NEURO-ONCOLOGY

Abstracts

2. GASS/AXL-FAMILY RECEPTORS IN SCHWANNOMA PATHOLOGICAL PROLIFERATION, ADHESION AND SURVIVAL
S Ammoun, L Zhou, M Barczyk, D Hilton, S Hafizi, and CO Hanemann;
University of Plymouth

INTRODUCTION: Tumour suppressor merlin deficiency leads to the development of schwannomas, meningeiomas and ependymomas. Using an in vitro model of human schwanna we have demonstrated that merlin-deficiency leads to increased cell proliferation, cell-matrix adhesion and survival involving ErbB2/3, platelet-derived-growth-factor-receptor-β (PDGFR-β), and Insulin-like growth factor receptor-1 (IGF-IR) acting via extracellular-signal-regulated kinase 1/2 (ERK1/2), AKT and JNK. We have also inhibited proliferation of schwannoma by treatment with sorafenib, imatinib, lapatinib and REZ-235. Since, schwannoma overexpress multiple receptors/signalling pathways the inhibition of a single target is not sufficient. Therefore, all relevant receptors/signalling pathways must be revealed. AXL-silenced RTKs (AXL, SKY, MDR and Ron) are over-expressed in cancers correlating with poor prognosis. Expression profiling and phosphoprotein arrays showed that AXL-family receptors are overexpressed/activated in human schwannoma. METHODS: Western blotting, immunohistochemistry, primary human cell culture, proliferation/adhesion assays. RESULTS: AXL, SKY and their ligand Gas-6 are over-expressed as well as activated in human schwannoma cells and tissues leading to increased proliferation and adhesion. CONCLUSIONS: AXL, SKY and Gas-6 constitute new potential therapeutic targets in merlin-deficient tumours.

3. CD133 GLYCOSYLATION IS AFFECTED BY HYPOXIA IN CULTURED GLIOMA STEM CELLS
Kristina S Lehnus, Laura K Donovan, Geoffrey J Pilkington, and Qian An;
Cellular and Molecular Neuro-Oncology Research Group, Institute of Biomedical and Biomolecular Sciences, School of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth, UK

INTRODUCTION: The widely employed cancer stem cell (CSC) marker CD133 has come under scrutiny in gliomas as the expression and poorly characterized glycosylation patterns of CD133 could affect immunological detection of CD133+ CSCs. The present study aims to investigate the effect of oxygen deprivation on CD133 expression and glycosylation in a glioma multiforme multiforme (GMM) cell line. Cells were cultured under normoxic (21% oxygen) and hypoxic (3% oxygen) conditions. Two CD133 antibodies, CD133+ (glycosylated) and ab19898 (unglycosylated), were used for CD133 immunodetection by flow cytometry and immunohistochemistry (ICC). RESULTS: Using flow cytometry, ab19898 detected 94.1% and 96.2% CD133+ cells were detected in hypoxia irrespective of antibodies used, a significantly higher geomean fluorescence intensity (GMI) was demonstrated by ab19898 (p < 0.005) in positive cells. ICC confirmed that ab19898 binds CD133 intracellularly while CD133+ binds the extracellular domain. CONCLUSIONS: This study demonstrates the shortcomings of CD133+ as the most used antibody to detect CD133+ CSCs. Our data indicate that 1) previously reported CD133− cells may have been misidentified using the glycosylation-specific CD133/1 as constitutive expression of CD133 was detected by the intracellular antibody ab19898; 2) hypoxia promotes glycosylation status of CD133. Therefore, the role of CD133 should be further explored in order to elucidate its biological significance. We are currently investigating CD133-associated signal transduction pathways and the impact of CD133-KD (knockdown) on cell biology.

4. COMPLICATIONS FOLLOWING GLIADEL WAFER INSERTION DURING SURGERY FOR GliOBLASTOMA MULTIFORME: A SINGLE CENTRE-EXPERIENCE
Ian A Anderson and Simon Thomon; Leeds Teaching Hospitals NHS Trust

INTRODUCTION & OBJECTIVE: Surgically implanted Carmustine-impregnated wafers (Gliadel®) confer a significant survival benefit in the surgical treatment of high grade glioma although complications have been reported including seizures, poor wound healing and infection. Complication rates are infrequently quantified in published reviews. We report the complication rates for all patients treated with Gliadel at a Yorkshire neurosurgical centre in a two-year period. METHODS: Retrospective review of case notes, electronic records and GP records. Outcome data collected on survival, wound breakdown, wound infection, post-operative length of stay, readmission rates, need for further surgery and seizure record. RESULTS: 11 patients identified: 8 male, 3 female; mean age 45.6 years; 10 glioblastoma multiforme, 1 anaplastic pleomorphic xanthoastrocytoma; 7 recurrent and 4 primary tumours; 7 frontal, 2 parietal. Estimated mean follow-up was 4.3 days (range 1–31) and 7 remain under follow-up (mean time since resection 8 months, range 2–17). 1 episode of suspected infected wound breakdown documented (9%); treated with oral antibiotics as an outpatient. No patients were re-admitted or required further surgery. Median post-operative length of stay was 4.3 days (range 1–15). CONCLUSIONS: Quoted rates may over-estimate the frequency of complications following insertion of Gliadel wafers after resection of high grade glioma. Just as clinicians must scrutinise the evidence in favour of a new treatment before implementing it, we must maintain objectivity when considering the significance of any adverse effects of a product before discounting it.

