involved remain to be unraveled. In vitro, GL261 cells were sensitive to NDV-mediated cytotoxicity in a dose- and time-dependent manner. Cell death showed features of necroptosis. NDV infection furthermore induced immunogenic cell death (ICD) in GL261 cells. The induced ICD route proved to be more effective than the apoptosis induced by viral infection in the tumor cells, which triggers the cells to activate the immune system. In ongoing work we are investigating the synergism between virotherapy and immunotherapy and expanding ND virotherapy to other brain tumor models such as DIPG.

HG-005. TARGETING RESISTANCE PATHWAYS IN BRAF-MUTANT PEDIATRIC GLIOMAS

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Mutational activation of BRAF is a common finding in pediatric gliomas. As many as 14% of pediatric high grade glioma and up to 66% of certain low grade pediatric glioma subtypes contain the BRAFV600E mutation. Small molecule inhibitors that selectively target BRAFV600E are FDA approved.
for melanoma and have shown significant efficacy in treating BRAFV600E glioma in pre-clinical trials. Despite showing initial efficacy, drug resistance significantly limits the use of BRAFV600E inhibitors in clinical settings. Here, we identified the molecular mechanisms of BRAFV600E inhibitor resistance in glioma and also provide strategies to overcome drug resistance. We have generated multiple BRAFV600E inhibitor resistant glioma cell lines and murine models to study the mechanisms of drug resistance in glioma. The BRAFV600E resistant ANSH and D17S535G cell lines and turkeys, respectively, to BRAF inhibitor induced MAP kinase inhibition, reduced cell viability and cell cycle arrest. Also, the BRAFV600E inhibitor resistant glioma xenografts do not respond to drug induced suppression of tumor growth, resulting in poor animal survival. By phospho-receptor tyrosine kinase array and phospho-protein array, we found that certain receptor tyrosine kinases, including EGF and Ax receptors, and pro-survival pathways such as Wnt signaling, are upregulated. Our data suggests novel therapeutic strategies to BRAFV600E -mutant pediatric glioma that bypass the insult from the drug.

Nimotuzumab was well tolerated with RT and CT. Our results show that Nimotuzumab containing regimens might be promising in patients with progressive disease. However, in primary DIPG patients, 42 months; but all are DOD. Nimotuzumab containing regimens might be promising in patients with progressive disease (PD) after RT + TMZ, received Nimotuzumab + chemotherapy (CT). After 2012 May, Nimototuzumab was given as primary treatment during and after RT. Nimototuzumab was administered 150mg/m2/ week for 6 weeks during RT and then bi-weekly with CT until PD. RESULTS: As of January 2014, Nimototuzumab was used in 15 children with DIPG, 7 with PD, 8 with primary diagnosis. Among PD patients, 4 had clinical improvement/stable disease for 18, 22 +, 8 and 6 months; one is alive with disease (AWD) at 42 months from initial diagnosis, 3 died of disease (DOD) at 24, 14 and 13 months from primary diagnosis. The other 3 had stable disease for 5, 4, and 1 month but progressed afterwards and all are DOD. In 8 patients who received Nimototuzumab + CT as primary treatment, all had clinical improvement at the end of RT and maintenance Nimototuzumab as expiratory five had clinical improvement for a duration of 19, 14 and 13, +, 9 and + months, 3 are alive, 2 are DOD. The other 3 were stable for 5, 6, and 6 months; but all are DOD. Nimototuzumab + carboplatin/vinorelbine/TMZ was well tolerated with no major adverse effects. Only one patient had hypersensitivity to Carboplatinum after Nimototuzumab that started after a year of use. The median survival is 14 months for PD patients. CONCLUSION: Nimototuzumab was well tolerated with RT and CT. Our results show that nimototuzumab containing regimens might be promising in patients with progressive disease. However, in primary DIPG patients, due to limited number and short follow-up, we cannot draw a conclusion on its efficacy.
HG-010. ONCOLYTIC VIROThERAPY FOR PAEDIATRIC HIGH GRADE GLIOMA; EVALUATION OF THE EFFECTS OF ONCOLYTIC VIRUS ON CELL VIABILITY, MIGRATION AND INVASION
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Paediatric high grade gliomas (pHGGs) are devastating tumours with five year survival outcomes between 15-35%. Despite aggressive management, tumours inevitably recur due to their diffuse and invasive nature and novel therapeutic approaches are needed. Oncolytic virotherapy, which uses viruses to selectively infect and destroy cancer cells as well as to stimulate an anti-tumour immune response, offers a novel treatment approach. Here we evaluate the in vitro cytotoxic effects of the oncolytic viruses, herpes simplex virus (HSV), measles, reovirus and vaccinia, on a panel of paediatric glioma cell lines. We also describe for the first time, the effects of oncolytic viruses on the migratory behaviour of pHGG cells. Cell viability was examined over 96 hours. The cytotoxic effect was particularly marked for cell lines infected and destroyed cancer cells as well as to stimulate an anti-tumour immune response, offers a novel treatment approach. Here we evaluate the in vitro cytotoxic effects of the oncolytic viruses, herpes simplex virus (HSV), measles, reovirus and vaccinia, on a panel of paediatric glioma cell lines. We also describe for the first time, the effects of oncolytic viruses on the migratory behaviour of pHGG cells. Cell viability was examined over 96 hours. The cytotoxic effect was particularly marked for cell lines treated with vaccinia, while sensitivity to other viruses was more cell-line dependent. Analysis of migratory and invasion assays indicated that HSV at multiplicity of infection 10, resulted in a near total blockade of both migration at 24 hours and invasion at 72 hours, in all cell lines tested. Some reduction in migration/invasion was also seen following vaccinia virus treatment, but this was not as effective as HSV. Our results demonstrate that pHGG cell lines are sensitive to the cytolytic effect of a range of oncolytic viruses. Moreover, we have shown that oncolytic viruses, particularly HSV, can block the migration and invasion of pHGG cells. We propose that oncolytic viruses may have therapeutic benefits for pHGG, not only as a cytotoxic and immunoaugmenting therapy, but also as an anti-invasive agents, improving outcome for this devastating disease.

HG-011. CENTRALLY REVIEWED GLIOMATOSIS CEREBRI IN CHILDREN AND ADOLESCENTS: A RETROSPECTIVE ANALYSIS FROM THE HIT-HGG DATABASE
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BACKGROUND: Gliomatosis cerebri (GC) is a rare, diffusely infiltrating glioma variant with little or no mass effect, predominantly WHO grade III/IV morphology and an almost uniformly fatal outcome. The aim of this retrospective analysis was to describe the clinical characteristics and outcome of children and adolescents with centrally reviewed GC registered in the HIT-HGG database in 2005 and 2010. MATERIALS AND METHODS: A total of nineteen patients (male, n = 13; median age at diagnosis 11.8 years [range, 0.2-18.3]) were identified. The majority of patients were treated according to the prospective multicenter trial (HT-HGG-2007; EudraCT No. 2007-001128-42) on locally administered TMZ followed by 12 cycles of radiobiologically optimized TMZ chemotherapy. Analysis of migratory and invasion assays indicated that HSV at multiplicity of infection 10, resulted in a near total blockade of both migration at 24 hours and invasion at 72 hours, in all cell lines tested. Some reduction in migration/invasion was also seen following vaccinia virus treatment, but this was not as effective as HSV. Our results demonstrate that pHGG cell lines are sensitive to the cytolytic effect of a range of oncolytic viruses. Moreover, we have shown that oncolytic viruses, particularly HSV, can block the migration and invasion of pHGG cells. We propose that oncolytic viruses may have therapeutic benefits for pHGG, not only as a cytotoxic and immunoaugmenting therapy, but also as an anti-invasive agents, improving outcome for this devastating disease.
for Angiocomb patients, and they were able to visit school or daycare and walk for a significantly longer time compared to controls (Log Rank 0.036 and 0.008, respectively). There was a trend towards less consumption of corticosteroids among study patients. Adverse effects were generally minor, mostly neutropenia and infections. In conclusion, the Angiocomb protocol resulted in a noticeable share of long-term survivors and created four extra-ordinary complete radiological responses. The treatment was generally well tolerated with acceptable adverse events and allowed a good quality of life of the patients. These results warrant more studies about antiangiogenic combination therapy for DIPGs in the future.

Diffuse intrinsic pontine glioma (DIPG) is a devastating paediatric brain tumour with no effective therapy and near 100% mortality. The failure of most therapies can be attributed to the delicate location of these tumours and choosing therapies based on assumptions that DIPGs are molecularly similar to adult disease. Recent studies have unravelled the unique genetic make-up of this brain cancer with nearly 80% harbouring a K27M-H3.3 or K27M-H3.1 mutation. However, DIPGs are still thought of as one disease with limited understanding of the genetic drivers of these tumours. Here we apply methylation profiling, whole genome sequencing, expression profiling, and single-cell analysis to further characterize these molecularly distinct diseases (H3-K27M, Silent, and MYCN) and uncover a mutation in a novel driver, ACVR1 in 20% of DIPGs. Mutations in ACVR1 were constitutively activating, leading to SMAD phosphorylation and increased expression of downstream activator genes such as ID1 and ID2. The H3-K27M subgroup is highly K27M-H3 mutated and associated with additional hits including activating mutations in ACVR1, frequent RB1 and TP53 deletions, PVT-1/MYC or PDGFRa gains/amplifications, genomic instabilities, and alternative lengthening of telomeres. Associated non-identical mutations and is instead characterized by hypermethylation and chromothripsis of chromosome 2p with high-level amplifications of MYCN and ID2. The Silent subgroup affects younger children, has genomes with minimal genomic instability and fewer mutations, over-expresses WNT pathway genes, as well as genes with known cancer association such as MDM2, MSMP and ADAM33. Our results show that this seemingly homogeneous entity in fact comprises three disease distinct subgroups with different demographic and molecular features. This complexity needs to be considered when designing new therapeutic approaches in order to improve outcome for these children.

Malignant transformation of pediatric low grade glioma is a very uncommon event. Radiotherapy seems to be the principal risk factor implicated. This has been widely described in adults with WHO grade 2 glioma and pediatric cases have also been reported. To our knowledge, spontaneous malignant transformation of WHO grade I to grade IV glioma has not yet been described. We report the case of a 7 year-old child, who presented with a pilocytic astrocytoma of spinal cord from C2 to C6 treated by surgical excision and adjuvant chemotherapy. The patient experienced local relapse treated with 4 cycles of Carboplatin with no benefit. Ten years later, patient consulted for neck pain and neurological disorders. Spinal MRI revealed tumor "relapse" presenting as a lesion from C1 to T1. Surgical resection was partial and histopathological examination concluded to a WHO grade IV glioblastoma. Tumor cells were labelled by anti-Olig2 (70%) but not anti-IDH1 (R132H) antibodies. Ki67 index reached 30% of tumor cell nuclei, p53 immunoreactivity was strong, 20% of tumor cells were EGFR positive. CD34 remained negative. V600E BRAF mutation was not found and tumor presented a H3 K27M sublime mutation. Treatment was composed by a spinal radiotherapy (40 Gy) followed by a tumor bed boost of 10 Gy, brain slices and 3D whole brain cultures, thus allowing assessment of drug distribution upon release from polymer matrices. CONCLUSIONS: We have developed two PLGA-based systems to deliver multiple chemotherapy agents simultaneously. Modification of drug release kinetics was achieved through tailoring the matrix formulation. The spatio-temporal distribution of drugs delivered from PLGA/PEG matrices within ex vivo brain cultures was dependent upon drug chemistry and release mechanism. The use of the modalities to measure drug distribution allowed the development of a laboratory-based screen for the selection of drug candidates with good distribution profiles and which are thus amenable for local delivery systems.

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with concomitant temozolomide, and maintenance chemotherapy with temozolomide. Five months later, a tumor progression was treated with irinotecan and bevacizumab. Unfortunately, patient died of tumor progression 12 months after diagnosis. Malignant transformation can occur without radiotherapy in pediatric pilocytic astrocytoma, and we described the first case of spontaneous malignant transformation of a cervical spinal cord pilocytic astrocytoma into glioblastoma in a child. HG-019. IN VITRO AND IN VIVO TARGETING OF NFKB BY DEHYDROXYMETHYLEPOXYQUINOMINIC (DHMEQ) IMPAIRS GROWTH IN PEDIATRIC GLOBLASTOMA Maria Gabriela Rodriguez, Gabriela Rivero, Clare Shilav, Lange Ana, Eduardo Rego, Carlos Scriddel, Kazuo Umezawa, and Luiz Tone; 1University of Sao Paulo, Ribeirao Preto, Brazil; 2Aichi Medical University, Nagakute, Japan INTRODUCTION: Primary brain tumors are the leading cause of cancer death in children. Despite advances in neurosurgery and post-operative radiation therapy and/or multimagent chemotherapy, pediatric patients with glioblastoma (GBM) have a dismal outcome, with 5-year survival rates that have remained between 5 and 15% for the last four decades. Vast evidence has indicated that the nuclear factor NF-kB is constitutively activated in cancer, playing key roles in growth and survival. Recently, Dehydroxymethyl-epoxyquinominic (DHMEQ) has shown to be a potent NF-kB inhibitor with anti-tumor properties in adult GBM, though there still no data on pediatric counterparts. METHODS: In the present study, the effects of DHMEQ on survival and its ability to surmount tumors invas-ive nature were explored in 2 pediatric GBM cell lines (SF188 and KNS-42) by means of qPCR, proliferation, clonogenicity, apoptosis and invasion assays on matrigel coated chambers. In vivo testing 2 x 10^6 cells were implanted subcutaneously in the back of 6-week-old nude mice (BALB/c nu). After tumor establishment, the experimental group was treated intraperi-tonally with 10 mg/kg DHMEQ as twice a week DHMEQ and the control group was administered vehicle solutions. RESULTS: Treatment with DHMEQ substantially impaired cell growth in dose and time-dependent manner when compared with control. Cell clonogenicity was diminished significantly with increased DHMEQ exposure, and was inhibited accordingly with lowered expression of invasion-related genes, such as MMP-12, MMP-14, MPP-23b, TIMP-2 and uPA. Moreover, subcutaneous tumors formed by SF188 or KNS-42 cells were reduced by ~20% and ~70% in size, respectively, by intraperitoneal adminis-tration of DHMEQ. CONCLUSION: Taken together, our results strength-en the potential usefulness of DHMEQ, in future therapeutic strategies for the treatment of pediatric GBM.

