induced significant cell death in all 4 cell lines *in vitro*. Temozolomide, difluoromethyornithine and chloroquine (CQ) were then tested together with pegArg-I in U87 *in vitro*. We found that only CQ showed additive effect with pegArg-I against glioma *in vitro*. Such additive cytotoxic effect may be associated with enhanced autophagy and necrosis as shown in transmission electron microscopy and autophagy markers' expression by Western blotting. PegArg-I prolonged the survival of glioma mice, suggesting its possible anti-glioma efficacy. However, CQ+pegArg-I didn't show further significant anti-cancer efficacy *in vivo*. CONCLUSION: PegArg-I may be useful in slowing the progression of glioma, but additional drug candidate which works synergistically with pegArg-I remains to be explored.

HGG-29. A CASE OF CIRCUMSCRIBED HIGH-GRADE ASTROCYTOMA WITH ATRX AND CDKN2A/B ALTERNATIONS WHO WAS INITIALLY DIAGNOSED AS GLIOBLASTOMA AND HAS 20 YEARS SURVIVAL

Yusuke Kobayashi, Yosuke Sato, Takashi Kon, Daisuke Tanioka, Katsuyoshi Shimizu, and Tohru Mizutani; Department of Neurosurgery. Showa University School of Medicine, Sinagawa-ku, Tokyo, Japan

Pediatric high-grade gliomas are rare and often hard to classify, which grow locally and show longer survival than diffuse high-grade gliomas in adults. We report a case of circumscribed high-grade astrocytoma who was initially diagnosed as glioblastoma and has 20 years survival. A 7-year-old girl suffered from epileptic seizure due to a left occipital lobe tumor. The tumor was resected in another hospital and diagnosed as glioblastoma. The tumor disappeared after extended local irradiation and chemotherapy using nimustine hydrochloride (ACNU) and cisplatin (CDDP). Eighteen years after initial onset, first recurrence was confirmed as the intra-tumoral hemorrhage. The tumor was resected and diagnosed as anaplastic oligoastrocytoma. After 6 courses of temozolomide (TMZ), the tumor disappeared. Twenty years after initial onset, the second local recurrence was confirmed. Although gamma knife and TMZ was performed, the tumor did not disappear. The tumor was surgically resected. Histopathology showed localized growth with some infiltration and mitosis but lacked pseudopallisading and microvascular proliferation. The tumor was diagnosed as circumscribed high-grade astrocytoma. Immunostaining revealed ATRX nuclear loss and CDKN2A / B homozygous deletion. After 10 courses of TMZ, the third local recurrence was confirmed. The tumor was completely removed and has not occurred recurrence more than 3 months after the last operation. Circumscribed high-grade glioma is expected to survive longer than invasive glioma. Pediatric gliomas should differ from adult gliomas in the genes of tumorigenesis. Care should be taken for its diagnosis and treatments. We also need a new classification based on histology and gene profile. HGG-30, ANALYSIS OF PEDIATRIC GLIOMAS IN OUR INSTITUTE *Kaoru Tamura*, Mai Fujioka, Masae Kuroha, Motoki Inaji, Yoji Tanaka, Tadashi Nariai, and Taketoshi Maehara; Tokyo Medical and Dental University, Tokyo, Japan. PURPOSE: Recent advances in genetic interrogation of pediatric glioma increase the importance of molecular diagnosis using surgical specimen. However, surgical resection may be avoided to preserve quality of life, especially in brain stem glioma cases. We retrospectively examined diagnosis and treatment of pediatric gliomas in our hospital. METHODS: This study includes 14 consecutive glioma patients under the age of 18 who underwent initial treatment at our hospital from 2000 to 2019. Histopathological diagnosis, clinical course and molecular status such as IDH, H3F3A and BRAF were analyzed. RESULTS: 5 patients (1 pilocytic astrocytoma (PA), 3 diffuse astrocytomas, 1 oligodendroglioma were treated only by surgical resection (group A). 7 patients (1 PA, 1 anaplastic oligodendroglioma, 2 diffuse midline gliomas and 3 glioblastomas (GBM)) received radiation and/or chemotherapy after surgical resection (group B). 2 diffuse intrinsic pontine gliomas (DIPG) received radiation and chemotherapy without surgical resection (Group C). No IDH mutation was observed in all pathological specimen obtained cases. BRAF alteration was observed in all PA cases. 1 case of GBM had *BRAF V600E*mutation and the other had H3K27M mutation. During a median of 7.7 years of follow-up, group A patients have no recurrence. Group B includes various diagnosis and prognosis. 2 group C patients diagnosed DIPG by MRI showed different clinical courses. CONCLUSION: Pediatric gliomas include diverse biological subgroups and show broad range of clinical behavior. Since pediatric glioma has a low incidence and a wide variety of genetic mutations, multicenter study is important to improve the treatment of pediatric glioma.