6. SURVIVAL OF PATIENTS WITH GliOBLASTOMA – HOW DO WE COMPARE TO STUPP?
M Barley, E Lekka, J Law, and C Davis; Royal Preston Hospital

INTRODUCTION: The publication of the results of the EORTC-NCIC trial in 2005 and 2009 changed the standard of care for patients with histologically proven glioblastoma. Their treatment paradigm is debulking surgery followed by concurrent 6 cycles of radiotherapy plus concomitant daily Temozolomide followed by up to six cycles of adjuvant Temozolomide has become known as the “Stupp regime”. The present study compares the survival of patients with Glioblastoma treated at our institution with the reported survival of the EORTC-NCIC trial. METHODS: We retrospectively collected and analysed data of treatment and survival of all patients with histologically proven glioblastoma for the five year period from 1/1/06 to 31/12/10. The Kaplan-Meier method was used to produce survival data and these were compared with the EORTC-NCIC trial results. RESULTS: In the 5 years from Jan 2006 to Dec 2010, 375 patients had a histological diagnosis of glioblastoma and 98 were treated with the “Stupp regime”. Of these, 68.4% underwent debulking surgery and 31.6% underwent biopsy. Median age was 56, median survival 13.0 months and the 2-year survival rate was 17.3% (compared to 14.6 months median survival and 2-year survival rate of 27.2% in the EORTC-NCIC trial). For the latter 3 years from Jan 2008 to Dec 2010 the estimated 2-year survival rate was 22%. In 2006 56% of patients underwent debulking surgery compared to 73% in 2009 and 82% in 2010. CONCLUSIONS: At our institution we have yet to achieve the survival rates achieved in the EORTC-NCIC trial. However, our estimated 2-year survival rate for glioblastoma patients has increased with time, and this may be due to an increased proportion of patients receiving surgical debulking rather than biopsy.

7. STEREOTACTIC RADIosurgery AT LEEDS GAMMA KNIFE CENTRE: INITIAL EXPERIENCE OF TREATING BRAIN METASTASES
KE Banfill, C Loughrey, and P Hatfield; St James’s Institute of Oncology

INTRODUCTION: Stereotactic radiosurgery for brain metastases has been carried out at the Leeds Gamma Knife Centre since March 2009. METHOD: A retrospective review of patients treated with radiosurgery between March 2009 and July 2010 was carried out (minimum 6 months follow up). Data on survival, primary tumour, Karnofsky performance status (KPS), previous whole brain radiotherapy (WBRT) and time from diagnosis to identification of brain metastases were recorded at initial presentation. Patients were then followed up with regular magnetic resonance imaging of the brain and data on toxicity and oral steroid dose were recorded. RESULTS: 58 patients (19 male/39 female) had a median survival of 50.4 weeks (95% CI, 32.6 to 68.2 weeks). Lung (36%) and breast (27%) were the most common primary tumours. 12% of patients had WBRT prior to radiosurgery and these patients had improved survival (possibly due to case selection). There was a clear but non-significant trend that patients with a disease-free interval longer than 1 year had a better outcome. The size of the largest treated metastases seemed a better predictor of outcome than number of metastases treated. 16% of patients had WBRT following radiosurgery. Data on steroid dose at first follow up were available for 45 patients. Steroid dose dropped significantly after radiosurgery (P < 0.01) and was the same or less in 91% of patients. There were only 3 cases of grade 3 toxicity (fatigue, weakness and seizure). CONCLUSION: Our

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study reports survival comparable with other series on radiosurgery and demonstrates a significant decrease in steroid dose following treatment.  

8. HIGH THROUGHPUT DRUG SCREENING OF PAEDIATRIC GLIOMA CELL LINES
Dorion, Bax, Ryan Bishop, Elliott, Ryan Bishop, Katy Taylor, Lyndel Marshall, Nathalie Gasper, Marta Viana-Pereira, Rui Reis, Jane Renshaw, Alan Ashworth, Chris Lord, and Chris Jones; Institute of Cancer Research

INTRODUCTION: The clinical outcome for children with high grade glioma is dismal, with little change in the past four decades. Novel therapies targeting the genetic alterations that drive the disease are therefore sorely needed. Recent molecular profiling data have demonstrated that paediatric high grade glioma cells differ biologically from their adult counterparts, and therefore may require different targeted agents. In order to identify candidates for targeted inhibition, we have performed an in vitro high-throughput drug screen on a series of well-characterised paediatric glioma cell lines. METHODS: The drug screen consisted of commercially available small molecule inhibitors and chemotherapeutic agents, at concentrations ranging from 1nM–1 µM. The compounds were screened in triplicate on the cell lines SF188, KNS42, UW479, Res259 and Res186. The primary endpoint was cell viability as measured using the CellTiter-Glo assay. Selected hits were then screened in more detail using MTS assay, immunoblotting, expression microarrays and cell cycle analysis. RESULTS: The drug screen data were compared to similar data generated on a panel of breast and ovarian cancer cell lines, identifying both generalisable and cell line specific hits. All glioma cell lines were found to be highly sensitive to inhibitors of aurora kinase, PI3-kinase/mTOR and the molecular chaperone HSP90. Individual cell lines were found to be sensitive to inhibition of distinct receptor tyrosine kinases related to the molecular profiles of the cells, including PI3-kinase, EGFR, MET and FGR families. CONCLUSION: These data demonstrate the suitability of high-throughput drug screening to identify novel classes of targeted therapies, which may be applied to paediatric high grade glioma models. In-depth validation of target inhibition related to the underlying biology of the tumour cells will provide rationale for selecting patients most likely to benefit from specific therapeutic regimens.  