HG-021. MOLECULAR INSIGHTS INTO HISTONE H3.3 MUTATIONS IN DIPE AND PEDIATRIC GLOBLASTOMA Farhana Haque, Ruman Rahman, Robert Layfield, and Richard Grundy; University of Nottingham, Nottingham, UK Diffuse intrinsic pontine glioma (DIPG) is an aggressive malignant astrocytoma in children, with poor survival rate and few treatment options.

HG-018. TEMOZOLOMIDE, BEVACIZUMAB WITH OR WITHOUT IRINOTECAN IN PEDIATRIC BRAIN TUMORS- MD ANDERSON CANCER CENTER EXPERIENCE Nisha Ramani, Wafik Zaky, Geoffrey Kannan, Ajaykumar Morani, David Sandberg, Leena Ketonen, Osama Maher, Fernando Corrales-Medina, Heather Meador, and Soumen Khatua; MD Anderson Cancer Center, Houston, TX, USA OBJECTIVE: To evaluate the outcome of brain tumors in children treated with temozolomide, bevacizumab with or without irinotecan. BACKGROUND: Even with advancing chemotherapy strategies for high grade gliomas (HGG) and diffuse intrinsic pontine gliomas (DIPG), the prognosis remains dismal. METHODS: Retrospective chart review from January 2011 to December 2013 in children who received treatment with temozolomide, bevacizumab with or without irinotecan was performed. Results: 7 patients were identified of which 2 had localized glioblastoma multiforme (GBM), 3 had features of gliomatosis cerebri (GC) and 2 patients had DIPG. They were treated with surgery and/or radiotherapy with temozolo-mide followed by maintenance chemotherapy with temozolomide, bevacizumab with or without irinotecan. RESULTS: The median progression free survival (PFS) was 41 weeks for localized GBM, 34 weeks for GC and 49 weeks for DIPG. A patient with GC and grade II pathology showing high-grade features on neuroimaging, continues to remain stable at 42 weeks. 1 patient developed a complete response. Intravenous ANP continued until the 40th month and then the patient was taking maintenance treatment with A10 and AS2-1 capsules (0.14 g/kg/d) each until the 56th month. At that time she underwent re-section of the scar tissue causing the seizures. The pathological examination did not demonstrate any presence of neoplastic process. The patient became asymptomatic and her follow-up MRIs between 6 and 15 years since the treatment start did not show tumor recurrence. She continues to live a normal life over 20 years later. This report indicates that it is possible to obtain long-term survival in PAA with a currently available investigational treatment.
HG-022. REIRRADIATION OF DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG) AT PROGRESSION AFTER FIRST LINE TREATMENT WITH NIMOTUZUMAB, VINORELBINE AND STEREOTACTIC RADIOThERAPY

Lorenza Gandola1, Emilia Pecor1, Veronica Biasson1, Elisabetta Schiavello1, Chiara Chiruzz1, Filippo Spreafico1, Piergiorgio Modena2, Ferdinand Bach3, Emanuele Pignoli1, and Maura Massimino1; 1Department of Oncology, Fondazione IRCCS Istituto Neurologico C. Besta, Milan, Italy; 2Department of Radiation Oncology, Children’s Memorial Health Institute, Warsaw, Poland; 3Department of Neurosurgery, University Hospital Heidelberg, Germany

A pilot phase 2 protocol was launched in our Institution in 2009 combining nimotuzumab with concomitant radiotherapy and vinorelbine for children with a diagnosis of DIPG confirmed by central radiological review. A protocol amendment in July 2011 introduced re-irradiation at relapse. Vinorelbine was administered weekly, with nimotuzumab in the first 12 weeks of treatment; radiotherapy was delivered from weeks 3 to 9, for a total dose of 54 Gy conventionally fractionated. Then vinorelbine and nimotuzumab were given every other week until the tumor progressed or for up to two years. Re-irradiation consisted of 19.8 Gy in 11 fractions of 1.8 Gy. The re-irradiated target volume included all tumor grossly visible on MRI, embracing previously-irradiated areas and any new areas of progressive disease. A 3D conformal technique was always adopted, and beam geometry for re-irradiation was chosen so as to avoid the entrance beam paths of the first-line treatment wherever possible. 25 children (mean age 7.4 years) were enrolled from August 2009 (median follow-up 29 months). Eleven of 12 locally-relapsing patients were re-irradiated, all as out-patients, at a median of 9 months after first radiotherapy (range 3-19 months). In 10 children symptoms improved enough to enable steroid suspension while in 7 tumor shrinkage was documented. Survival after re-irradiation ranged from 6 weeks to 14 months (median 6 months). Median OS was 16 months for re-irradiated children, and 12 months for the 5 children with local relapse but not re-irradiated (P = 0.03). One-year PFS and OS rates for the whole series were 30 ± 10% and 76 ± 9%, respectively. In our experience, re-irradiation of DIPG children progressing locally, was feasible without unexpected side-effects or worsening of neurological symptoms. This approach points to a real chance of a longer life expectancy after relapse that warrants assessment in appropriate, larger clinical trials.

HG-023. TREATMENT RESULTS OF DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG) IN CHILDREN. ONE-CENTER EXPERIENCE

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AIM: Presentation of treatment results of children with DIPG treated in one center according to Polish Pediatric Neuro-Oncology Group (PPOG) protocol. MATERIAL AND METHODS: Between 2005–2013 73 patients with DIPG diagnosed by MRI were treated. There were 47 girls and 27 boys, age range from 2yrs3mos to 17yrs11mos (mean 8yrs8mos, median 6yrs). The time from first symptoms of disease to diagnosis ranged from 5 to 24 weeks. Cranial nerve palsy (80%) and balance disorders (76%) were the most common symptoms. At diagnosis 33% of patients presented with classical brainstem syndrome.On MRI 35 tumors had no contrast enhancement, in 34 patients the tumor showed ring-like enhancement and in 38 enhancement was homogenous. All patients were treated with 3 courses of temozolomide and cisplatin followed by irradiation (54 Gy). Post-irradiation chemotherapy was also administered. It’s type depended on tumor response to pre-irradiation chemotherapy. Patients with good response and stabilization received 6 courses of temozolomide/cisplatin and in not other individualized protocols. Analysis included; tumor response to 2 courses of temozolomide/cisplatin and other chemotherapy protocols when given in case of progression. Good response was defined as CR, PR and minor response. Treatment results are presented (1,2,5 and 7 yr PFS and OS) RESULTS: 8 patients showed good response, 37 stable disease and 28 progression. 19 patients completed the protocol, 3 are still treated. 51 patients had progression and were treated with second-line chemotherapy consisting of irinotecan/carboplatin (26 pts), etoposide/dacarbazine (26 pts), doxorubicin (1pt). Packer protocol (7pts). Best responses were achieved with etoposide/dacarbazine (42.3% good responses, 30.8% stabilization, 26.9% progression, with time to consecutive progression from 0–108 weeks median-18 weeks). PFS and OS were as follows; at 1 year: 21.1% and 56.3%, at 2 years - 7% and 23.9%, at 5 years - 7% and 7.5%. Prolonged survival can be achieved with post-irradiation chemotherapy in children with DIPG.

HG-024. MULTIPLE NOVEL FUSION GENES WITHIN THE RTK-RAS-PI3K SIGNALLING AXIS HIGHLIGHT ITS CENTRAL ROLE IN THE TUMORIGENESIS OF PEDIATRIC GLIOBLASTOMA

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Oncogenic activation of receptor tyrosine kinases (RTKs) as well as deregulated downstream signalling through the RAS/RAF and PI3K pathways are hallmarks of glioblastoma (GBM) tumorigenesis, giving rise to the most frequent and aggressive malignant brain tumor in children and adults. Genomic alterations including somatic mutations, gene amplifications as well as structural aberrations are widely visible in GBMs and their role in tumourigenesis is still unknown. Here we describe a strategy for the genome wide detection of single amino acid substitutions in H3.3, is considered. For example, in control cell lines (HeLa and wild type histone pHGG/DIPG) and recombinant pHGG cases. These mutant-specific antibodies will also be utilized to identify novel downstream targets and pathways of the different mutants which, in turn may have a role in DIPG/HGG genesis and progression. The strategy used for identifying cooperating partners leads to further insights into spatial/anatomical aspects of the mutations in GBMs, which, in turn may have a role in DIPG/pHGG genesis and progression. For example, in control cell lines (HeLa and wild type histone pHGG/DIPG) and recombinant pHGG cases. These mutant-specific antibodies will also be utilized to identify novel downstream targets and pathways of the different mutants which, in turn may have a role in DIPG/HGG genesis and progression.
DIPG and 10 adjacent normal) and validated significant NG2 expression in DIPG, the impact of adjuvant temozolomide on PsP, and the impact of radiation on the outcome. METHODS: Demographic, clinical, and radiological data from patients aged 1-21 years with a radiological diagnosis of DIPG was collected and assessed for evidence of radiological and/or clinical PsP. Results: Of 53 patients identified, 12 had PsP. Conclusion: PsP on the outcome. METHODS: Demographic, clinical, and radiological data from patients aged 1-21 years with a radiological diagnosis of DIPG was collected and assessed for evidence of radiological and/or clinical PsP. Results: Of 53 patients identified, 12 had PsP. Conclusion: PsP on the outcome.
benefit in untreated KO compared to untreated WT mice with DIPG (median survival 43 days vs. 34 days, p = 0.02). We observed that dasatinib almost doubled the survival in the KO model; 44 days vs. 80 days (p = 0.0004). Cancer cells isolated recently and from all the harvesting sites showed significant decrease in their doubling time in the WT model: 42 days vs. 59 days (p < 0.0001). Pharmacokinetic analysis demonstrated that genetic ablation or pharmacological inhibition of ABC transporters significantly increased dasatinib delivery into normal brain tumor. TRD studies further corroborated a discrepancy between BBDs in both DIPG models. CONCLUSIONS: (1) ABC transporters promote brainstem gliomagenesis. (2) ABC transporters regulate dasatinib delivery into normal brain but not into the tumor as BBD is disrupted in our DIPG mouse model. (3) ABC transporter activity limits the efficacy of dasatinib in vivo. Our results suggest that ABCG2 and ABCB1 inhibitors may have a therapeutic role in the treatment of DIPG.

**HG-030. THE TYPE OF HISTONE H3 VARIANT WITH LYS27MET (K27M): CHANGE IS A PROGNOSTIC MARKER ASSOCIATED WITH TWO DIFFERENT PHENOTYPES OF DIFFUSE INTRINSIC PONTINE GLIONE GLIOMAS (DIPG)**

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BACKGROUND: Histone H3 variant K27M mutations are the most frequent alterations observed in DIPG. They impair trimethylation of the K27 repressive histone mark by interfering with the PRC2 complex together with a general hypomethylation of the DNA. The mutations are twice more frequent in the histone H3F3A gene than in the HIST1H3B gene. Since these two proteins have different functions on the chromatin organization, we studied if they were associated with different biological characteristics.

PATIENTS AND METHODS: Fifty-one DIPG were characterized clinically, radiologically and biologically (mutations, gene expression, CGH/haray) along with 90 non-brainstem high-grade glioma. Tumor material was obtained from stereotactic biopsies performed at diagnosis. RESULTS: All DIPG showed a loss of the H3K27me3 mark by immunohistochemistry. HIST1H3B K27M mutations were exclusively seen in DIPG while H3F3A K27M ones were distributed in all the midline structures of the central nervous system. Median overall survival of H3.1 mutated tumors was longer (17 vs 11 months, p = 0.006). HIST1H3B mutations were associated with lower expression of circadian genes and lower expression of angiogenic markers. CONCLUSION: Despite similar clinicopathological characteristics, H3F3A and HIST1H3B mutations are associated with different biological characteristics that may suggest different therapeutic approaches.

**HG-031. THE PITFALLS OF MSI-H DETECTION IN BRAIN CANCERS DIAGNOSED IN THE CMMR-D (CONSTITUTIONAL MISMATCH REPAIR DEFICIENCY) SYNDROME**

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BACKGROUND: New treatments are urgently needed for Diffuse Intrinsic Pontine Gliomas (DIPG). Methylation in brain cancer is an attractive therapeutic target. The presence of DNA methylation modifies gene expression and could provide targets of therapy. In glioblastomas, loss of promoter methylation has been linked to tumor progression and atherapeutic strategy for DIPG.

**HG-032. TARGETING MITOCHONDRIA AND CELL METABOLISM AS A NOVEL THERAPEUTIC APPROACH IN THE TREATMENT OF DIFFUSE INTRINSIC PONTINE GLIONE GLIOMAS (DIPG)**

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BACKGROUND: Histone H3 variant K27M mutations are the most frequent alterations observed in DIPG. They impair trimethylation of the K27 repressive histone mark by interfering with the PRC2 complex together with a general hypomethylation of the DNA. The mutations are twice more frequent in the histone H3F3A gene than in the HIST1H3B gene. Since these two proteins have different functions on the chromatin organization, we studied if they were associated with different biological characteristics.

PATIENTS AND METHODS: Fifty-one DIPG were characterized clinically, radiologically and biologically (mutations, gene expression, CGH/haray) along with 90 non-brainstem high-grade glioma. Tumor material was obtained from stereotactic biopsies performed at diagnosis. RESULTS: All DIPG showed a loss of the H3K27me3 mark by immunohistochemistry. HIST1H3B K27M mutations were exclusively seen in DIPG while H3F3A K27M ones were distributed in all the midline structures of the central nervous system. Median overall survival of H3.1 mutated tumors was longer (17 vs 11 months, p = 0.006). HIST1H3B mutations were associated with lower expression of circadian genes and lower expression of angiogenic markers. CONCLUSION: Despite similar clinicopathological characteristics, H3F3A and HIST1H3B mutations are associated with different biological characteristics that may suggest different therapeutic approaches.