HGG-31. UNIQUE BIOLOGICAL CHARACTERISTICS OF RADIATION-INDUCED GLIOMAS

Kateřina Váňová[1,2], Aleš Vícha[1,2], Lenka Krsková[3], Josef Zámečník[3], Běla Malinová[3], David Sumerauer[1,2], Adéla Mišove[4,2], Petr Libý[5], and Michal Zápotocký[1,2]; [1]Prague Brain Tumor Research Group, Second Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic, [2]Department of Pediatric Haematology and Oncology, Second Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic, [3]Department of Pathology and Molecular Medicine, Second Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic, [4]Prague Brain Tumor Research Group, Second Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic, [5]Department of Neurosurgery, Second Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic

Radiation-induced gliomas (RIGs) are the most common secondary solid tumours with very unfavourable prognosis. We aimed to describe different clinical and molecular biological characteristic of RIGs from primary gliomas. We reviewed clinical data of ten patients with RIGs. In patients with available samples, we used the whole genome methylation array and performed targeted sequencing for specific mutations. Between 2000–2018, we diagnosed RIG in 10 patients (M/F 2/8) aged 5–12 years at primary diagnosis of different solid tumours and acute leukaemia. These patients developed RIG with a median 9.5 years (ranging 3–31) after primary diagnosis. Eight patients died within 1 year after diagnosis of RIG and 2 patients are still alive more than 4 years from this diagnosis. According to Heidelberg DNA methylation-based classification, most RIGs belong to the IDH-wild type glioblastoma subclass midline which biologically corresponds to diffuse midline glioma (DMG). However, compared to primary DMGs they do not carry the characteristic H3K27M mutation. One patient developed anaplastic ganglioglioma with BRAF-V600E mutation and methylation profile identical to pleomorphic xanthoastrocytoma (alive for 4 years after diagnosis of RIG). In half of the patients from the group DMGs IDH wild type, examined by methylation array, PDGFRA amplification was found. Our data shows that most RIGs are midline IDH-wild type glioblastomas with poor prognosis that are biologically different from primary DMGs. PDGFRA amplifications are potentially targetable by kinase inhibitors in order to order to prognosis of these patients.

HGG-32. UNCOVERING THERAPEUTIC VULNERABILITIES IN MISMATCH REPAIR-DEFICIENT GLIOMAS

Adam Boynton[1], Sangita Pal[1], Mehdi Touat[1], Naomi Currimjee[1], Kenin Qian[1], Charlotte Bellamy[1], Patricia Ho[1], Jim Berstler[2], Keith Ligon[1], Rameen Beroukhim[1], and Pratiti Bandopadhayay[1]; [1]Dana-Farber Cancer Institute, Boston, MA, USA, [2]Broad Institute, Boston, MA, USA

INTRODUCTION: We have observed that approximately 26% of recurrent gliomas acquire hypermutation following treatment with temozolomide (TMZ). Intriguingly, 91% of these tumors harbor mutations in mismatch repair (MMR) genes. Strategies to target MMR-deficient gliomas thus stand to impact a large number of patients. METHODS: We ablated the MMR genes *MSH2, MSH6, MLH1, and PMS2* using an all-in-one sgRNA-CRISPR/Cas9 expression vector to generate panels of isogenic MMR knockouts in patient-derived glioma cell lines. We have characterized the phenotype of these MMR-deficient glioma cells, and leveraged high-throughput drug screens to identify therapeutic vulnerabilities induced by loss of MMR. RESULTS: We demonstrate that sgRNA-CRISPR/Cas9 targeting of either *MSH2* or *MLH1* – the two obligatory components of the MutSα and MutLα complexes, respectively – also results in loss of protein expression of their respective binding partner MSH6 or PMS2. Moreover, we show that loss of each MMR component confers resistance to TMZ while maintaining sensitivity to the alkylating nitrosourea CCNU. Furthermore, we show that long-term TMZ treatment of *MSH2* and *MSH6* knockouts in an *MGMT*-methylated line induces hypermutation with enrichment of C > T mutations but not in MMR wild-type controls. Lastly, loss of MSH2 or MLH1 confers differential dependencies to small molecule inhibitors. CONCLUSIONS: CRISPR/Cas9 knockout of individual MMR pathway members allows us to systematically study the response of MMR-deficient cells to alkylating agents in an isogenic context. MMR deficiencies in glioma confer dependencies to small molecule treatment, which may inform future therapies for MMR-deficient tumors.