9. THE SELECTIVE ACTION OF TRICHOSTATIN A IN COMBINATION WITH THE DNA DAMAGE AGENTS, TEMOZOLOMIDE AND CISPLATIN
CA Bellamy, I Shaw, JF Alder, AJ Shorrocks, and RW Lea; Brain Tumour North West, School of Pharmacy and Biomedical Sciences

INTRODUCTION: Changes to the epigenome of a cell precede genetic alterations during transformation to a malignant state; hence enzymes involved in epigenetic modulation are a potential therapeutic target. One such target is histone deacetylase (HDAC), an enzyme involved in regulation of gene expression by deacetylation of histones. HDAC inhibitors (HDACi) promote DNA damage in normal and cancerous cells however, due to aberrant genetics of neoplasms, only normal cells can repair this damage. The aim of this study was to assess whether HDACi combined with the DNA damaging agents cisplatin and temozolomide, could work selectively and synergistically to increase cytotoxicity in a glioma cell line compared to a normal astrocytic culture. METHODS: 0.1 nM Trichostatin A (TSA) was used in combination with 1 µM temozolomide or 1 µM cisplatin to treat U87-MG glioblastoma and SVG-P12 normal cells. Single treatments of each drug were included for comparison. An MTS cell proliferation assay was performed to compare the cytotoxic effects of the drug combinations on the cell lines. RESULTS: A significant decrease in cell proliferation for both combinations, compared to single drug treatment, was seen in the U87-MG cell line. There was no significant difference between combination treatments and single drug treatments in the SVG-P12 cell line. CONCLUSIONS: TSA showed a significant synergistic and selective effect on the U87 glioma cell line in combination with DNA damaging agents. This highlights the potential of novel combination therapies to increase efficacy of all glioma cell lines, which may lead to dose-reduction of chemotherapeutic agents and hence reduced side-effects.  

10. MODULATING IMMUNOSUPPRESSIVE BEHAVIOUR IN GLIOBLASTOMA
Suzanne Binks, Michael Burnet, and Geoffrey Pilkington; University of Portsmouth

INTRODUCTION: Glioblastoma are remarkably adept at evading immune responses mounted against them and have been shown to manipulate immune components into promoting tumourigenesis. They achieve this through a number of synergistic methods, including the release of inhibitory cytokines, that converge to inhibit T-cell proliferation, activation and migration as well as inducing T-cell apoptosis. Administering compounds that up-regulate the expression of tumour-suppressive cytokines whilst decreasing the immunosuppressive cytokines may show some therapeutic benefit. METHODS: Using SYGA (Synovo GmbH), which regulate macrophage phenotype through NFκB activation and translocation promotion, at 10, 3, 1 and 0.3 µM for 72 hours and subsequently performed the same analyses as described above. RESULTS: Using MAC387 antibody, we have shown the macrophage chemokine expression of our early passage glioblastoma cells to be around P1 and P2. We have analysed the macrophage and myeloid components of the population as well as assessing IL-10, IL-12, IL-23 and IFN-γ expression and secretion by Western blot, flow cytometry and ICC. After treating the cells with 9 compounds (SYGA and temozolomide), which regulate macrophage phenotype and have been shown to upregulate tumour suppressor cytokines, we observed a significant decrease during the expression of the tumour-suppressor cytokines IL-12 and IL-12 and IFN-γ. Preliminary studies show that several of the compounds enhance the expression of the tumour-suppressor cytokines IL-12 and IFN-γ while decreasing amounts of IL-10 and IL-23. CONCLUSIONS: Initial data suggest that modulating macrophage phenotype might increase the anti-tumour immune response. Further investigation is required on how this manipulation affects tumour cell behaviour and survival; studies will also determine the effect on other immune components such as myeloid cells.  

12. A SINGLE CENTRE CASE CONTROL STUDY OF THE EFFICACY AND SAFETY OF 5-AMINOLEVULINIC ACID GUIDED RESECTIONS OF GRADE IV (WHO) GLIOBLASTOMAS
Julius D Bruch, Jasmine Ho, Colin Watts, and Stephen J Price; University of Cambridge

INTRODUCTION: This is a study to assess the safety and efficacy of using 5-aminolevulinic acid (5ALA) to guide the resection of grade IV glioblastomas based on single data obtained in a single UK centre. METHODS: A primary histologically-confirmed grade IV glioblastoma who underwent 5ALA guided resection were included in the study. Each patient was matched by age and tumour location to a control patient with similar performance status who had a resection without 5ALA. Outcome measures include residual tumour on post-op MRI, post-op deficits and access to chemoradiotherapy. RESULTS: The proportion of gross total resections (GTRs) in 5ALA patients was 53% (n = 74) and thus significantly better compared to the control group with 30% (p = 0.0001). The median GTR rate at the end of the resection predicted a significantly better GTR rate of 72%, compared to a GTR rate of 19% in the presence of residual fluorescence (p < 0.0001). Two cases with mild adverse effects could be attributed to 5ALA. Outcome measures include residual tumour on post-op MRI, post-op deficits and access to chemoradiotherapy. CONCLUSIONS: 5ALA guided resection of grade IV glioblastoma results in a significantly better GTR rate compared to conventional surgery. This result reflects the findings of the previous stage III clinical trial. The procedure also appears to be safe with no significant increase in hospital stay and new neurological deficits between the two groups. 5ALA treatment did significantly not compromise access to chemoradiotherapy (p = 0.05). CONCLUSIONS: 5ALA guided resection of grade IV glioblastoma results in a significantly better GTR rate compared to conventional surgery. This result reflects the findings of the previous stage III clinical trial. 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14. THROMBOCYTOPENIA DURING CHEMORADIOThERAPY FOR GLIOBLASTOMA: POTENTIAL IMPLICATIONS FOR ADJUVANT TREATMENT AND OUTCOME
B Clark, M Mackinnon, N MacLeod, W Stewart, and A Chalmers; Beatson West of Scotland Cancer Centre

INTRODUCTION: Concurrent chemoradiation with oral temozolomide, followed by up to 6 cycles of adjuvant temozolomide is standard of care following resection of glioblastoma (GBM) in fit patients under 70 years old. Myelosuppression, particularly thrombocytopenia, is common during and after concurrent treatment. We investigated the incidence of thrombocytopenia during concurrent chemoradiotherapy and its impact on subsequent delivery of adjuvant chemotherapy and overall survival.