**HG-033. TARGETED INHIBITION OF THE FACT (FACILITATES CHROMATIN TRANSCRIPTION) COMPLEX IS A NOVEL AND EFFECTIVE THERAPEUTIC APPROACH IN DIPG**

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BACKGROUND: Novel therapeutic strategies are urgently needed for Diffuse Intrinsic Pontine Gliomas (DIPG). Methylation in brain cancer is an attractive therapeutic target. The presence of DNA methylation modifies gene expression and could provide targets of therapy. In glioblastomas, loss of promoter methylation has been linked to tumor progression and a therapeutically exploitable feature of DIPG.

**HG-034. THE 2% OF COMPOUNDS THAT DEMONSTRATE SIGNIFICANT CYTOTOXIC ACTIVITY IN DIPG MODELS**

Nicolas Lacroix1, Marie Lemaire1, Marie-Helene Briand1, and David Ziegler1; 1Children’s Cancer Institute Australia, Sydney, NSW, Australia; 2Stanford Cancer Institute, Stanford, CA, USA; 3Roswell Park Cancer Institute, Buffalo, NY, USA.

BACKGROUND: New treatments are urgently needed for Diffuse Intrinsic Pontine Gliomas (DIPG). Methylation in brain cancer is an attractive therapeutic target. The presence of DNA methylation modifies gene expression and could provide targets of therapy. In glioblastomas, loss of promoter methylation has been linked to tumor progression and a therapeutically exploitable feature of DIPG.

**HG-035. TARGETED INHIBITION OF THE FACT (FACILITATES CHROMATIN TRANSCRIPTION) COMPLEX IS A NOVEL AND EFFECTIVE THERAPEUTIC APPROACH IN DIPG**

Sandy Simon1, Maria Tsoi1, Anna Vanniasinghe1, Michelle Hiber3, Katerina Gurova3, Andreu Gudkov1, Michelle Haber5, and David Ziegler1; 1Children’s Cancer Institute Australia, Sydney, NSW, Australia; 2Stanford Cancer Institute, Stanford, CA, USA; 3Roswell Park Cancer Institute, Buffalo, NY, USA; 4Children’s Cancer Institute Australia, Sydney, NSW, Australia; 5Lowy Cancer Research Institute, Sydney, NSW, Australia.

BACKGROUND: Novel therapeutic strategies are urgently needed for Diffuse Intrinsic Pontine Gliomas (DIPG). We performed a high-throughput drug screen of over 3,500 FDA-approved clinically active compounds against DIPG neurospheres. Two antimalarial drugs quinacrine and mefloquine were amongst the top 2% of compounds that demonstrated significant cytotoxic activity. Their anti-tumor activity was found to be related to the activation of the tumor suppressor protein p53 and induction of apoptosis. Curaxins are a new class of anti-cancer drug which are structurally related to quinacrine and mefloquine p53 while suppressing NF-kB and HSF1. They are potent inhibitors of the chromatin remodeling complex FACT (Facilitates Chromatin Remodeling) and show promising results in DIPG models.
Transcription. We have examined FACT expression in DIPG, as well as efficacy of the curamin clinical candidate, CBL0137, as a novel DIPG therapy. METHODS: FACT expression was measured by PCR and Western blot in DIPG neurospheres compared with normal fetal brain astrocyes, and in primary tumor specimens compared with normal brainstem or cerebellum. The anti-tumor effects of CBL0137 alone or in combination with radiotherapy were measured in DIPG neurospheres using alamar-blue assays, clono- genic assays, and TUNEL activity visible using anaplasia 3’/7’ activity assays. We used western blot to measure the effect of CBL0137 on p53 expression, NFkB activity and epigenetic methylation status. RESULTS: FACT expression was significantly increased in both DIPG neurosphere cultures and primary DIPG tumors. CBL0137 inhibited the proliferation of DIPG neurospheres in short term culture assays and clonogenic assays at nanomolar concentrations, and enhanced the activity of radiotherapy. Treatment with CBL0137 led to increased p53 levels and suppression of NFkB-mediated transcription, activating caspase 3/7 and inducing apoptosis. Initial results suggest that treatment with CBL0137 increases H3K27me3 status. CONCLUSIONS: Our findings indicate that CBL0137 represents a novel, potentially effective therapy for children with DIPG. A Phase I COG trial of CBL0137 is planned that will include a cohort of DIPG patients.

HG-034. HIGH-GRADE GLIOMAS – EXTENT OF RESECTION SIGNIFICANTLY IMPACTS THE PROGNOSIS OF PEDIATRIC PATIENTS TREATED IN PRAGUE CENTRE  
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1Department of Paediatrics, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic; 2Department of Radiotherapy and Oncology, University Hospital Motol, Prague, Czech Republic; 3Department of Pathology and Molecular Medicine, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic; 4Department of Neurosurgery, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic; 5Department of Biology and Medical Genetics, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic.

High-grade gliomas (HGGs) encompass the WHO Grade III and Grade IV gliomas (glioblastoma multiforme, GBM). To evaluate outcome of children with HGG in our institution we analyzed 37 patients diagnosed with primary supra or infratentorial HGG (excluding DIPG) within years 2005-2012. Patients cohort consisted of 25 boys and 12 girls (MF = 2:1:1) with median of age 10.8 years (0.5 to 19.6 years). Only two patients were younger than 3 years. 17 patients were diagnosed with grade III HGG, 15 patients with grade IV and 5 patients with gliomatosis cerebri or multifocal gliomas. In younger than 3 years. 17 patients were diagnosed with grade III HGG, 15 patients with grade IV and 5 patients with gliomatosis cerebri or multifocal gliomas. Infiltrative tumors were predominantly treated by chemotherapy and surgery. But, five of them ultimately succumb to disease progression within 2,5 years. Survival time significantly impacted with extent of resection. Survival in patients undergoing gross total resection or biopsies in all our patients was 29%, with significant improvement for patients undergoing subtotal resection in 13 pts. Therapy consisted of irradiation plus chemotherapy: 2xtemozolomide (TMZ), 2xTMZ + CCNU, 2xTMZ + bevazumab, 5xCCNU + CDPD + VCR. Patients under 3 years were treated with chemotherapy alone. Probability of 5 years overall survival (OS) in all our patients was 29%, with long-term survivors in 5 cases. All patients with gliomatosis or multifocal HGG died with median survival time 0.9 years (0.3 - 2.5 y) as well as patients with leptomeningeal disease. Resection over 90% of tumor volume (STR, GTR) was accompanied with significantly better prognosis compared to biopsy and partially resected HGGs (40% vs 8%, p = 0.01). No significant difference in OS was observed between Grade III and Grade IV HGGs. Our results support the importance of complete resection in HGG patients. Long-term survivors in pediatric HGG patients is achievable. Supported by MZCR-DRO, University Hospital Motol, Prague, Czech Republic 00064203.

HG-035. NOVEL ORTHOTOPIC PAEDIATRIC GLIOMA XENOGRAFTS EVALUATED WITH MAGNETIC RESONANCE IMAGING MIMIC HUMAN DISEASE  
Jessica Boult1, Maria Vinci1, Katy Taylor1, Lara Perryman1, Gary Box1, 
Craig Cummings2, Suzanne Eccles2, Jeffrey Bamber1, Ralph Sinkus2, 
Jan Yarr1, and Simon Robinson1; 1The Institute of Cancer Research, London, UK; 2Ataturk Training and Research Hospital, Ankara, Turkey; 3Ataturk Training and Research Hospital, Ankara, Turkey.

Central nervous system tumors (CNS) are the second most common malignancies in children. Brain stem gliomas are particularly aggressive and difficult to treat. Combining both genetic and epigenetic data, we have developed a novel pGBM models in situ is vital and requires sensitive functional imaging. Established paediatric GBM cell lines SF188 (H3F3A wild-type) and KNS42 (H3F3A G34V) produce well-defined tumours, with minimal local invasion, when implanted supratentorially in mice. Tumours are clearly T1- and T2-weighted MRI hyperintense (kPa) tissue mechanical properties in vivo following application of a vibration-ally stress. Paediatric glioblastomas (pGBM) grow in a partially infiltrative manner, and co-option of host vasculature leaves the blood brain barrier (BBB) intact. Evaluation of novel therapeutic approaches in vivo is challenging. Using pGBM models in situ is vital and requires sensitive functional imaging. Established paediatric GBM cell lines SF188 (H3F3A wild-type) and KNS42 (H3F3A G34V) produce well-defined tumours, with minimal local invasion, when implanted supratentorially in mice. Tumours are clearly T1- and T2-weighted MRI hyperintense (kPa) tissue mechanical properties in vivo following application of a vibration-ally mechanical stress. Paediatric glioblastomas (pGBM) grow in a partially infiltrative manner, and co-option of host vasculature leaves the blood brain barrier (BBB) intact. Evaluation of novel therapeutic approaches in vivo is challenging. Using pGBM models in situ is vital and requires sensitive functional imaging. Established paediatric GBM cell lines SF188 (H3F3A wild-type) and KNS42 (H3F3A G34V) produce well-defined tumours, with minimal local invasion, when implanted supratentorially in mice. Tumours are clearly T1- and T2-weighted MRI hyperintense (kPa) tissue mechanical properties in vivo following application of a vibration-ally mechanical stress. Paediatric glioblastomas (pGBM) grow in a partially infiltrative manner, and co-option of host vasculature leaves the blood brain barrier (BBB) intact. Evaluation of novel therapeutic approaches in vivo is challenging. Using pGBM models in situ is vital and requires sensitive functional imaging. Established paediatric GBM cell lines SF188 (H3F3A wild-type) and KNS42 (H3F3A G34V) produce well-defined tumours, with minimal local invasion, when implanted supratentorially in mice. Tumours are clearly T1- and T2-weighted MRI hyperintense (kPa) tissue mechanical properties in vivo following application of a vibration-ally mechanical stress. Paediatric glioblastomas (pGBM) grow in a partially infiltrative manner, and co-option of host vasculature leaves the blood brain barrier (BBB) intact. Evaluation of novel therapeutic approaches in vivo is challenging. Using pGBM models in situ is vital and requires sensitive functional imaging. Established paediatric GBM cell lines SF188 (H3F3A wild-type) and KNS42 (H3F3A G34V) produce well-defined tumours, with minimal local invasion, when implanted supratentorially in mice. Tumours are clearly T1- and T2-weighted MRI hyperintense (kPa) tissue mechanical properties in vivo following application of a vibration-ally mechanical stress. Paediatric glioblastomas (pGBM) grow in a partially infiltrative manner, and co-option of host vasculature leaves the blood brain barrier (BBB) intact. Evaluation of novel therapeutic approaches in vivo is challenging. Using pGBM models in situ is vital and requires sensitive functional imaging. Established paediatric GBM cell lines SF188 (H3F3A wild-type) and KNS42 (H3F3A G34V) produce well-defined tumours, with minimal local invasion, when implanted supratentorially in mice. Tumours are clearly T1- and T2-weighted MRI hyperintense (kPa) tissue mechanical properties in vivo following application of a vibration-ally mechanical stress. Paediatric glioblastomas (pGBM) grow in a partially infiltrative manner, and co-option of host vasculature leaves the blood brain barrier (BBB) intact. Evaluation of novel therapeutic approaches in vivo is challenging. Using pGBM models in situ is vital and requires sensitive functional imaging. Established paediatric GBM cell lines SF188 (H3F3A wild-type) and KNS42 (H3F3A G34V) produce well-defined tumours, with minimal local invasion, when implanted supratentorially in mice. Tumours are clearly T1- and T2-weighted MRI hyperintense (kPa) tissue mechanical properties in vivo following application of a vibration-ally mechanical stress. Paediatric glioblastomas (pGBM) grow in a partially
infiltrative manner, with boundaries that are often difficult to define by conventional MRI. MRI was utilised to assess the viscoelastic properties of D212-MG orthotopic pGBM xenografts. D212-MG cells were derived from a supratentorial giant-cell GBM, serially propagated subcutaneously before intracranial inoculation. MRI and MRE were performed using a 7T Bruker Micro-imaging system; tumours were identified on T2-weighted images and 3D steady-state MRE data were acquired using a vibration frequency of 1000 Hz. Models of orthotopic (elastancy) and Gl (viscoelasticity) were reconstructed using 300 μm isotropic pixels. Tumour extent and growth characteristics were assessed on H&E stained sections. D212-MG tumours were less elastic (lower Gd), viscous (lower Gl), and therefore softer than the surrounding brain tissue. Tumours were most clearly distinguishable in maps of Gl. D212-MG tumours were significantly less elastic and viscous than well-circumscribed U87-MG human adult GBM tumours in the mouse brain. However, D212-MG did not significantly differ from RG2 rat glioma xenografts, which also grew as well-defined masses with locally invasive growth at the periphery. Histopathological evaluation of vascularity and extracellular matrix composition are being assessed to better understand the basis for the observed dissimilarities in the viscoelastic properties between intracranial tumours and the surrounding brain tissue, and the differences between the tumour models studied. We will extend this work to diffuse infiltrative pGBM models and identify whether local viscoelastic properties as assessed by MRE may provide accurate, non-invasive biomarkers to assess pGBM growth, delineate tumour margins, and monitor therapeutic response.

HG-038. TARGETING MYC/MYCN BY INHIBITION OF CHECKPOINT KINASE 1 (CHK1) SENSITIZES PAEDIATRIC GLOBLASTOMA (pGBM) CELLS TO TEMOZOLOMIDE
Meera Nandhabalan1, Lynn Bjerve2, Mara Vinci1, Anna Burford1, Wendy Ingram1, K02288) revealed selective growth inhibitory effects in vitro. ACVR1/ALK2 appears to be an attractive therapeutic target in at least a subset of DIPG patients, not least due to its presence on the ‘trunk’ of an individual tumour’s branching evolutionary history.