HGG-34. DETECTION OF ONCOGENIC FUSION EVENTS IN SUPRATENTORIAL GLIOBLASTOMAS OF YOUNG CHILDREN

Torsten Pietsch[1], Christian Vokuhl[2], Gerrit H. Gielen[1], Andre O. von Bueren[3], Everlyn Dörner[1], Glen Kristiansen[2], Andreas Waha[1], and Christof Kramm[4]; [1]Department of Neuropathology & DGNN Brain Tumor Reference Center, University of Bonn, Bonn, Germany, [2]Department of Pathology, University of Bonn, Bonn, Germany, [3]Department of Pediatrics, Obstetrics and Gynecology, Division of Pediatric Hematology and Oncology, University Hospital of Geneva, Geneva, Switzerland, [4]Department of Pediatric Hematology / Oncology, University of Göttingen, Göttingen, Germany

INTRODUCTION: Glioblastoma in infancy and early childhood is characterized by a more favorable outcome compared to older children, a stable genome, and the occurrence of tyrosine kinase gene fusions that may represent therapeutic targets. METHODS: 50 glioblastomas (GBM) with supratentorial location occurring in children younger than four years were

retrieved from the archives of the Brain Tumor Reference Center, Institute of Neuropathology, University of Bonn. DNA and RNA were extracted from FFPE tumor samples. Gene fusions were identified by FISH using break-apart probes for *ALK, NTRK1, -2, -3, ROS1* and *MET*, Molecular Inversion Probe (MIP) methodology, and targeted RNA sequencing. RESULTS: 37 supratentorial GBM occurred in the first year of life, 13 GBM between one and four years. 18 cases showed fusions of *ALK* to different fusion partners; all occurred in the first year of life (18/37 cases, 48.6%). Fusions of *ROS1* were found in 5, *MET* in 3, *NTRK1*, -2, -3 in 10 cases. 12 cases showed no and two novel fusions. The different methods led to comparable results; targeted RNA sequencing was not successful in a fraction of cases. Break-apart FISH led to reliable results on the next day, MIP technology represented the most sensitive method for analysis of FFPE samples. CONCLUSIONS: Gene fusions involving the tyrosine kinase genes *ALK, MET, ROS1* and *NTRK1, -2, -3* occurred in 72% of glioblastomas of children younger than four years; the most frequent were *ALK* fusions occurring in infant GBM. DNA based MIP technology represented the most robust and sensitive assay.

HGG-35. PEDIATRIC PLEOMORPHIC XANTHOASTROCYTOMA WITH ANAPLASIA TREATED WITH SURGERY AND ADJUVANT CHEMOTHERAPY: A CASE SERIES OF 3 LONG-TERM SURVIVORS

Rebecca Ronsley[1], Christopher Dunham[1], Stephen Yip[2], Juliette Hukin[1], and Sylvia Cheng[1]; [1]British Columbia Children's Hospital, Vancouver, BC, Canada, [2]British Columbia Cancer Agency, Vancouver, BC, Canada