METHODS: We retrospectively examined the medical records of 130 consecutive patients treated with concurrent chemoradiotherapy for GBM at a single institution between 2005 and 2010. Survival was calculated from date of surgery and analysed by the Kaplan-Meier method using SPSS version 16. RESULTS: Complete haematological results were available for 117 patients. Median duration of follow-up from date of surgery was 14.4 months. The incidence of any grade thrombocytopenia during the concurrent phase was 29.9%, grade 3 or 4 incidence was 6.0%. Of patients who developed grade 1 or higher thrombocytopenia during concurrent treatment, 14.3% completed 6 adjuvant cycles, median number of cycles was 1. Median OS was 13.5 months, 2-year OS 15.2%. Of those who maintained normal platelet counts, 42.7% completed 6 cycles, median number of cycles was 5. Median OS was 19.3 months, 2-year OS 31.0% (log rank p = 0.02). CONCLUSIONS: Thrombocytopenia is common during concurrent chemoradiotherapy for GBM and is associated with a poorer outcome. The incidence of thrombocytopenia in GBM is higher than previously reported. Using co-morbid factors such as PS, prior debulking and subsequent lines of palliative chemotherapy, it also lends support to the importance of the adjuvant phase of treatment.

15. ADHERENCE TO NICE GUIDANCE IMPROVES SURVIVAL FOR GBM PATIENTS IN NORTHERN IRELAND
AJ Cole, GG Hanna, K Bailie, DS Conkey, and JH Arney; Northern Ireland Cancer Centre

INTRODUCTION: Outcomes following radiotherapy in the treatment of Glioblastoma Multiforme (GBM) are poor. A recent phase III study comparing concurrent chemoradiotherapy with temozolomide versus radiotherapy alone has demonstrated increased median survival with the use of chemoradiotherapy. NICE guidance advises radical radiotherapy with the use of concurrent temozolomide in the treatment of histologically proven WHO Grade IV primary brain tumours and in those patients with ECOG performance status (PS) of <2. We report our two year institutional experience.

METHODS: In a retrospective audit of neurooncology registrations in Northern Ireland from June 2007 to March 2009, patients with grade IV Glioblastoma were identified. Baseline patient characteristics, prior surgical management (biopsy/debulking/radiological) and treatment received were recorded. The completion rate of chemoradiotherapy was documented. Time from diagnosis to radiotherapy, number of cycles (PS >3 received 30Gy), and overall survival (OS) were calculated and compared using the log rank test. RESULTS: We identified 86 patients with GBM, 19 received chemoradiotherapy, 9 had radical radiotherapy. There was 100% completion of concomitant temozolomide. 12/19 (63.1%) completed the adjuvant component of treatment. In those treated with chemoradiotherapy, good PS and prior debulking surgery were associated with improved OS (p < 0.0001). Median OS for those receiving chemoradiotherapy was 22.9 months versus 7.5 months for radical radiotherapy alone (p < 0.0001). CONCLUSION: In our series, baseline PS, prior surgical debulking and chemoradiotherapy are associated with improved overall survival. Concurrent chemoradiotherapy should be offered to appropriate patients with grade IV malignant brain tumours.

16. THE EFFECT OF CORTICOSTEROIDS ON THE T2 ENHANCING REGION OF THE T2 APPEARANCE OF GLIOBLASTOMA MULTIFORME
CA Darlow, S Chapman, LA Mohsen, and SJ Price; Addenbrookes Hospital

BACKGROUND: Glioblastoma multiforme (GBM) are known for their invasion, which is a key reason for their poor prognosis. On MRI they are characterised by T1 enhancing lesion, surrounded by a peri-tumoural T2 enhancing region, which is a key reason for their poor prognosis. On MRI they are characterised by T1 enhancing lesion, surrounded by a peri-tumoural T2 enhancing region. Measurements of the T2 weighted area were measured at the axial level of the largest T1 enhancing lesion cross-section area. RESULTS: The change in size of the T2 weighted lesion was not significant in the non-invasive GBM cohort. The T2 enhancing region doesn’t significantly reduce with corticosteroid therapy. This is evidence supporting our hypothesis that the T2 enhancing lesion may be invasive tumour. This is supported by histological studies that tumour cells are present at the periphery of the T2 enhancing region. Further work needs to be done assessing how the T2 abnormalities affect outcome and recurrence patterns.

17. INDUCING GlioBLASTOMA CANCER STEM CELL DIFFERENTIATION WITH DIBUTYRYL CYClic-AMP AND THEOPHYLLINE, PITUTIARY ADENYlATE CYCLASE ACTIVATING POLYPEPTIDE AND ALL-TRANS RETINOIC ACID
Laura Donovan, Suzanne Birks, and Prof. Geoffrey Pilkington; University of Portsmouth