HG-039. THE ROLE OF ACVR1 MUTATIONS IN DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG)
Katy Taylor1, Alan Mackay1, Mara Vinci1, Sergey Popov1, Wendy Ingram1, Natacha Entz-Werle1, Michelle Monje1, Nagore Olaciregui1, Jaume Mora1, Institute of Cancer Research, London, UK; 4University Hospital of Navarra, Pamplona, Spain; 5Hospital Sant Joan de Deú, Barcelona, Spain; 6Structural Genomics Consortium, Oxford, UK
There is an urgent need for novel therapeutic strategies for children with diffuse intrinsic pontine glioma (DIPG), as outcome remains dismal with a median overall survival of 9-12 months. We have recently identified recurrent mutations in the gene ACVR1, which encodes the receptor serine/threonine kinase ALK2, in 25% of DIPG patients. These somatic mutations are located at the same residues (R206H, Q207E, R258G, G328E/V/W, G356D) that were present in the activating somatic germline malformation syndrome fibrodysplasia ossificans progressiva (FOP), and ALK2 inhibitors are currently in development for this disease of abnormal cellular differentiation. We sought to investigate the role of ACVR1 mutations in the context of DIPG and to evaluate preclinically the potential of targeting the receptor in these tumours. ACVR1 mutations mark a distinct subgroup of DIPG, as they strongly co-segregate with histone H3.1 (HIST1H3B) K27M mutations, younger age of onset, slightly improved clinical outcome, and a female predominance. ACVR1 mutant tumours have an enrichment of gene expression associated with an astrocytic lineage, a phenotype observed histologically, sometimes in conjunction with areas of cartilaginous differentiation. In studies of subclonal variation, ACVR1 mutations are always found to be present in the earliest common ancestral clone, implying an important role early in tumour development. ACVR1 mutant tumours are associated with a gene expression signature indicative of downstream pathway activation (ID2-4) and overexpression of the mutations into wild-type DIPG cells confers a weak activation of phospho-Smad1/5/8. Screening a series of DIPG primary cell cultures with a panel of ALK2 inhibitors (LDN-193189, LDN-212854, dorsomorphin, DMH1, K02288) revealed selective growth inhibitory effects in vitro. ACVR1/ALK2 appears to be an attractive therapeutic target in at least a subset of DIPG patients, not least due to its presence on the ‘trunk’ of an individual tumour’s branching evolutionary history.

HG-040. PAEDIATRIC GLOBLASTOMA (pGBM) AND DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG) HARBOUR MULTIPLE CPU-400x400 (1661x1661) alongside multiple single cell-derived colonies expanded in vitro, we observed private events (ID2-4) and overexpression of the mutations into wild-type DIPG cells confers a weak activation of phospho-Smad1/5/8. Screening a series of DIPG primary cell cultures with a panel of ALK2 inhibitors (LDN-193189, LDN-212854, dorsomorphin, DMH1, K02288) revealed selective growth inhibitory effects in vitro. ACVR1/ALK2 appears to be an attractive therapeutic target in at least a subset of DIPG patients, not least due to its presence on the ‘trunk’ of an individual tumour’s branching evolutionary history.

HG-040. PAEDIATRIC GLOBLASTOMA (pGBM) AND DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG) HARBOUR MULTIPLE CPU-400x400 (1661x1661) alongside multiple single cell-derived colonies expanded in vitro, we observed private events (ID2-4) and overexpression of the mutations into wild-type DIPG cells confers a weak activation of phospho-Smad1/5/8. Screening a series of DIPG primary cell cultures with a panel of ALK2 inhibitors (LDN-193189, LDN-212854, dorsomorphin, DMH1, K02288) revealed selective growth inhibitory effects in vitro. ACVR1/ALK2 appears to be an attractive therapeutic target in at least a subset of DIPG patients, not least due to its presence on the ‘trunk’ of an individual tumour’s branching evolutionary history.
HG-041. TREATMENT RESULTS OF PILOT STUDY WITH IRONOTECAN AND CARBOPLATIN IN CHILDREN WITH NEWLY DIAGNOSED HIGH GRADE GLIOMAS (HGG). ONE INSTITUTION EXPERIENCE

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INTRODUCTION: Treatment results in children with high grade gliomas remain poor. Since responses to ironotocan in HGG have been reported we have introduced a combination of ironotocan and carboplatin at first as a second line/salvage treatment, then as preirradiation chemotherapy in patients with newly diagnosed HGG. AIM: To assess treatment outcome of pilot study with ironotocan and carboplatin in children with HGG in comparison to historical group treated according to own protocol. PATIENTS AND METHODS: 17 pts: 9 girls and 8 boys treated between 2010 and 2013 were assessable for response. Two were diagnosed with anaplastic oligoastrocytoma, 2 anaplastic oligodendroglioma, 3 anaplastic astrocytoma and 10: glioblastoma, 2 pts underwent complete tumour resection, 1 subtotal, 8-partial resection and 5 biopsy only. At the onset of treatment three patients had disseminated disease. Chemotherapy protocol consisted of 5-day courses of ironotocan 50 mg/m² and carboplatin 250 mg/m² given every 3 weeks before the initiation of adjuvant radiotherapy. Chemotherapy was evaluated according to WHO criteria, toxicity- according to CTC. RESULTS: 17 pts received a cumulative number of 60 cycles of chemotherapy. Out of 17 pts with newly diagnosed HGG 3 pts achieved CCR, 1 pt- CR, 7 pts- PR, SD was observed in 3 pts and disease progression in 1 pt. Time of pilot study pts- CCR, CR, or PR compared to 30 % in historical group. 3 yrs OS was 46,5 % in pilot study vs 31,8 % in historical group, PFS- 22,8 % vs 22,7 % (3 yrs) and 49,9 % vs 38,6 % (1 yr) consecutively. In patients after complete resection (2 with CR, 1 with anaplastic oligoastrocytoma) according to tailor-made chemotherapy in 2 pts OS and PFS were estimated at 100%. Myelosuppression and gastrointestinal toxicities were the most common and manageable. CONCLUSION: Ironotocan with carboplatin regimen shows activity against HGG in children and has acceptable toxicity.

HG-042. HYPOXIC CONDITIONS TRANSFORM PEDIATRIC HIGH GRADE GLIOMA CELLS TO A HIGHLY RESISTANT PROGENITOR-LIKE PHENOTYPE IN VITRO

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Intratumoral hypoxia plays a fundamental role in tumor progression and resistance to therapies. Tumor cell adaptation to a hypoxic environment is regulated partially by the Hypoxia Inducible Factor-1alpha (HIF-1α), which is involved in gene expression of energetic metabolism, angiogenesis and other processes. HIF-1α accumulation in cells is in particular driven by oncogenic pathway activation, mainly PI3K-AKT-mTOR and RAS-MAPK-mTOR pathways. In high grade gliomas (HGGs), both hypoxia and oncogenic pathways increase during treatment allowing the selection adaptation of tumor cells to different local environments. To demonstrate the role of hypoxia in the HGG, we first, characterized in 23 paediatric HGG samples the expression of the proteins involved in the two main signaling pathways. Secondly, we studied in three well-established cell lines (UW479, SF188 and KNS42) the impact of 1% hypoxia on proliferation, gene expression, metabolomic profiles and hypoxia-induced protein modulations. Thirdly, targeting key proteins in the hypoxia pathways (e.g. mTOR and HIF-1α) was a dynamic approach in the three cell lines to understand the inhibition and the potential therapeutical possibilities in these poor outcome tumors. More than 75% of the tumors had activated PI3K-AKT-mTOR or RAS-MAPK-mTOR pathways, and this protein modulation seemed to confer specific hypoxic profiles. In each of the cell lines, hypoxia was associated with a unique metabolomic protein and gene expression signature indicative of three specific adaptive processes. These signatures are linked to a specific response to mTOR/ HIF-1α inhibition and could be predictive profiles defining sensitive HGGs to therapies targeting hypoxia. In the more drug resistant cells, grown under hypoxic conditions appear to be transformed to a progenitor-like phenotype, with a reduced proliferation rate and induced HIF-1α/HIF-2 expression. This study demonstrates the importance of the intratumoral conditions in determining therapeutic response and provide clues towards a mechanistic understanding of the inherent resistance of pediatric HGG.

HG-043. IMMUNE RESPONSES AND CLINICAL OUTCOME IN A PILOT STUDY OF VACCINATION WITH GLIOMA-ASSOCIATED ANTIGEN PEPTIDES AND POLY-ICLC IN CHILDREN WITH NEWLY DIAGNOSED MEDULLOBLASTOMA AND NON-BRAINSTEM GLIOMAS

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BACKGROUND: Diffuse brainstem gliomas (BSGs) and other high-grade gliomas (HGGs) of childhood are among the most deadly brain tumors of childhood, and new therapies are needed. Immunotherapy is a particularly promising approach in this regard. Having identified a series of glioma-associated antigens (GAAs) commonly overexpressed in pediatric gliomas, we initiated a pilot study of subcutaneous vaccinations with GAAs peptide epitopes in HLA-A2+ children with newly diagnosed BSGs or HGG.

METHODS: Children received subcutaneous vaccinations with synthetic peptides derived from EphA2, interleukin (IL)-13 receptor-a2, and survivin, emulsified in Montanide-ISA-51 and administered every 3 weeks for 8 doses, in conjunction with intramuscular poly-ICLC. Booster vaccinations were administered every 6 weeks for up to 2 years for children with at least stable disease following the initial phase of therapy. Primary endpoints were safety and T cell responses against vaccine-targeted GAAs. Treatment response was evaluated by MR imaging, and by MR imaging, RPS.

Twenty-six children were enrolled in the initial pilot phase of the study, 14 with newly diagnosed BSG treated with irradiation, and 12 with newly diagnosed BSG or HGG treated with irradiation and concurrent chemotherapy. No safety or efficacy was met, and this study was stopped. Results were compared to historical group treated according to their own protocol. PATIENTS AND METHODS: 3 yr OS and PFS were estimated at 100%. Myelosuppression and gastrointestinal toxicities were the most common and manageable. CONCLUSION: Irinotecan with carboplatin regimen shows activity against HGG in children and has acceptable toxicity.

HG-044. INCREASED 5-HYDROXYMETHYLCYTOSINE AND DECREASED 5-METHYLCYTOSINE ARE INDICATORS OF GLOBAL EPIGENETIC DYSREGULATION IN DIFFUSE INTRINSIC PONTINE GLIOMA

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Diffuse intrinsic pontine glioma (DIPG) is a universally fatal tumor. Eighty percent of DIPG patients have a mutation in the H3F3A gene resulting in missense mutation at position 27 of Histone 3.3 (H3K27M) which inhibits methylation at position 27 and leads to global chromatin changes. DIPG is also associated with global DNA hypo-methylation. Evidence suggests that 5-methylcytosine (5mC) can be converted to cytosine in a multistep enzymatic process with 5-hydroxymethylcytosine (5hmC) being the initial intermediate. 5hmC represents an important epigenetic mark and with 5mC is thought to mediate. 5hmC represents an important epigenetic mark and with 5mC is thought to mediate. 5hmC represents an important epigenetic mark and with 5mC is thought to mediate. 5hmC represents an important epigenetic mark and with 5mC is thought to mediate. Since 5mC and histone methylation plays a key role in neural development. Due to the global hypomethylation noted in DIPGs, we hypothesized that levels of 5mC, 5hmC and H3K27 trimethylation would be altered. We used formalin-fixed paraffin embedded tumor samples in tissue microarrays to perform immunohistochemical studies, comparing DIPGs to adult and pediatric glioblastoma (GBM) and normal brain. Tumors were scored for histone 3 lysine 27 trimethylation (H3K27me3), histone 3 lysine 9 trimethylation (H3K9me3), 5-hydroxymethylcytosine (5hmC), and 5-methylcytosine (5mC). H-score was derived by multiplying intensity (0-2) by percentage of positive tumor nuclei (0-100%). We identified decreased levels of 5mC, 5hmC and H3K27me3 in DIPGs compared to pediatric GBM (p < 0.001), adult GBM (p < 0.0001) and normal brain (p < 0.0001). H3K9me3 was not significantly different between tumor types. Global DNA methylation as measured by 5mC levels was significantly lower in DIPGs compared to pediatric GBM (p < 0.001), adult GBM (p < 0.001), and normal brain (p < 0.001). Surprisingly,
ShmC levels were significantly higher in DIPGs compared to pediatric GBM (p < 0.0001) and adult GBM (p < 0.0001). This data indicates that DIPGs are different from their GBM counterparts with derangements in both histone methylation. The identification of hndbrain progenitors into a pathological state. The presence of high-levels of ShmC in the context of global hypomethylation may provide an opportunity for therapeutic intervention.

**Method:**

Between 02/2012-03/2013 28 patients were recruited (3 AA, 9 GBM, 15 DIPG, one anaplastic oligoastrocytoma), 27 after first-line temozolomide radiochemotherapy (HIT-HGG-2007 protocol).

**Results:**

Among 159 GBM, 158 DIPG, 100 other histologies. Median overall survival in HIT-HGG-CilMetro was 0.397 (0.181-0.613) vs. 0.507 (0.451-0.562) years (not significant), OS and EFS after relapse were nearly identical. However, the three GBM survivors in the HIT-HGG-CilMetro group might represent true responders. CONCLUSION: HIT-HGG-CilMetro offered a feasible salvage treatment approach with tolerable toxicity for pediatric patients with relapsed HGG and might induce in contrast to previous cilegilde monotherapy trials an increased progression-free survival in a small relapsed GBM subgroup.