OBJECTIVE: Pleomorphic xanthoastrocytoma (PXA) with anaplasia is a rare histological subtype of central nervous system astrocytoma and generally treated as high grade gliomas. The optimal extent of therapy required is unknown. Here we report on 3 pediatric cases of PXA with anaplasia. We also describe molecular features and methylation profile of PXA with anaplasia compared to age-matched PXA without anaplasia. METHODS: Our institutional database was queried for cases of PXA since 1998 and 3 cases with anaplasia were identified and records reviewed. RESULTS: 2/3 patients were male and all were aged 12 at diagnosis. All underwent a gross total resection (GTR), where the diagnosis of PXA with anaplasia was made. Immunohistochemistry demonstrated that two cases were BRAF V600E positive and two were CD34 positive. Methylation profiling revealed unique pattern of CpG methylation/unmethylation. All patients underwent 5400cGy radiation to the surgical bed. 2/3 patients received concurrent temozolamide with radiation followed by maintenance chemotherapy with temozolamide and lomustine for 6 cycles as per the Children's Oncology Group Protocol ACNS0423. These two patients had a continued complete response. The third patient received temozolamide following radiation and subsequently had recurrent disease at the end of treatment and went on to have a re-resection GTR and achieved complete response after 6 cycles of lomustine, vincristine and procarbazine. All are alive with no evidence of disease at more than 2 years post treatment completion (OS=100%,EFS=67%). CONCLUSIONS: This rare pediatric tumor is not well understood. The genetic landscape may be informative for optimizing treatment and prognosis.

HGG-36. HIF-2: A NEW DRUG TARGET IN PEDIATRIC HIGH-GRADE GLIOMA WITH PROMISING PRECLINICAL RESULTS

Quentin Fuchs[1], Marina Pierrevelcin[1], Christophe Papin[2], Monique Dontenwill[1], and Natacha Entz-Werlé[1,3]; [1]UMR CNRS 7021, Strasbourg, France, [2]IGBMC, Strasbourg, France.,[3]University Hospital of Strasbourg, Strasbourg, France

Pediatric high-grade gliomas (pHGGs) have a very dismal prognosis and need new innovative strategy for treatment. Despite the past discovery of histone H3 driver mutations, we are not able for instance to stop this induced epigenetic remodulation. Therefore, proactive translational studies wish to go further discovering new targetable proteins in pHGG. In our past clinical work, we were able to link significantly HIF-2alpha to a worse pHGG outcome and to their treatment resistance. We designed this new work to determine in several patient-derived cell lines (6 PDCLs) with or without H3.3 mutation the variation of HIF-2alpha, its role, its induction in normoxic and hypoxic microenvironment and its transcriptional targets using RNAseq, metabolomics and ChipSeq analyses. Complementary functional analyses were performed using siRNA strategy during cultures and migration assays. Finally, preclinical drug testing involving commercialized and non-commercialized HIF-2alpha specific inhibitors in the same PDCLs were evaluating their antiproliferative and pro-apoptotic effect. Our results confirmed the central role of HIF-2alpha in cell resistance to treatment, in pHGG stemness features and its direct link with metabolism adaptation and histone interaction. After the confirmation of its frequent presence in multiple PDCLs initiated from thalamic pHGGs and DIPG, we were using inhibitors in a single and combinatorial strategy targeting HIF-2alpha plus another hypoxia biomarker (mTor). This preclinical targeting was highly effective to favor cell arrest, apoptosis and to stop cell migration. In conclusion, HIF-2alpha seem to be a major biomarker in pHGGs that might be targeted giving a useful new opportunity for pHGG treatments.

HGG-37. PAEDIATRIC GLIOBLASTOMA CELLS SHOW CRITICAL DEPENDENCIES ON EPIGENOMIC AND EPITRANSCRIPTOMIC CONTROL OF GENE EXPRESSION BY H3.3G34R/V MUTATIONS

Lynn Bjerke[1], Alan Mackay[1], Rebecca Rogers[1], Yura Grabovska[1], Valeria Molinari[1], Sara Temelso[1], Kristina Cole[2], Angela Waanders[3], Angel Montero Carcaboso[4], Maria Vinci[5], and Chris Jones[1]; [1]The Institute of Cancer Research, London, United Kingdom, [2]Children's Hospital of Philadelphia, Philadelphia, PA, USA, [3]Ann & Robert H Lurie Children's Hospital, Chicago, IL, USA, [4]Hospital Sant Joan de Deu, Barcelona, Spain, [5]The Bambino Gesù Children's Hospital, Rome, Italy