INTRODUCTION: Glioblastomas represent a considerable therapeutic challenge due to their remarkable heterogeneity and adaptability and potent ability to evade or resist current treatment strategies. Recent studies suggest that some small cell lines are tumorigenic and that their tumorigenicity is mediated by a stem cell population. We investigated the hypothesis that certain differentiation agents, in particular db-cAMP and theophylline, may induce such a lineage switch, which may lead to much of the tumorigenic behaviour associated with these cells but render them more resistant to therapy. METHODS: Using an early passage biopsypreserved human paediatric cell line grown in neurobasal medium under non-necrotic conditions, we have treated the cells for 72 hours with either db-cAMP and theophylline (1mM), PACAP (1nM) or ATRA (10mM) before analysing the expression of GFAP, O4, Tu-20, CD133, Nestin, Mushashi 1, Olig 2 and Sox2 by flow cytometry and immunocytochemistry. Viability, proliferation, live cell imaging and cell cycle analysis were also carried out. RESULTS: Of the three agents, db-cAMP and theophylline had the most striking effect, with cells differentiating along an astrocytic lineage within 48 hours as shown by the increased expression of GFAP and down-regulation of the stem cell markers. Treatment with ATRA and PACAP showed similar but less pronounced effects (where p = 0.05). CONCLUSIONS: Differentiation agents, in particular db-cAMP and theophylline, appear to reduce the stem cell like behaviour of glioblastoma cells treated in vitro. Future studies will include elucidating signal transduction pathways as well as determining post-differentiation therapeutic sensitivity to nuclear and mitochondrial agents.
19. AN INTERPRETATIVE PHENOMENOLOGICAL ANALYSIS OF THE PATIENT EXPERIENCE OF AWAKE CRANIOTOMY - FROM DIAGNOSIS TO DISCHARGE
Kimberley Fletcher, Roshan das Nair, Jamie MacNiven, Surajit Basu, and Paul Byrne; University of Nottingham

INTRODUCTION: Previous research exploring the patient experience of awake craniotomy has yielded inconsistent results. In the current study the authors aimed to explore the lived experience of having undergone an awake craniotomy in the United Kingdom. METHODS: Seven participants took part in the study, which used a qualitative research methodology. Participants were recruited from a Department of Neurosurgery in the United Kingdom. Each participant was interviewed using a semi-structured interview schedule. Interviews were audio recorded and transcribed verbatim. Transcripts were analysed using interpretative phenomenological analysis. RESULTS: Analysis of transcripts yielded three super-ordinate themes: self-preservation, operation environment and information. The super-ordinate themes were interpreted as interconnected with each other, as well as embedded in a core theme: relationship with the neurosurgeon. CONCLUSIONS: The relationship with the neurosurgeon appears crucial to the patient experience of awake craniotomy. The relationship could impact on the patients’ decision to have the awake craniotomy, their experience of the awake operation and their acceptance of the information given. This knowledge requires consideration in clinical settings and could lead to improved delivery of care in the future.

20. SURGICAL TREATMENT OF CEREBRAL METASTASES
LJ Glancz and G Critchley; Brighton & Sussex NHS Trust, Hurstwood Park Neurological Centre, Lewes Road Haywards Heath, West Sussex RH16 4EX, United Kingdom

INTRODUCTION: The aim of our study was to elucidate the proportion of cancer operations performed for metastatic disease and the survival of these patients. METHODS: Data were collected on the number of operations performed for treatment of cerebral malignancy between January 2007 and December 2010 at our institution. All patients undergoing surgery for excision of cerebral metastases were included. This information along with basic demographics, location of lesion, presentation and histology was ascertained from histology forms. Survival was calculated from date of surgery to date of death or last follow-up, updated to March 2011. RESULTS: 410 operations were undertaken for treatment of cerebral malignant tumours over this 4 year period. Of these 71 (17.4%) were for metastatic disease. The most common histology of metastases was bronchial carcinoma (42%), followed by breast carcinoma (17%), malignant melanoma (13%), gastro-intestinal (11%) and renal cell carcinoma (9%). Greatest survival was observed in the breast cancer group (median survival of 9.5 months) followed by the renal cell carcinoma (8.5 months), melanoma (6 months), gastro-intestinal (5 months) and lung carcinoma groups (4 months). CONCLUSIONS: A significant proportion of operations are performed at our unit for treatment of intracranial metastases. This reflects an increasing number of patients with cerebral metastases being offered surgery. Although survival is poor, our data corroborate an increasing literature that surgery in combination with chemotherapy or best supportive care may provide additional prognostic information on patients presenting with cerebral metastases.
INTRODUCTION: To the rarity and inconsistent criteria for defining atypical meningioma prior to the WHO 2000 classification, its management and prognostic factors are poorly understood. Only few articles addressed survival rates of WHO-classified atypical meningiomas; with small numbers or disproportionate representation of irradiated patients. Thus, it was difficult to assess the impact of radiotherapy treatment. This study evaluated whether extent of surgical resection and receiving postoperative radiotherapy along with other patient characteristics had influenced recurrence and survival rates of atypical meningiomas. METHODS: Clinical and surgical data of the 79 patients with grade II atypical meningioma treated at our centre between 1996 and 2009 were retrospectively evaluated. The histology grading was consistent with WHO 2000 classification. Simpson grading system was used to assess the extent of surgical resection. Kaplan Meier analysis, Cox multivariate regression analysis and Log-rank test were conducted. RESULTS: The average age at the time of initial operation was 58 years, 54 % were males. The mean follow up period was 50 months. In Cox multi-variate analysis, only Simpson grading was predictive of recurrence (hazard ratio = 2.22/1 increase in Simpson grade, p = 0.003). Simpson grade I patients had a relapse-free survival rate of 97%, 74% at 1 and 3 years respectively compared with 88%, 32 % in the subtotal resection group (Simpson grades II to IV). There was no statistically significant correlation between recurrence and subjecting patients to postoperative radiotherapy. Apart from Simpson grade I patients, there was a general trend for worse outcomes in irradiated patients. CONCLUSIONS: The most important prognostic factor in determining recurrence was Simpson grading. There was no statistically significant impact of adjuvant radiotherapy on the recurrence of atypical meningiomas. Meta-analysis for the existing literature is needed.