**Abstracts**

**HG-045. MANGO GINGER: A CURCUMA SPECIES HIGHLY SYNERGISTIC WITH CHEMOTHERAPY AGAINST GLIOMA CELL LINES**

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Pediatric brain tumors are the most common form of solid tumors in children accounting for about 20-25% of all pediatric cancers. Chemotherapy options for brain tumor treatment are very much limited because of the blood brain barrier and emergence of drug resistance in brain tumor cells. Combining nutraceuticals or botanical drugs with cancer drugs is one of the ways to improve the efficiency of chemotherapy and quality of life in an integrative oncology setting. In the present investigation, cytotoxicity of anticancer drugs [Etoposide (ETP), Temozolomide, TMZ] and supercritical extracts of Curcuma amada (CA-CO), C. xanthorrhiza (CX-CO) and C. longa (CL-CO), curcumin and Turmeric Force either as single agent or their combinations in glioma cell lines (U87MG, U188 MG) were analyzed by MTT assay. Synergism, additiveness or antagonism between cancer drugs and supercritical extracts were determined using Compusyn analysis of cytotoxicity data. Apoptosis and necrosis induced by different agents on their combinations were analyzed using Roche Annexin-V-FLUOS staining kit in a flow cytometer. The expression of genes associated with apoptosis and cell proliferation (p53, p21, Bcl-2, Bax, and P10) were determined by RT-PCR assay. Both glioma cell lines are generally resistant to cancer drugs such as TMZ. CA-CO showed superior cytotoxic effects as compared to CX-CO and CL-CO. CA-CO also had significantly better cytotoxic effects than curcumin and Turmeric Force 160. Compusyn analysis of cytotoxic data showed that the combination of ETP and/or TMZ with CA-CO produced very high synergistic effects on cytotoxicity. The combination of cancer drugs with CA-CO induced higher percentage of apoptosis and necrosis than single agents. Gene expression studies showed that CA-CO down regulated the expression of P10 and P53 genes and increased the ratio of Bax/Bcl-2 mRNA. These positive results suggest the need for continuous evaluation of CA-CO in xenograft models and clinical trials in brain tumor patients.

**HG-046. CILEGILDE AND METRONOMIC TEMOZOLOMIDE FOR RELAPSED OR REFRACTORY HIGH GRADE GLIOMAS OR DIFFUSE INTRINSIC PONTINE GLIOMAS IN CHILDREN AND ADOLESCENTS – PRELIMINARY RESULTS OF THE GERMAN PHASE II STUDY HIT-HGG-CILMETRO**

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**Introduction:** Relapsed high-grade gliomas (HGG) in pediatric patients like glioblastoma multiforme (GBM), anaplastic astrocytoma (AA), diffuse intrinsic pontine glioma (DIPG) represent a dismal prognosis group currently without standard salvage therapy. In this population, combined cilegilde and metronomic temozolomide was investigated: Cilegilde 1800 mg/m² twice weekly; temozolomide: 75-80 mg/m²/7d for 8 weeks with one week rest, in total over one year. **Patients and Methods:** Between 02/2012-03/2013 28 patients were recruited (3 AA, 9 GBM, 15 DIPG, one anaplastic oligoastrocytoma), 27 after first-line temozolomide radiochemotherapy (HIT-HGG-2007 protocol). RESULTS: Ten months after recruitment closure, 3/28 relapse patients are still alive and progression-free (all GBM) with a follow-up of 11 to 22 months. The following serious adverse events (SAE) were observed: Intracranial hemorrhage (n = 4); neurological deterioration (n = 4) and seizures (n = 3), pneumonia (n = 2), relapse (n = 1), severe anemia (n = 1), pain (n = 1), and cytokines (n = 1). No suspected unexpected serious adverse reaction (SUSAR) occurred. However, recruitment was prematurely stopped due to an altered risk benefit assessment after two negative clinical trials employing cilegilde in pediatric and adult HGG had been published. The present cohort was compared to a historical control of HGG relapse patients from our database, all treated individually for their relapse (n = 417 patients: 159 GBM, 158 DIPG, 100 other histologies). Median overall survival in HIT-HGG-CilMetro was 0.397 (0.181-0.613) vs. 0.507 (0.451-0.562) years (not significant), OS and EFS after relapse were nearly identical. However, the three GBM survivors in the HIT-HGG-CilMetro group might represent true responders. CONCLUSION: HIT-HGG-CilMetro offered a feasible salvage treatment approach with tolerable toxicity for pediatric patients with relapsed HGG and might induce in contrast to previous cilegilde monotherapy trials an increased progression-free survival in a small relapsed GBM subgroup.

**Diffuse Intrinsic Pontine Glioma (DIPG) is a universally fatal cancer with only a 9-month median survival. The present study represents an international, multi-institutional collaboration to pool DIPG tissue resources and evaluate comprehensive molecular and functional targets of DIPG therapy. We performed whole exome sequencing of 17 DIPG tumors, RNAseq expression analysis and a chemical screen against 14 DIPG cell lines. For subsets of DIPG cell lines, we performed whole exome sequencing of 17 DIPG tumors, RNAseq expression analysis and a chemical screen against 14 DIPG cell lines. For subsets of DIPG cell lines, we performed whole exome sequencing of 17 DIPG tumors, RNAseq expression analysis and a chemical screen against 14 DIPG cell lines. For subsets of DIPG cell lines, we performed whole exome sequencing of 17 DIPG tumors, RNAseq expression analysis and a chemical screen against 14 DIPG cell lines. For subsets of DIPG cell lines, we performed whole exome sequencing of 17 DIPG tumors, RNAseq expression analysis and a chemical screen against 14 DIPG cell lines. For subsets of DIPG cell lines, we performed whole exome sequencing of 17 DIPG tumors, RNAseq expression analysis and a chemical screen against 14 DIPG cell lines.**
HG-048. PRECLINICAL BIO-DISTRIBUTION OF 89Zr-BEvacizumab in DIPG and Supratentorial GBM Xenograft Models Using Positron Emission Tomography (PET)

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INTRODUCTION: Diffuse intrinsic pontine glioma (DIPG) is a devastating therapy-resistant tumor and children diagnosed with this disease have a dismal prognosis. Bio-distribution of therapeutic agents in DIPG is unknown, and poor tumor penetration may be the reason for therapeutic failure. Bevacizumab is a monoclonal antibody that neutralizes VEGF, a growth factor involved in angiogenesis and cell proliferation, which is overexpressed in DIPG. The role of bevacizumab in the treatment of DIPG is unclear. The aim of this study is to investigate bio-distribution and tumor-uptake of zirconium-89 (89Zr) labeled bevacizumab in a xenograft model, in relation to a radiologic or biopsy proven diagnosis of DIPG. METHODS: Human DIPG, mouse GBM, and human glioblastoma (GBM) cell lines, known for their diffuse growth pattern in vivo, were injected into the subcutis, pons, or striatum of 30 athymic mice. A sub-therapeutic dose of 89Zr-bevacizumab was administered 18 or 35 days after xenografting. The location of injection and timing of treatment represented different tumor localizations and disease stages. Seventy-two to 96 hours after administration of 89Zr-bevacizumab, mice were imaged using Positron Emission Tomography (PET) and biodistribution was analyzed ex vivo. VEGF expression was confirmed by in situ hybridization (ISH). RESULTS: 89Zr-bevacizumab uptake in the mouse brain nor in the E98 gliomas located in the pons and striatum at either stage of disease. Instead, the subcutaneous tumors showed high accumulation of 89Zr-bevacizumab. VEGF expression was confirmed by ISH in the subcutaneous tumors, but was absent in the majority of intracranial tumors. CONCLUSIONS: The uptake of 89Zr-bevacizumab in intracranial E98FM tumors is poor, while 89Zr-bevacizumab accumulates in subcutaneous E98FM tumors. We hypothesize that the lack of blood-brain-barrier in these subcutaneously growing xenografts. These results stimulate the use of PET studies in children with brain tumors, to investigate correct drug targeting and to personalize treatment.

HG-049. HIGHLY SELECTIVE INTRA-ARTERIAL CHEMOTHERAPY FOR THE TREATMENT OF PROGRESSIVE DIFFUSE INTRINSIC PONTINE GLIOMAS (DIPG)

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BACKGROUND: DIPG remains a largely fatal diagnosis. Radiation therapy remains the only intervention that alters the natural history of the disease, but is not curative. Given the frequent imaging feature of a non-contrast enhancing mass at diagnosis, the extent of systemic delivery of chemotherapeutics to the pons is uncertain. Strategies to improve delivery include: convection-enhanced delivery utilizing intrapontine catheters, blood-brain barrier disruption, and intra-arterial (IA) administration of chemotherapy into the vascular circulation. This pilot study explores the safety, tolerability, and early efficacy of highly selective intra-arterial administration of melphalan via the basilary artery at the time of clinical progression following initial radiotherapy for DIPG. METHODS: Subjects with a radiologic or biopsy proven diagnosis of DIPG are eligible, known for time of progression following initial irradiation. Subjects with a documented hypercoagulable disorder or vasculopathy or who have been re-irradiated are not eligible. Two cycles of IA chemotherapy are planned 4 weeks apart. Each cycle of chemotherapy is catherized with a 4-French guide catheter. Following a baseline angiogram, a 1.5-French microcatheter is advanced into the mid basilary artery. IA delivery of 4 or 6 mg of melphalan is delivered over 30 minutes. Subjects are observed overnight. Subjects are monitored for toxicity between, and following, IA administrations. RESULTS: To date, 2 children, of a planned cohort of 5 children, have been treated. Both children tolerated the actual IA administrations without any procedural associated toxicity including the absence of stroke, hemorrhage, or other technical complications. The first child received one of the planned IA administrations but clinically deteriorated shortly before the second IA infusion was planned. The second child received both planned IA administrations and is in early follow-up. CONCLUSIONS: Highly selective IA administration of melphalan to the basilary artery is feasible and safely tolerated. Updated experience and follow-up with enrolled patients will be presented.

HG-050. INVESTIGATIONAL AURORA A KINASE (AAK) INHIBITOR MLN8237 (ALISERTIB) SUPPRESSES NEUROSPHERE PROLIFERATION OF PEDIATRIC GLOBLASTOMA AND PROLONGS ANIMAL SURVIVAL IN PATIENT TUMOR-DERIVED ORTHOTOPIC XENOGRAFT MOUSE MODEL

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BACKGROUND: Pediatric glioblastoma (GBM) is highly aggressive and development of novel therapy is needed. AAK is critical for mitosis and its overexpression results in malignant transformation of normal cells. MLN8237 (Alisertib) is an investigational orally available reversible selective inhibitor of the Aurora A serine/threonine kinase. We investigated the effects of MLN8237 on glioma stem cell proliferation and animal survival. METHODS: Levels of AAK expression were screened in a panel of patient tumor-derived orthotopic xenograft mouse models. Anti-tumor effects were examined by Cell Counting Kit-8 assay in paired monolayer cell lines (FB5-based media) and neurosphere cultures (serum-free media containing EGFbFGF) from two xenograft mouse models, IC-4686GBM (previously untreated) and IC-R0315GBM (established from the therapy-resistant autopsy sample). Susceptibility to cancer stem cell population was analyzed by flow cytometric analysis. Activity was examined in vivo by orallyadministered MLN8237 (30 mg/kg) for 3 days in IC-4686GBM glioma cell bearing two weeks post intra-cerebral tumor cell implantation. RESULTS: Overexpression of AAK mRNA (4-10 fold over normal control) was observed in our pediatric GBM models. Tumor growth in monolayer cells was suppressed in the previously untreated line (IC-4686GBM) but not in the therapy-resistant model (IC-R0315GBM) cell line. Cell growth in neurosphere cultures was suppressed in both lines in a time- and dose-dependent manner. Tumor killing activity in neurospheres was confirmed in cancer stem cell marker (CD133/CD15) expressing cells in both IC-4686GBM and IC-R0315GBM. In IC-4686GBM, 3 days treatment with MLN8237 led to significant decreased tumor mass in the treatment group (p < 0.05). In the therapy-resistant model IC-R0315GBM, no significant prolongation was observed, which correlates with the in vitro lack of effect on the monolayer cells. CONCLUSION: We detected high levels of AAK expression in pediatric GBM models and MLN8237 appears active in targeting GBM in vitro and in vivo. Our data in the therapy-resistant model IC-R0315GBM support the future research into potential combination regimens in therapy-resistant GBMs.

HG-051. HIGH FREQUENCY OF MISMATCH REPAIR GENE DEFICIENCY AMONG PEDIATRIC HIGH GRADE GLIOMAS IN JORDAN

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BACKGROUND: Biallelic mismatch repair deficiency (BMMRD) is a cancer predisposition syndrome affecting individuals from consanguous families with multiple childhood cancers; most commonly malignant brain tumors. The impact of the syndrome on malignant gliomas in countries where consanguinity is high is unknown. We determined the prevalence of clinical findings suggestive of BMMRD and mismatch repair dysfunction among pediatric patients diagnosed with these cancers in two centers in Jordan and Canada. PATIENTS AND METHODS: All patients aged 18 years and diagnosed with intracranial high grade glioma (HGG) between 2003 and 2013 were included. Clinical data regarding presence of cafe au lait spots (CAL), family history of cancer, consanguinity, diagnosis and treatment were collected. Tumors were centrally reviewed by two pathologists. RESULTS: 120 pediatric patients with HGG were evaluated (HGG)
proteins. RESULTS: Thirty-five patients fulfilled the inclusion criteria; twenty-two of them (63%) had available clinical data. Fifteen tumors (41%) lost at least one MMR stain and 30% also lost MMR stain in the corresponding normal brain tissue. Tumor DNA sequenced revealed two intracranial tumors each; with identical MMR gene loss in both tumors. P53 immunopositivity was highly enriched in MMR deficient tumors (p = 0.003). Clinical data suggestive of BMMRD was present in 36% of patients. Frequency of MMR-deficiency was significantly lower in the Seattle cohort (23%; p = 0.03). Importantly, genetic testing confirmed BMMRD in 50% of these patients. Five year overall survival for patients with MMRD tumors in both cohorts was 17% ± 10%. The only long term survivors with BMMRD whose tumors were discovered by surveillance. CONCLUSIONS: In Jordan, the frequency of clinical and immunohistochemical alterations suggestive of BMMRD in pediatric HGG is high. Genetic testing is needed to confirm an underlying BMMRD in order to perform counseling and cancer screening to improve survival of these patients.