H3.3G34R/V mutations are restricted to glioblastomas of the cerebral hemispheres, and occur predominantly in adolescents and young adults. We had previously shown these mutations to result in a global re-organisation of the activating mark H3K36me3 to drive transcription of key developmental transcription factors and oncogenes such as MYCN, however the precise mechanism was unclear. Using multiple H3G34R/V samples and ChIP-seq with antibodies specific to both wild-type and mutant histone H3.3, we show a high degree of incorporation of mutant histone into nucleosomes, with only a minority (<15%) remaining wild-type only. Heterogenous G34-mutant nucleosomes displayed significantly elevated H3K36me3 binding, the majority apparently in *trans* to the mutation on the wild-type H3.3, and expression signatures associated with chromatin modification, cell cycle progression, DNA repair and gene transcription. Super-enhancer analysis by H3K27ac ChIP-Seq highlighted lineage-dependent transcription factors and previously identified targets MYCN and NOTCH1 (both stabilised by FBXW7, down-regulated by loss of chromosome 4q), as well as specific H3K36 lysine demethylases and splicing factors. Whole-genome CRISPR-Cas9 screening of patient-derived H3.3G34R/V cells identified critical dependencies on these latter targets, in addition to a general essentiality for genes involved in RNA processing. Assessment of RNA methylation by MeRIP-seq revealed a strong concordance of m6A-modified RNA and H3K36me3 binding, with differentially modified transcripts in mutant cellsassociated with the 3'-UTR but also the promoter and gene bodies. These data highlight the critical nature of the epitranscriptome in H3.3G34R/V-mutant paediatric glioblastoma, and highlight novel targets for therapeutic intervention.

HGG-38. A COMPARATIVE PROTEOMIC-ANALYSIS OF THE CELL MEMBRANE FRACTIONS OF HISTONE 3 MUTATED BRAIN TUMOURS TO IDENTIFY NOVEL THERAPEUTICS

James Pickering, Ruman Rahman, Richard Grundy, Robert Layfield, and Farhana Haque; University of Nottingham, Nottingham, United Kingdom

Improvements in the treatments for childhood and adolescent brain tumours, High-Grade Glioma (pHGG) and Diffuse Intrinsic Pontine Glioblastoma (DIPG), have not advanced much and they continue to carry a very poor prognosis. These brain tumours are now defined by mutations affecting histone 3 proteins, indeed 80% of DIPGs harbour histone H3.1 and H3.3 K27M somatic mutations whilst 30% of pHGGs exhibit H3.3 G34R or G34V mutations. We hypothesized that the histone 3 mutant tumours will have distinct mutation specific surfactome. We therefore analysed the cell surface proteomics of pHGG and DIPG, in order to identify novel targets for therapy. We have at first isolated the cell membrane fractions from a range of patient cells carrying different histone 3 mutations (G34R, G34V, K27M), relative to wild type histone 3. A comparative quantitative mass-spectrometry analyses of these cell surface membrane fractions is then performed to identify specific targetable factors, which can be then be used for tumour specific precision-therapy. Results of these experiments will be presented.

HGG-39. CLINICAL CHARACTERISTICS AND OUTCOME OF PATIENTS WITH RADIATION-INDUCED GLIOMA

Makoto Ohno[1], Yasuji Miyakita[1], Masamichi Takahashi[1], Takaki Ohmura[1], Natsuko Satomi[1], Yukie Tamura[1], Yuko Matsushita[1], Koichi Ichimura[2], and Yoshitaka Narita[1]; [1]National Cancer Center Hospital, Chuo-ku, Tokyo, Japan, [2]National Cancer Center Research Institute, Chuo-ku, Tokyo, Japan

The development of gliomas subsequent to therapeutic cranial irradiation is a rare but serious complication. The purpose of this study is to understand the clinical characteristics and outcome of patients with radiation-induced glioma (RIG). Between 2001 and 2018, we identified 10 patients with RIG, which satisfied the Cahan's criteria in our data base. There was no sex predominance (M: 5, F: 5), and the median age of the primary diseased was 13.5 years (range: 1–39). The primary diseases included 2 germinoma, 2 acute lymphoblastic lymphoma, 2 medulloblastoma, 1 diffuse astrocytoma, 1 pilocytic astrocytoma, 1 pituitary adenoma and 1 metastatic tumor from