26. THE USE OF INTRA-OPERATIVE CARMUSTINE CHEMOTHERAPY WAFERS IN THE MANAGEMENT OF HIGH GRADE GLIOMA: AN ASSESSMENT OF COMPLICATIONS AND ACCESS TO FURTHER THERAPY

J Ho, J Bruch, C Watts, and SJ Price; Addenbrooke’s Hospital

INTRODUCTION: NICE have approved the use of Carmustine wafers and Temozolomide in the treatment of high-grade gliomas, but have not commented on the combined use. The concern is that Carmustine wafers will lead to increased complications that will prevent patients receiving Temozolomide. This study aims to see if this is happening. METHODS: Retrospective review of high-grade glioma patients in whom the intention was to insert Carmustine wafers. Outcome data assessed complication rates and subsequent access to oncological treatment. Those patients whose Carmustine wafers was not inserted as intended RESULTS: 83 patients were included, 35 received Carmustine wafers and 48 did not. Out of the 48, 10 had diagnoses that were not high-grade gliomas (HGG). Comparing HGG patients only, 9/35 (25.7%) in the Carmustine group and 7/38 (18.4%) in the control group did not receive Carmustine wafer experienced complications, showing no significant difference between the two groups (p = 0.574). Subsequently, 30/35 (85.7%) of those receiving Carmustine wafers went on to have chemoradiotherapy compared to 24/38 (63.2%) of those not receiving Carmustine wafers. CONCLUSION: In our series, we found that there is no difference in complication rates between patients given Carmustine wafers and those who were not. The use of Carmustine wafers also did not compromise the subsequent access to radical oncological follow-up treatment.

27. IMPLEMENTATION OF VMAT FOR INTRA-CRANIAL TUMOURS: A PLANNING COMPARISON

G Lamb, S Smith, A James, and M Glegg; BwsOCC

This planning study compared conventional RT with multiple VMAT (Varian Solution) treatment plans for intra-cranial sites with planning target volumes (PTVs) overlapping organs at risk (OARs). METHODS: 6 patients were planned using conformal RT, & using 3 VMAT techniques. The first VMAT planning technique utilised a single complete arc, the 2nd used 2 concentric coplanar arcs & the 3rd technique utilised 4 non coplanar semi arcs, positioned to avoid entry through eyes. The PTV was separated into high & lower dose regions receiving 60Gy & 54Gy in 30 fractions respectively. The lower dose region encompassed overlapping OARs. The objects were treated to over 95% of the PTVs to 95% (CI95) of the dose & 99% of the PTVs to 90% (CI90) of the dose, without violating OAR constraints. Dose volume histograms for PTV & OARs were compared. RESULTS: Semi arc treatment plans achieved all CI90 & CI95 PTV & OAR dose constraints for all patients. Double arc technique achieved all the constraints for only 1 out of 6 patients while the single arc plans did not achieve all constraints for any patient. Double & single arc plans were able to achieve the mean brain dose constraint for only 1 patient, other OAR constraints were significantly exceeded. Conformal RT plans never achieved PTV dose constraints. Normal tissue conformity index (NI CI 95%) was comparable for all VMAT treatment plans (0.97−0.99) & significantly reduced for conformal RT (0.65−0.75). CONCLUSIONS: VMAT achieved better PTV coverage when compared to Single arc VMAT. Similarly normal tissue was achieved except for normal brain tissue, where the dose was greater with conventional RT, single & double arc VMAT plans. Semi arc non-coplanar treatment plans produced better PTV coverage than single arc VMAT plans while achieving OAR dose constraints. Semi arc non-coplanar treatment plans will now be used as a class solution when planning primary brain tumours that have PTVs overlapping OARs.

28. IMMUNOHISTOCHEMICAL ANALYSIS OF EMMPRIN AND CYCLOPHILIN A EXPRESSION IN ADULT GLIOMA

T Jeffcote, S Boulus, P Robbins, and N Knuckey; Kings College Hospital

INTRODUCTION: Extracellular matrix metalloproteinase inducer (EMMPRIN), a member of the immunoglobulin superfamily, is present on the surface of tumor cells. It is closely associated with Cyclophilin A (CypA) a cytoplasmic and extracellularly expressed molecule upregulated in cerebral hypoxia. We have analyzed the clinicopathological characteristics of EMMPRIN and CypA expression in normal brain tissue, low grade glioma, high grade glioma tissue. METHODS: 10 paraffin embedded samples were sectioned and processed following the standard automated immunohistochemistry protocols at Sir Charles Gardiner Hospital (Pathwest). 20 high grade, 20 low grade and 10 normal samples were treated with commercially available antibodies and scored by two trained investigators. RESULTS: Both EMMPRIN and CypA were expressed in normal brain and glioma tissues with different levels of malignancy. The intensively positive expression rates of EMMPRIN in anaplastic astrocytoma and glioblastoma tissues were significantly higher (92% of samples/ Mean Allred score – 4.5) than those in normal brain and low-grade astrocytoma tissues (0%/0 and 7.5%/0.9, respectively). No consistent relationship between glioma grade and CypA expression could be established. The positive expression of EMMPRIN was associated with higher grade gliomas. DISCUSSION: Based on these results, we conclude that EMMPRIN is avidly expressed in high grade glioma alone. A correlation between CypA expression and glioma grade could not be established by this study. The detection of EMMPRIN expression is feasible using standard high throughput immunohistochemical techniques and may facilitate diagnosis of high grade glioma where histological differentiation is inconclusive.