HG-052. INTRANASAL DELIVERY OF CHEMOTHERAPEUTIC AGENTS IN A NON-HUMAN PRIMATE MODEL
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BACKGROUND: Achieving adequate tissue concentrations of chemotherapeutic agents for the treatment of brain tumors is limited by the blood-brain barrier (BBB) and systemic toxicities. We hypothesized that intranasal (IN) administration could overcome the BBB by providing direct access to the CSF. In this study, we evaluated the feasibility of the plasma and cerebrospinal fluid (CSF) pharmacokinetics of IN-delivered temozolomide (TMZ) compared to intravenous (IV) administration in a non-human primate (NHP) model. METHODS: In separate experiments, four adult rhesus monkeys received TMZ IN (dose = 20 mg, bolus) and IV (dose = 7.5 mg/kg, 1 hr infusion). Following both IN and IV dosing, serial paired plasma and CSF samples were collected. TMZ was quantified using HPLC/tandem mass spectrometry. Pharmacokinetic (PK) parameters were estimated using non-compartmental methods. Exposures, under the same concentration x time curves (AUCCs) were dose-corrected. RESULTS: No significant toxicities were observed after IN or IV administration. PK parameters are reported as median (range) values: IN (n = 4): CSF peak concentration (Cmax) was 2.39 (2.03-2.72) μM; time to Cmax (Tmax) was 1.50 (1.00-3.00) hr; AUCCs was 6.80 (4.36-7.53) μM·hr/mg/kg. Plasma Cmax was 11.73 (7.87-12.80) μM; Tmax was 0.28 (0.18-1.00) hr; AUCCp was 11.26 (6.32-15.06) μM·hr/mg/kg. Ratio of exposure (AUCCp/AUCCs) was 57.05% (38.31-99.10). IV (n = 3): Cmax was 18.96 (14.60-23.10) μM; Tmax was 1.53 (1.50-2.00) hr; AUCCs was 7.31 (7.04-11.42) μM·hr/mg/kg. Plasma Cmax was 86.20 (83.60-127.80) μM; Tmax was 1.00 (1.00-1.50) hr; AUCCp was 35.09 (34.16-54.42) μM·hr/mg/kg. Ratio of exposure (AUCCp/AUCCs) was 20.90% (20.07-21.40). RESULTS: CSF exposure IN was 65.90% (59.68-91.80). Ratio of CSF exposure IN vs. IV AUCCp was 27.68% (18.59-33.33). CONCLUSIONS: Compared to IV-administered TMZ, IN administration could overcome the BBB and systemic toxicities. IN dosing provides direct access to the CSF, providing dose comparable systemic exposure.

HG-054. BI-ALLELIC MISMATCH REPAIR MSH6 GENE MUTATIONS IN A PATIENT SURVIVING A CHILDHOOD MALIGANT BRAIN TUMOR
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Pediatric gliomas comprise a diverse group of malignancies accounting for more than half of childhood CNS-tumors. Here we apply next-generation-Sequencing to the exome-profiling of an unusually high malignantly oligodendroglioma operated from a 13 years old child who achieved long term survival. Radical tumor extirpation was performed and the treatment included 8-drugs-in-1-day, followed by radiotherapy. Today, 24 years later, the patient is still healthy. Patient’s blood and tumor DNA were exome enriched and sequenced resulting in ~26X target region coverage. Surprisingly, we detected >260 variants enriched by a search for germline mutations. This high somatic mutation rate was similar to that observed in some sporadic colorectal tumors and tumors developing in autosomal dominant Lynch syndrome (LS). It is known that germline mutations in DNA mismatch repair (MMR) genes: MLH1/2, MSH6 and PM2D lead to LS. Childhood cancers are though not part of the LS tumor spectrum. In rare occasions however, germline bi-allelic MMR genes mutations may lead to a condition called constitutive MMR deficiency. The syndrome is characterized mainly by colorectal cancer, hematological neoplasms and brain tumors. In contrast to LS, these malignancies occur in the childhood or adolescence. Interestingly, the search for germline mutations revealed compound heterozygous mutations in MSH6. One of the mutations, rs637508977973 predicts a predicted damaging a substitution at position 533. The second one is a novel, nonsense mutation at an amino acid position 201. Immunohistochemistry for MSH6 showed no protein expression in the tumor. Very few cases of brain tumors due to bi-allelic MMR genes defects have been reported, however, they might have clinical, pathological or molecular characteristics that allow early diagnosis. It has also been proposed that alkylating agents should be avoided in such cases and that radiation should be used with caution. It is therefore important to document cases like this particularly with regard to the excellent outcome.

HG-053. PI-3 KINASE PATHWAY INHIBITION IN A GENETIC MOUSE MODEL FOR HIGH-GRAGE ASTROCYTOMA
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BACKGROUND: High-grade astrocytomas (HGA) are aggressive brain cancers that affect thousands of patients each year and carry a dismal prognosis. The phosphatidylinositol-3-kinase (PI3K)/Akt pathway is a core mutated pathway in gliomagenesis and is therefore an attractive therapeutic target. METHODS: We evaluated the mTOR inhibitor rapamycin (3 days, 2.5 mg/kg) in the wild-type (WT) mouse, the irreversible PI3K inhibitor PX-866 (5 days, 25 mg/kg) po alone and in combination in a genetically engineered mouse model for HGA lacking Pten, a negative regulator of the PI3K pathway. Alterations of the PI3K signaling pathway and indices of proliferation and apoptosis were assessed in HGA by immunohistochemistry and western blotting. RESULTS: Rapamycin treatment had little effect on signaling upstream of mTOR but led to decreased phospho-S6. The proliferative index in rapamycin treated tumors (22.8%) was significantly lower than in vehicle treated HGA (45.3%, p = 0.003) whereas apoptosis was significantly higher in rapamycin treated (7.5%) compared to vehicle treated (0.03%). Treatment with PX-866 effectively decreased phospho-S6 however effects on other signaling intermediates of the PI3K/AKT pathway and on proliferation and apoptosis were variable and modest. Combination treatment was well tolerated; decreased survival (31 vs 33 days, p = 0.017) and p-4EBP1 by two fold (p = 0.19) compared to either drug alone. The proliferative index in combination treated tumors (16.3%) was significantly lower than in rapamycin treated HGA (22.8%, p = 0.041). Interestingly, the apoptotic index was 37.1% in tumors treated with the combination, a significant increase in cell death compared to rapamycin treatment alone (7.5%, p = 0.004). CONCLUSION: The combination of rapamycin and PX-866 demonstrates cooperative effects on downregulation of PI3K signaling, suppression of cell proliferation and induction of apoptosis in a genetically engineered mouse model for HGA. Studies to evaluate the survival benefit of drug treatments are ongoing.

HG-055. AN INDIVIDUAL PATIENT DATA META-ANALYSIS ON CHARACTERISTICS, TREATMENT AND OUTCOME OF PATIENTS WITH METASTATIC HIGH-GRAGE GLIOMAS
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BACKGROUND AND PURPOSE: Dissemination of high-grade gliomas (WHO IV) is rare. The aim of the study was to delineate the patient characteristics, treatment, and outcome of patients with metastatic glioblastoma multiforme (GBM)/gliosarcoma reported in the literature until April 2013. METHODS: PubMed and Web of Science were searched for case
series and cohort studies reporting on metastatic GBM and GS. RESULTS: 215 publications reporting on 357 patients met our inclusion criteria. There was a significant increase in the number of reported cases over the last 10 years with a median age at initial diagnosis of 18.5 years (range 3-84 years). Thirty-four patients (9.8%) were children or adolescents, aged <18 years. We estimated a median overall survival (OS) from diagnosis of metastasis (in the majority of the patients at time of relapse; in 17% at time of initial diagnosis) of 3.0 months for supratentorial HGG (range 0.1-12 months). Interestingly, OS was similar for patients with metastatic disease at initial diagnosis when compared to patients with disseminated disease at time of relapse (3-months OS: 42 ± 10% versus 46 ± 5%). By univariable analyses, gender, age (cutoff 18 years), the histological subtype and the time interval between initial diagnosis and the occurrence of metastases did not influence outcome. In contrast, metastatic disease restricted to areas outside the central nervous system was associated with longer survival. The overall survival after diagnosis of metastasis was independent of the date of publication. Median survival was 42 months for supratentorial HGG (range 4 months to 147 months) with a 5% 10-year survival. In adults, the addition of TMZ results in a modest improvement in overall survival. We retrospectively review our series of paediatric patients with metastatic GBM from a children's cancer center in Singapore. METHODS: Data was collected from the Singapore Children's Cancer Centre (SCCR) and hospital chart records. All patients aged 0-19 years with HGGs diagnosed and treated in KKH from July 1997-June 2012 (15 years) were included. Information regarding demographics, clinical presentation, diagnosis, treatment and outcomes were studied. RESULTS: 27 patients with HGG were identified of which 15 (56%) were boys. The age at diagnosis ranged from 3 months to 14.7 years (median 6.8 years; mean 7.2 years). All patients were of Asian ethnicities - 17 (63%) Chinese, 6 (22%) Malay and 4 (15%) Indian. Most common presentations included vomiting (50%), cranial nerve palsies (46%), headache (43%) and unsteady gait (36%). The tumour locations were supratentorial in 12 patients, infratentorial in 13, and multifocal in 2 (including one with multifocal gliomatosis cerebri). Biopsy and/or resection were done for 19 patients (70%). Of these, 10 (53%) were WHO Grade III and 9 were Grade IV. The most common histologies were anaplastic astrocytoma (26%), glioblastoma NOS (16%), glioblastoma with mesenchymal differentiation (11%) and anaplastic ependymoma (11%). Of the 12 supratentorial HGG, 11 underwent resection, 7 underwent radiotherapy and 4 underwent chemotherapy. Of the 13 infratentorial HGG, 4 underwent resection, 10 underwent radiotherapy and 3 underwent chemotherapy. Median survival was 42 months for supratentorial HGG (range 4 months to 16 years), and 16 months for infratentorial HGG (range 1-54 months). The 5-year overall survivals were: 31.3% (supratentorial HGG), 7.7% (infratentorial HGG), 17.3% (entire cohort). CONCLUSION: Survival outcome for HGG remains poor, especially for infratentorial HGG.

**HG-056. HIGH GRADE GLIOMAS IN CHILDREN: REPORT FROM A CHILDREN'S CANCER CENTRE IN SINGAPORE**

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BACKGROUND: High grade glioma (HGG) in children, while not as common as in adults, are also aggressive disease with very poor overall survival. We retrospectively review our series of paediatric patients with HGGs in KK Women’s and Children’s Hospital (KKH). METHODS: Data was collected from the Singapore Childhood Cancer Registry (SCCR) and hospital chart records. All patients aged 0-19 years with HGGs diagnosed and treated in KKH from July 1997-June 2012 (15 years) were included. Information regarding demographics, clinical presentation, diagnosis, treatment and outcomes were studied. RESULTS: 27 patients with HGG were identified of which 15 (56%) were boys. The age at diagnosis ranged from 3 months to 14.7 years (median 6.8 years; mean 7.2 years). All patients were of Asian ethnicities - 17 (63%) Chinese, 6 (22%) Malay and 4 (15%) Indian. Most common presentations included vomiting (50%), cranial nerve palsies (46%), headache (43%) and unsteady gait (36%). The tumour locations were supratentorial in 12 patients, infratentorial in 13, and multifocal in 2 (including one with multifocal gliomatosis cerebri). Biopsy and/or resection were done for 19 patients (70%). Of these, 10 (53%) were WHO Grade III and 9 were Grade IV. The most common histologies were anaplastic astrocytoma (26%), glioblastoma NOS (16%), glioblastoma with mesenchymal differentiation (11%) and anaplastic ependymoma (11%). Of the 12 supratentorial HGG, 11 underwent resection, 7 underwent radiotherapy and 4 underwent chemotherapy. Of the 13 infratentorial HGG, 4 underwent resection, 10 underwent radiotherapy and 3 underwent chemotherapy. Median survival was 42 months for supratentorial HGG (range 4 months to 16 years), and 16 months for infratentorial HGG (range 1-54 months). The 5-year overall survivals were: 31.3% (supratentorial HGG), 7.7% (infratentorial HGG), 17.3% (entire cohort). CONCLUSION: Survival outcome for HGG remains poor, especially for infratentorial HGG.

**HG-058. INCREASE HISTONE METHYLATION FOR THE TREATMENT OF H3 K27M MUTANT BRAINSTEM GLIOMAS**

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INTRODUCTION: Histone gene mutations occur in pediatric brainstem gliomas. The most common mutation, a substitution of methionine for lysine at position 27 (K27M) of H3.3 protein, one of the histone family members, causes substantial reduction in histone H3 tri- and di-methylation of K27 in cellular chromatin. We hypothesize that increase histone methylation would be a unique therapeutic approach for treating this cancer. METHODS: Histone H3 lysine 27 (H3K27) methylation status was evaluated by western blotting. Cell proliferation assays were performed to assess the response to pharmacological inhibition with GSK-J4, a selective inhibitor of H3K27 demethylase JMJD3 and genetic suppression using JMJD3 siRNA. Gene expression changes and sequence association of K27me3, as a result of GSK-J4 treatment, were examined in cells with the K27M mutation using CHIP sequence and gene expression array. In vivo tumor growth and response to therapy were quantitatively measured by bioluminescence imaging and animal survival. RESULTS: H3.3 K27M mutant brainstem glioma cells showed global reduction of H3K27 methylation compared to H3.3 wild-type glioma cells. Pharmacologic inhibition and genetic suppression of the H3 K27 demethylase JMJD3, had anti-tumor activities that are specific to glioma cells with K27M mutation. Inhibition of JMJD3 opposed the suppressive effect of K27M mutation on histone tri- and di-methylation of K27 in cellular chromatin. We hypothesize that increase histone methylation would be a unique therapeutic approach for treating this cancer.