29. INTERSTITIAL RADIOSURGERY FOR CEREBRAL METASTASES

Adonye Banigo, Andrew R Brodbelt, and Michael D Jenkinson; The Walton Centre

INTRODUCTION: Brain metastases are a significant cause of morbidity and mortality. Treatment modalities include surgery, stereotactic radiosurgery and whole brain radiotherapy. We investigated the outcome of interstitial radiosurgery with radio-sodium seeds (brachytherapy) for metastatic brain tumours. METHODS: Retrospective analysis of patients treated with interstitial radiosurgery between 2001-2004. Patient demographics, ECOG performance status, Recursive Partitioning Analysis (RPA) class, primary tumour type, size/number of brain metastases, seed dose, duration of implant and overall survival were determined. RESULTS: 19 patients, median age 60.6 years (range: 41–77), were treated. 18 had a single brain metastasis, 1 had two metastases. Median tumour diameter was 15mm (range: 10–30). Four patients had extracranial metastases. 2 patients had an ECOG of 1, the remainder were ECOG 2. Primary tumour types were: lung (n = 10), melanoma (n = 3), breast (n = 2), oesophagus (n = 2), renal (n = 1); colorectal (n = 1). 4 patients were RPA class I, 15 were RPA class II. Radio-sodium125 seeds (median radioactivity 19.5 mCi) were implanted for 3 to 5 days to deliver a reference dose of 60Gy. Complications included seizures (n = 2) and transient arm monoparesis (n = 1). 4 patients had adjuvant WBRT. Median overall survival from date of implant to death was 4.3 months (range: 0.5–47.1 months). There was no statistically significant difference in overall survival according to RPA class or ECOG. CONCLUSION: Interstitial radiosurgery is an alternative local therapy to resection or stereotactic radiosurgery. In our experience, it has a low complication rate, but does not afford a survival advantage compared to other treatment options.
30. MICRORNA EXPRESSION IN PAEDIATRIC BRAIN TUMOURS
Jennie N Jeyapalan, Muhammad A Mumin, Tim Forschew, Andrew RJ Lawson, Ruth G Tatavosian, Thomas S Jacques, and Denise Sheer; Queen Mary University of London
INTRODUCTION: MicroRNAs are key players in the regulation of gene expression and their low proliferation rate has been identified in a number of cancers but few studies have looked in paediatric brain tumours. The aim of this study is to improve our understanding of the role of miRNAs in paediatric brain tumours. METHODS: MiRNA and mRNA expression were analysed on paediatric brain tumours (n = 16) using Illumina MicroRNA expression array (v2) and Human HT-12 Beadchips. The Illumina Genome Studio software, Sylarray and Sylamer algorithms (http://www.ebi.ac.uk/enright/sylamer) used for data analysis. RESULTS: MicroRNA profiles were produced for each tumour type, a 28 gene signature for ependymomas and 30 gene signature in medulloblastomas showed the greatest differential expression compared to control brain. MiR-124 was identified as a key player in all tumours. MiR-124 and other miRNAs involved in neuronal differentiation, miR-128, MiR-129 and miR-133b were also down-regulated. MiR-216 cluster was up-regulated specifically in medulloblastoma, which has recently been shown to target PTEN. MiR-450a was up-regulated in astrocytomas and miR-31 up-regulated in ependymomas. MiR-146, linked to inflammation and senescence, was up-regulated in pilocytic astrocytomas. With characteristics microRNAs in adult glioblastoma and medulloblastoma, plus novel microRNAs were identified in the paediatric glioblastomas. CONCLUSIONS: This study identified aberrantly expressed miRNAs in a range of paediatric brain tumours. These novel findings will be investigated further to elucidate the targets and pathways altered in the tumours.

31. CHARACTERISATION OF GENOMIC IMBALANCES IN PAEDIATRIC EPENDYMOMA IDENTIFIES A PROGNOSTIC ROLE FOR PRUNE
JP Kilday, K Wright, S Leavy, J Lowe, EC Schwalbe, SC Clifford, R Gilbertson, B Coyle, and RG Grundy; Children’s Brain Tumour Research Centre
INTRODUCTION: Paediatric ependymomas remain a clinical challenge with a relatively poor prognosis. An improved understanding of ependymoma biology may identify new correlates of outcome and potential therapeutic targets. METHODS: Affymetrix 500K SNP arrays were used to characterise microRNAs in adult glioblastomas, plus novel microRNAs were identified in the paediatric glioblastomas. CONCLUSIONS: This study identified aberrantly expressed miRNAs in a range of paediatric brain tumours. These novel findings will be investigated further to elucidate the targets and pathways altered in the tumours.

32. MiR-23B TARGETS THE X-LINKED INHIBITOR OF APOPTOSIS GENE IN GLOBLASTOMA
Paula Kinsella, Martin Clynes, Verena Amberger-Murphy, and Niall Barron; National Institute for Cellular Biotechnology
INTRODUCTION: micro (mi) RNAs are highly conserved noncoding RNAs that control gene expression post-transcriptionally, by degradation of target miRNAs or the inhibition of protein translation. As they control important processes such as differentiation, cell growth and cell death, miRNAs hold great promise for targeted cancer therapy. METHODS: using the Firefly luciferase reporter system, we identified miR-23b as a key regulator of Bax, a key player in all the differentia- 

tional expression compared to control brain. MiR-124 was identified as a key player in all tumours. MiR-124 and other miRNAs involved in neuronal differentiation, miR-128, MiR-129 and miR-133b were also down-regulated. MiR-216 cluster was up-regulated specifically in medulloblastoma, which has recently been shown to target PTEN. MiR-450a was up-regulated in astrocytomas and miR-31 up-regulated in ependymomas. MiR-146, linked to inflammation and senescence, was up-regulated in pilocytic astrocytomas. With characteristics microRNAs in adult glioblastoma and medulloblastoma, plus novel microRNAs were identified in the paediatric glioblastomas. CONCLUSIONS: This study identified aberrantly expressed miRNAs in a range of paediatric brain tumours. These novel findings will be investigated further to elucidate the targets and pathways altered in the tumours.