**HG-059. A CASE OF DIFFUSE INTRINSIC PONTINE GLIOMA IN A 5 YEAR OLD FEMALE**

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Brainstem tumors occur in approximately 10% to 15% of pediatric patients with central nervous system tumors. The median age at diagnosis is 7 to 9 years. Intrusive diffuse pontine gliomas (DPG) are a unique subset of brainstem tumors with a dismal prognosis, and accounts for 70–80% of all brainstem tumors. Current standard of care is primarily radiation therapy. We report a case of a 5 year old female diagnosed with DPG who has completed conventional radiotherapy and has shown marked clinical improvement was ataxic for 2 weeks later. She suddenly developed right-sided hemiparesis, decreased sensorium, headache and vomiting prompting consultation at our institution. She could follow commands with spontaneous eye-opening but inappropriate responses and disorientation. There was notoreflex with level rectus palsy and a weak gag reflex. Right pupil was lower and irreducible.
weakness (MMT 1/5) with 100% sensory deficit, and loss of bowel and bladder control. Cranial MRI with contrast revealed a large necrotic mass in the pons with surrounding edema consistent with brainstem glioma. The patient was described for lung and intracranial carcinomatous cell lines established on Mammot 0.6 mg/kg and Dexamethasone 0.6 mg/kg. He received primary radiation treatment with 54 Gy in 1.8 Gy fractions. After two weeks of radiotherapy, her sensorium and articulation improved, headache and vomiting resolved, swallowing done with ease, complete bowel and urinary bladder control regained, and motor function of the right extremities improved to MMT 4/5. Improvement in clinical status is usually evident in 2 to 3 weeks of radiation therapy alone, but progression-free interval is short (median 6 months) with median survival less than 1 year and survival rates at 2 years of less than 20%. Studies have shown that adjuvant chemotherapy does not improve overall survival, although there are still ongoing investigations in the role of newer chemotherapeutic agents.

Combination with dasatinib (inhibitor of MET and other RTKs) was studied according to the Chou-Talalay method. RESULTS: DIPG primary tumors and cell lines exhibited the signature of sensitivity to dasatinib prevalent in adult high grade gliomas (HGG). Patient was initially started on dasatinib treatment with 100 mg in 1.1 mg fractions. The activity of downstream effectors of dasatinib targets was strongly reduced. Multiple RTKs were activated simultaneously in DIPG cell lines, including MET, suggesting benefit of combination with other RTK inhibitors. Since MET was shown to be altered (amplification/mutation) in DIPG, we tested combination treatment with dasatinib and cabozantinib. Interestingly, synergistic effects were observed in 3 out 4 cell lines. CONCLUSIONS: Dasatinib exhibits anti-tumor effects in vitro on DIPG cell lines that could be increased by the combination with another RTK inhibitor targeting MET.

Chimeric antigen receptor (CAR)-modified T cells have shown considerable efficacy in the treatment of cancer. Targeting a single glioma-restricted antigen, however, carries an inherent risk of creating antigen loss tumor cell variants owing to the significant intra- and inter-tumor heterogeneity that exists in High Grade Glioma (HGG). We have demonstrated that simultaneously targeting of multiple glioma-restricted antigens offsets this escape mechanism leading to improved therapeutic efficacy. Using in silico modeling, we designed a novel bispecific CAR that incorporates a HER2-specific scFv and a mutated IL13Rα2 molecule, joined in tandem (TanCAR), to target the glioma-restricted antigens, HER2 and IL13Rα2, respectively. The intra-cellular T-cell activating domain consisted of a T-cell signaling chain and a signaling moiety from the co-stimulatory molecule CD28. T cells from HGG patients were genetically engineered to express the TanCAR using a retroviral system and the surface expression of the extracellular antigen-binding domain was confirmed by flow cytometry using HER2- and IL13Rα2-specific strategies. In vitro functional assays, TanCAR T cells distinctly recognized HER2 and IL13Rα2 antigens individually, and interestingly, exhibited enhanced functionality upon encountering both targets simultaneously. HER2/IL13Rα2 bispecific TanCAR T cells showed improved killing of HER2 and IL13Rα2 positive autologous HGG cells and glioma cell lines over HER2- or IL13Rα2-specific CAR T cells from the same patient. Further, adoptively transferred HER2/IL13Rα2 bispecific TanCAR T cells induced regression of established and vasculatized human HGG xenografts and improved tumor control compared to their HER2-specific or IL13Rα2-specific counterparts. This demonstrates the potential of TanCAR T cells as a more effective therapeutic T-cell product for future clinical application.

**Purpose:** The platelet-derived growth factor (PDGF) pathway and in particular its receptor A (PDGFRα) is a possible therapeutic target in DIPG. We explored in vitro the efficacy of a multi-tyrosine kinase inhibitor, dasatinib on new DIPG models derived from stereotactic biopsies performed at diagnosis. **Experimental Design:** Gene expression profile were obtained from 41 DIPG biopsies before treatment and compared with the profiles associated with sensitivity/resistance to dasatinib. A panel of 12 new DIPG cell lines were established, characterized by gene expression microarray and immunohistochemistry. Effects of dasatinib on proliferation, invasion and cytotoxicity were determined on 4 of these cell lines with a range of concentrations from 1 nM to 10 μM using live-cell imaging and cytometric assays. Downstream signaling and receptor tyrosine kinases (RTK) were assessed by Western blot and phospho-RTK array.

**Conclusion:** Dasatinib exhibits anti-tumor effects in vitro on DIPG cell lines that could be increased by the combination with another RTK inhibitor targeting MET.
samples from 159 HGG pediatric patients (age range, 0-18 years; 31 patients <3 years at diagnosis) treated with an age adapted postoperative (radio)chemotherapy according to the German HIT-HGG/GBM or HIT-GBM protocols. The presence of H3.3K27 mutations, and these mutated cases were mostly located in the midline and occurred in non-infants. A significant superior overall survival in diffuse pediatric HGG patients graded as AA III in contrast to GBM IV was detected (for all patients, p < 0.002, for non-infants only, p = 0.025, log-rank-test). The presence of H3.2K7 mutations was a significant adverse prognostic marker. Multivariable analysis showed that WHO grading and H3.3K27 mutational status were independent prognostic markers. By combining these two features in Kaplan-Meier analysis for grouping non-infant patients, overall survival was best for patients with H3.3K27 wildtype (wt) AAlII (n = 19, 5y-OS, 30.5 +/- 13.6 %, followed by H3.3wt GBM IV (n = 40, 5y-OS, 10.6 +/- 5.7 %), and H3.3K27 mutated cases did worse with lowest survival rates, irrespective to WHO grading (AAlII H3.3K27 mutated, n = 11, 5y-OS, 9.1 +/- 8.7 %; GBMV H3.3 mutated, n = 30, 5y-OS, 0 %). These data suggest that WHO histological grading and H3.3 mutational analysis allows a three-tier prognostic stratification of pediatric HGG patients and risk-adapted therapeutic design.

HG-064. MicroRNA PROFILING REVEALS TWO SUBGROUPS OF DIFFUSE INTRINSIC PONTINE GLOMA
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BACKGROUND: Progress in understanding the biology of diffuse intrinsic pontine glioma (DIPG) has been limited by a lack of tumor specimens. Recent work has focused on protein, mRNA and methylation profiles as well as mutations in the genes encoding Histone 3.3. MicroRNAs are dysregulated in cancer, including pediatric CNS tumors. We aim to describe microRNA expression patterns in DIPG and their relationship to H3F3A K27M mutation status. METHODS: Tumor samples from patients DIPG at autopsy (n = 14) were obtained. RNA purification from FFPE was performed using RecoverAll® RNA isolation kit was performed and used for cDNA synthesis. TaqMan® miRNA assays were used to quantify the levels of 762 mature miRNAs from each sample using the Applied Biosystems 7900HT Fast Real-Time PCR system in 384-well low density arrays (TLDAs). Evaluation of H3K27me3 is underway by immunohistochemistry. Clinical and pathological data were collected when available. RESULTS: Tumor tissue was available for 14 patients. Histologically, 13 samples were malignant glioma and 1 was described as an astrocytoma. The presence of a midline location and age were added to an existing DIPG molecular diagnostic panel of 43 miRNAs. DNA sequencing data was available for 12 tumors. 11 tumors carried H3F3A K27M mutations, and 1 was identified as a H3F3A wildtype (wt) AAlII (n = 19, 5y-OS, 30.5 +/- 13.6 %, followed by H3.3wt GBM IV (n = 40, 5y-OS, 10.6 +/- 5.7 %), and H3.3K27 mutated cases did worse with lowest survival rates, irrespective to WHO grading (AAlII H3.3K27 mutated, n = 11, 5y-OS, 9.1 +/- 8.7 %; GBMV H3.3 mutated, n = 30, 5y-OS, 0 %). These data suggest that WHO histological grading and H3.3 mutational analysis allows a three-tier prognostic stratification of pediatric HGG patients and risk-adapted therapeutic design.

HG-065. INTEGRATIVE ANALYSIS OF THE CODING AND NON-CODING GENOME REVEALS NOVEL TARGETS OF TRANSCRIPTIONAL REGULATION IN DIFFUSE INTRINSIC PONTINE GLOMA (DIPG)
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BACKGROUND: The outcome of diffuse intrinsic pontine glioma (DIPG), based on clinical symptoms and imaging appearance rather than tissue, has not improved in the last 30 years. With dramatic advances in MRI-imaging, neurosurgical techniques and molecular profiling of very small samples, there is a growing understanding that DIPG patients’ needs will require novel therapeutic approaches to be revisited. METHODS: A collaboration of 22 clinical institutions throughout the United States joined together into a DIPG treatment consortium called BATS (Biologic and Treatment Strategies) DIPG. Each neurosurgeon involved in the protocol is required to undergo specific operative (or surgical) training and all patients are monitored with extensive clinical and regulatory oversight to ensure that biopsies can be performed safely in a multi-institutional practice. RESULTS: To date, 28 patients with newly diagnosed DIPG (as defined by < 6 months of clinical symptoms and diffuse expansion of the pons on MRI) have undergone biopsy. Tumor tissue for molecular analysis was successfully obtained in 27 of 28 cases. Only one patient experienced significant morbidity possibly associated with the biopsy. Immunohistochemical profiling and genomic sequencing has identified high quality tumor tissue in the majority. Further investigation of somatic non-non-synonymous coding variants compared to other cancers (8-22), and a mutational signature characterised by a predominance of C>T substitutions at NpCpG trinucleotides. 757 recurrent non-coding variants (NCVs) were identified, with 15% located in regulatory regions, including 79 genes overexpressed in DIPG including HOXA3, FGFR3, and BMP1R. A total of 3777 NCVs (9.2%) were identified within ENCODE consensus transcription factor binding sites (TFBS), with 977 regulatory motifs in 377 NCVs. 27.7% of NCVs were carried H3.2K7 mutations, and these mutated cases were mostly located in the midline and occurred in non-infants. A significant superior overall survival in diffuse pediatric HGG patients graded as AA III in contrast to GBM IV was detected (for all patients, p < 0.002, for non-infants only, p = 0.025, log-rank-test). The presence of H3.2K7 mutations was a significant adverse prognostic marker. Multivariable analysis showed that WHO grading and H3.3K27 mutational status were independent prognostic markers. By combining these two features in Kaplan-Meier analysis for grouping non-infant patients, overall survival was best for patients with H3.3K27 wildtype (wt) AAlII (n = 19, 5y-OS, 30.5 +/- 13.6 %, followed by H3.3wt GBM IV (n = 40, 5y-OS, 10.6 +/- 5.7 %), and H3.3K27 mutated cases did worse with lowest survival rates, irrespective to WHO grading (AAlII H3.3K27 mutated, n = 11, 5y-OS, 9.1 +/- 8.7 %; GBMV H3.3 mutated, n = 30, 5y-OS, 0 %). These data suggest that WHO histological grading and H3.3 mutational analysis allows a three-tier prognostic stratification of pediatric HGG patients and risk-adapted therapeutic design.
HG-067. MUTATIONS IN ACVR1 ALONG WITH K27M H3.1 INDUCES ECTOPIC PROLIFERATING LESIONS IN VIVO
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BACKGROUND: Diffuse intrinsic pontine gliomas (DIPG) are the leading cause of death for children diagnosed with brain tumors. As there are currently no effective treatments, it is important to develop a mouse model that mimics the human disease in order to understand the biology of these tumors. Recently it has been shown that activating mutations in ACVR1, which encodes for the Activin A receptor (ALK-2), are found in 20% of DIPG patients. However, how these mutations affect tumors remains unknown. METHODS: Through the use of the RCAS/t-va system, we were able to study the effects of ACVR1 mutations in vitro and in vivo. Neurospheres generated from p3 Nestin t-va p53 Boxed (Np53Em1) mice were infected in vitro with mutated RCAS K206H-ACVR1, G328V-ACVR1, G328E-ACVR1, or WT-ACVR1 along with RCAS K27M H3.1 and RCAS Cre were injected intracranially into Np53Em1 mice to study possible glioma formation. RESULTS: Neurospheres infected with R206H-ACVR1 or G328V-ACVR1 demonstrated increased proliferation in vitro as compared to WT-ACVR1. Likewise, mice that were injected with R206H-ACVR1 or G328V-ACVR1, K27M H3.1, and Cre exhibited ectopic proliferating lesions in the upper or middle brainstem in vivo. Interestingly, mice injected with RCAS R206H-ACVR1 and Cre did not exhibit such lesions, suggesting a cooperation with K27M H3.1. Characterization of these lesions reveals that they are Olig2 negative and Nestin positive. CONCLUSIONS: Based on our results, mutations in ACVR1, in particular R206H and G328V, lead to increased proliferation in vitro. More importantly, these mutations along with K27M H3.1 and p53 loss lead to the formation of ectopic proliferating lesions in vivo. Further characterization of these lesions and determining the mechanism of lesion formation is ongoing and will be useful in treating DIPG.

HG-068. TARGETING mTOR AND NOTCH IN DIFFUSE INTRINSIC PONTINE GliOMA
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Diffuse intrinsic pontine glioma (DIPG) is known to harbor mutations in upstream activators of the mammalian target of rapamycin (mTOR) such as PI3K, PDGFR and EGFR, and our high-throughput analysis of autozygous-derived DIPG samples identified increased expression of Notch pathway effectors. However, while Notch is important for proliferation and self-renewal of adult GBM, and mTOR controls metabolism and growth in multiple cell types, their roles in DIPG are unknown. We confirmed that both primary DIPG samples and DIPG cell lines expressed high levels of Notch pathway using western blot and qPCR assays. Because mTOR and Notch pathways are known to interact in other contexts, we examined the effects of blocking them alone and in combination in the established DIPG neurosphere lines JHH-DIPG1, SF7761, SUDIPG2 and SUDIPG4. Gamma-secretase inhibitor (GSI) treatment inhibited NOTCH activity in a dose dependent manner, as measured by decreased expression of pathway targets Hes1 and Hey1. We also detected significant reductions in cellular proliferation and growth. To verify that the Notch pathway was being targeted, short hairpin RNAs targeting the core binding factor (CBF) were utilized. CBF/p300 is the common transcriptional modulator essential to canonical Notch signaling. Western blots showed that CBF and Hes1 were both decreased compared to the scrambled control. shCBF also led to increased mutant K27M H3.1 expression measured by BrdU (p < 0.05) and increased cell death as measured by cleaved caspase 3 (p < 0.05). We observed a similar decrease in proliferation and increase in apoptosis using the dual TORC1/TORC2 inhibitor PP242. Combination therapy with MKR003 and PP242 led to a significant 2-fold increase in apoptosis compared to either treatment alone. These data suggest that dual targeting of the mTOR and NOTCH pathways with GSI will decrease DIPG cell growth and proliferation, increase cell death, and reduce tumorigenicity.

HG-069. NIMOTUZUMAB EXPERIENCE IN PEDIATRIC HIGH-GRADe GLIAL TUMOURS
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Treatment of high-grade brain tumors, especially diffuse intrinsic pontine gliomas or recurrent gliomas is a major problem. Irradiation plus temozolomide (TMZ) is the current combination approach for high grade gliomas, but with poor results. We, therefore, need to have new treatment strategies with novel drugs. In this study we shared our experience with nimotuzumab in high grade gliomas. We used nimotuzumab 150 mg/m2 in combination with vinorelbine (VNR) in 5 DIPG, one glioblastoma multiforme and one recurrent anaplastic ependymoma. In DIPG group median age was 9 years-old (3.5-17), there were 4 female and 1 male. Diagnosis of DIPG was made with MR, CT and 4, and except one who had biopsy. All were irradiated after diagnosis. TMZ was used in 4 out 5 patients, prior to NMZ-VNR. Upon clinical or radiological progression, NMZ-VNR was started at median 7 (3-13) months. Median time of NMZ-VNR use was 6 months (1.5-15). One patient did not receive TMZ, but still on NMZ-VNR. She is alive for 17 months. Three out of other 4 patients died of progressive disease, but one is still alive for 30 months. We have also used NMZ-VNR in a patient with GBM who progressed at 13 months. Use of NMZ-VNR for 4 months unfortunately failed to stop progression in this patient. Last patient was anaplastic ependymoma who relapsed 2 years after initial diagnosis. After second surgery NMZ-VNR combination was started at 27 months. During 9-month-use of this combination she stayed progression-free, however she later progressed and died of metastatic lesions. Nimotuzumab plus vinorelbine combination seems to have some benefit in high-grade gliomas. Our results with two patients are encouraging. In children, we need to have more clinical trials with these targeted therapies of high-grade gliomas or recurrent gliomas.

HG-070. HISTOLOGICAL AND MOLECULAR ANALYSIS OF A DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG) WITH UNUSUAL METASTATIC RECURRENCE IN A 9-YEAR OLD PATIENT
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INTRODUCTION: Diffuse intrinsic pontine glioma (DIPG) is an aggressive pediatric brainstem tumor with inadequate therapeutic options. Although recurrence for DIPG is typically localized, a small proportion of DIPGs will have metastases. Recent molecular analysis of DIPGs has led to an increase in understanding of the pathogenesis of the disease. We report a patient with an unusual synchronous intracranial and local recurrence. We present post-mortem targeted histone sequencing and methylation analyses of both primary and metastatic lesions. CASE: A 9-year old female with localized DIPG was treated on the COG protocol ACNS0927 with radiation therapy and vorinostat. Seven months after completing radiation, despite symptomatic stability, her DIPG recurred synchronously both locally and along the undersurface of the anterior corpus callosum, septum pellucidum, and even with subsequent therapy, she ultimately progressed and died. METHODS: Pathologic tissue was obtained after consent. Brainstem and metastatic tumors were divided into subsections that were alternatively frozen or formalin-fixed. Sections were stained and reviewed by a neuropathologist. DNA was extracted from both primary and metastatic tumors and used for methylation and directed histone sequencing. Data were analyzed using Partek Genome software. RESULTS: The metastatic lesions were of a higher grade, SUDIPG2 and SUDIPG4 demonstrated rapid growth compared to the primary lesion (30% vs. 8%, respectively). Genomic analysis showed both tumors were H3.1 and H3.3 wild type. Methylation analysis showed the metastatic tumor exhibited a distinct methylation pattern from the brainstem tumor components. CONCLUSIONS: This case demonstrates that disseminated DIPG lesions may be more aggressive although similar in genetic
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makeup to the primary lesion. However, the markedly different methylation patterns may have implications regarding appropriate treatment options in the context of disseminated disease.

HG-071. RE-IRRADIATION PLUS BEVACIZUMAB FOR PROGRESSIVE DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG) - ASSESSMENT OF FEASIBILITY AND TOLERABILITY OF A TREATMENT STRATEGY
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BACKGROUND: For DIPG patients, radiation is the only modality shown to impact the natural history of their disease, and patients universally succumb to their disease. Bevacizumab has been shown to sensitize tumor cells from radiosensitive niches in pre-clinical models. We now summarize our institutional experience using a re-irradiation strategy and bevacizumab for patients with progressive DIPG. METHODS: With an IRB and Privacy Board waiver, a retrospective analysis of patients with DIPG who had undergone re-irradiation with concomitant bevacizumab was performed. Initial treatment comprised dose, time to progression, field and modality of re-irradiation, details of bevacizumab dosing, and acute and post-treatment toxicities were assessed. RESULTS: Five patients fit selection criteria. All received initial RT, two with concomitant biological agents. One patient received post-RT convection enhanced delivery of radio-immunotherapy. All patients recurred with worsening clinical symptoms prompting MRI investigation. Median time to progression for re-RT + bev patients (completion of radiation to radiographic progression) was five months (range 3-5 months). Re-irradiation was to the entire tumor - including areas of radiographic progression - via intensity modulated RT (IMRT). Median dose was 2400 cGy (range 2000-2400 cGy) in 200 cGy fractions. Bevacizumab (10 mg/kg every two weeks) was initiated during the first week of re-irradiation, continuing until progression. Common acute toxicities of bevacizumab (headache, hypertension) were not severe enough to stop treatment. All patients experienced some palliation of their symptoms noted at progression. Two patients imaged post-re-RT had radiographic response. All patients again developed symptomatic progression prompting MRI. Median radiographic clinical stability was five months (range 2-7 months). CONCLUSIONS: In our series, re-irradiation with bevacizumab was tolerable and led to clinical stability in all patients treated and with some evidence of radiographic response. Limited survival time precludes long term toxicity assessment. Further study is needed.

HG-072. EPIDEMIOLOGY OF MALIGNANT PONTINE GLIOMAS (MPG) IN THE PAEDIATRIC POPULATION IN CANADA: A STUDY OF THE CANADIAN PAEDIATRIC BRAIN TUMOUR CONSORTIUM (CPBTC)
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BACKGROUND: The International Diffuse Intrinsic Pontine Glioma (DIPG) Registry may have limited impact due to the low prevalence of DIPG and the poor outcome of patients with typical features of DIPG can be long-term survivors. However, reasons for such unexpected outcomes are unknown.

HG-073. ELEVATED PD-1 EXPRESSION IN TUMOR AND PERIPHERAL BLOOD LYMPHOCYTES OF PEDIATRIC AND ADULT GLIOMA PATIENTS
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INTRODUCTION: The dismal outcome for pediatric high-grade gliomas with conventional therapy highlights the need for additional treatment modalities. Immunotherapy through PD-1 directed monoclonal antibody blockade has produced durable responses in several adult malignancies. To investigate the potential role of PD-1 blockade in glioma, we analyzed the immunologic profile of adult and pediatric patients in tumors and peripheral blood. METHODS: Flow cytometric analysis of tumor infiltrating lymphocytes (TILs) and peripheral blood mononuclear cells (PBMCs) was performed in 8 patients. RESULTS: The percentage of CD8+ TILs expressing PD-1 was significantly elevated in the CD3+ CD8+ T cell population (mean ± SE: 61.90% ± 4.85%) as compared to patient PBMCs (33.10% ± 8.74%, p < 0.05) or donor PBMCs (20.22% ± 8.41%, p = 0.001). Similarly, the percentage of CD3+ CD8+ cells expressing PD-1 was also higher in TILs (56.08% ± 8.08%) as compared to PBMCs (24.39% ± 5.73%, p < 0.05) or donor PBMCs (16.56% ± 3.48%, p < 0.01). Activation marker CD69 expression on CD3+ CD8+ cells was elevated in TILs compared to patient or donor PBMCs (57.28% ± 15.91% vs. 3.67% ± 2.63% vs. 2.25% ± 0.65%) with p-values <0.05. The CD4 to CD8 ratio was significantly lower in TILs (mean ± SE: 0.49 ± 0.22) than donor PBMCs (1.577 ± 0.23, p < 0.01). CONCLUSION: TILs isolated from glioma patients express a significantly increased proportion of PD-1 as compared to donor PBMCs. A similar increase in PD-1, although not significant, was also observed between patient and normal donor PBMCs. These results suggest that a significant proportion of T lymphocytes are functionally impaired in glioma patients and represents a verifiable target for checkpoint inhibitor blockade in these patients.

HG-074. ESTABLISHMENT OF AN INTERNATIONAL DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG) REGISTRY
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BACKGROUND: The International Diffuse Intrinsic Pontine Glioma (DIPG) Registry is a collaborative effort by physicians and researchers from North America, Europe, and Australia to centralize and standardize the collection of clinical, radiographic, pathology and genomics data. The goal is to develop new approaches to diagnosis, response assessment, treatment and follow-up to improve patient outcome. METHOD: The
International DIPG Registry website (dipgregistry.org) facilitates education, recruitment and enrollment. Data are collected by registry staff, working directly with patient families and institutions. Living patients are referred through the website. Institutions providing deceased patient records after institutional approval. Demographic, clinical, treatment, and outcome data are abstracted by DIPG Registry staff using standardized case report forms (CRFs), coded and stored in an Oncore® clinical data base. Anonymized MRIs are uploaded utilizing Amicas® PACS Research System. Studies at diagnosis, and each progression or response are requested. A pathology repository stores tissue samples for central review and future research and will be linked to a bioinformatics repository of DIPG genomics data (dipg-progenetix.org). Collectively these repositories form the DIPG Registry. Overseeing the strategic direction of the registry are scientific and steering committees who also review and approve proposals from investigators around the world. RESULTS: Since April 2012, 195 patients have been enrolled; with 530 additional patients committed from 27 participating institutions internationally. The radiology repository contains over 700 studies from 92 patients. The pathology repository contains tissue on 14 patients, (10 fresh-frozen), with 36 specimens pending submission. Central review of specimens and imaging is ongoing. Two approved research proposals include a study of long-term DIPG survivors and an epidemiological study to determine DIPG incidence patterns in North America. CONCLUSIONS: The International DIPG registry provides a highly collaborative, international, hypothesis-driven research infrastructure that can support a wide spectrum of interdisciplinary and translational projects in DIPGs for all investigators.

### HGD-075. TUMOUR LYSE PULSED DENDRITIC CELL VACCINATION FOR CHILDREN WITH HIGH GRADE GLIOMA

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We have developed a phase I trial will assess the safety and feasibility of delivering tumour lysis pulsed dendritic cell vaccine in paediatric patients with a primary diagnosis of high grade glioma. The principle of autocrine immunotherapy is to generate potent anti tumour responses, resulting in the generation of effector cells capable of specifically killing tumour cells. In diseases such as melanoma this approach has been used successfully to generate responses against defined tumour associated antigens (TAA). An alternative approach, for tumours in which TAA’s have either not been defined, or are not sufficiently immunogenic, is to generate responses against a number of different but undefined tumour antigens using a autologous tumour lysate. We have evaluated immune responses following vaccination with alpha type 1 polarised dendritic cells matured with both type 1 and 2 interferons in serum free medium, which have previously been shown to generate high levels of IL-12. The trial also is also evaluating the strength of immune responses following maturation in the presence or absence of prostaglandin E2. Dendritic cell vaccines are given in relapse or as primary treatment in combination with temozolomide chemotherapy. Although dendritic cell vaccination has been used before in children with cancer including high grade glioma, the incorporation of vaccinations into the context of standard chemotherapy is relatively novel. Hence the trial is predominantly addressing the question of the feasibility of delivering such a vaccination strategy in this setting. Additionally the study will also provide data on the nature of the immune responses against the tumour and is designed to answer questions regarding the optimal way to activate autologous dendritic cells in the laboratory. Immune response data from the first 4 patients will be presented.

### HGD-077. REDUCTION IN H3K27ME3 IS A MOLECULAR AND PROGNOSTIC SURROGATE IN PEDIATRIC GLOBLASTOMAS

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Pediatric glialomas (GB) are highly aggressive and lethal tumors. Recent sequencing studies have shown that ~30% of pediatric GB show mutations in the H3F3A gene, a variant encoding histone H3.3, H3F3A K27M mutations lead to global reduction in H3K27me3. Our goal was to evaluate the utility of this reduction in H3K27me3 as a clinical and histopathologic biomarker. To address this question we assessed H3K27me3 by immunohistochemistry in 160 (118 pediatric and 42 adult) brain tumors of various subtypes and grades and 45 non-neoplastic reactive brain tissues. Global reduction in H3K27me3 was specific to H3F3A K27M mutant GB and was not observed in all other examined conditions. Further, H3F3A K27M mutant GB with reduced H3K27me3 exhibited a significantly poor prognosis compared to H3F3A wild type tumors with preserved H3K27me3 (Log-rank (Mantel-Cox) test hazards ratio = 2.9, p = 0.0021). These results suggest that global reduction in H3K27me3 is a histopathologic molecular surrogate for H3F3A K27M mutant GB and defines a prognostically poor subset of pediatric GB.