33. GENE EXPRESSION PROFILING OF PILOCYTIC ASTROCYTOMA
SR Lambert, DTw Jones, D Pearson, I Ichimura, and VP Collins; University of Cambridge
INTRODUCTION: Pilocytic astrocytoma (PA) are the most frequent central nervous system tumour in paediatric patients. They are most common among cases characterised by RAS fusion genes (with occasional gene mutations) and are associated with a relatively poor prognosis. An improved understanding of ependymoma biology may identify new correlates of outcome and potential therapeutic targets. METHODS: Affymetrix 500K SNP arrays were used to characterise microRNAs in adult glioblastomas, plus novel microRNAs were identified in the paediatric glioblastomas. CONCLUSIONS: This study identified aberrantly expressed miRNAs in a range of paediatric brain tumours. These novel findings will be investigated further to elucidate the targets and pathways altered in the tumours.

34. GLUCOSE-RESPONSIVE MICRORNA NETWORKS ARE ESSENTIAL FOR AGGRESSIVE TUMOUR GROWTH IN GLOBLASTOMA
LP Steele, P Sinha, P Chumas, J Tyler, D Ogawa, EA Chiocca, M DeLay, A Bronisz, M Nowicki, J Godlewski, and SE Lawler; University of Leeds
INTRODUCTION: In order for aggressive tumours such as glioblastoma multiforme to grow rapidly, cells must be able to survive periods of stress such as hypoxia, acidity and nutrient deprivation. We previously identified a pathway in which miR-451 acts as a glucose responsive switch regulating glioma proliferation, migration and survival. METHODS: Microarray analysis of microRNA expression was performed using custom arrays, and validated by qRT-PCR. Target identification was done using bioinformatics followed by qRT-PCR, Western blotting, and analysis of 3’UTR reporters. Cell-based assays were performed using synthetic microRNA mimics, and in vivo studies were carried out using X12 glioma cells stably expressing miR-451/144. RESULTS: We have identified a miR-451 regulated network that mediates responses to glucose alterations in glioblastoma. We also report that miR-451 is co-expressed with miR-144 in patient samples (R2 = 0.718), and that miR-144 may support the miR-451 network. Overexpression of miR-451/144 in X12 patient-derived glioblastoma cells causes unusual tumour growth patterns and completely blocks invasion into brain tissue in vivo, suggesting that fluctuating miR-451/144 levels in glioblastoma in response to glucose are necessary for rapid growth. We have also identified several additional glucose-regulated microRNAs using microarrays. The significance of these alterations and emergence of a glucose-responsive microRNA network in glioblastoma will be discussed. CONCLUSIONS: The dynamic regulation of microRNAs in response to fluctuating glucose levels in the glioblastoma
microenvironment is essential for rapid tumour growth and dissemination, and is revealing novel targets for therapeutic intervention.

35. IS STEREOTACTIC RADIOSURGERY UNDER-UTILIZED IN THE TREATMENT OF SURGICALLY EXCISABLE CEREBRAL METASTASES? Maggie K Lee, Mohsen Javadpour, and Michael D Jenkinson; The Walton Centre for neurology and neurosurgery NHS Foundation Trust

INTRODUCTION: The limited availability of stereotactic radiosurgery (SRS) in the UK means surgical resection is the only local treatment option for most patients. Recent capitol investment in new SRS services has increased availability. We determined the proportion of surgically resected metastases that would be suitable targets for SRS. METHODS: Patients undergoing surgery for cerebral metastases between April 2007 and March 2009 were identified. Primary tumour type, adjuvant therapy, complications and length of stay were recorded. Estimated targets for elective SRS and craniotomy were obtained. Pre-operative CT/MRI were reviewed to determine tumour size (mm), composition (solid vs cystic) and location, presence of hydrocephalus or midline shift and number of metastases. Suitable SRS targets were defined as: <50mm diameter, no hydrocephalus, no symptomatic mass effect, solid tumour. RESULTS: 116 patients (median age 63 years) underwent surgical resection; 86 received adjuvant whole brain radiotherapy (WBRT); 30 did not receive WBRT due to poor performance status. 102 cases (88%) were solitary metastases; 14 (12%) had multiple metastases. Median tumour diameter was 34mm (range: 12–70mm). 41 cases (35%) were suitable targets for SRS. Size (n = 65), cystic tumour (n = 18) and hydrocephalus (n = 7) alone and in combination were the commonest reasons for lack of suitability. Seven patients (6%) had surgical complications. Median post-operative length of stay was 7 days (range: 2–51). Median overall survival (OS) was 7.7 months for those suitable for SRS and 5.4 months for those not (Log Rank: P = 0.52). The current targets for elective SRS and craniotomy are £7777 and £6129 respectively. CONCLUSIONS: One third of surgically excisable brain metastases are suitable targets for SRS. Day case SRS would minimise length of stay and complication rates, but there would be no crude financial benefit. Full health economic analysis warrants further investigation.

36. CYTOKINE MEASUREMENT IN PRE AND IMMEDIATE POSTOPERATIVE SERUM SAMPLES OF HIGH-GRADE GLIOMA PATIENTS USING LUMINEX TECHNOLOGY

E Lekka, P Abel, T Dawson, B Lea, and C Davis; Royal Preston Hospital

INTRODUCTION: There is a growing interest in the potential use of cytokine measurement as a biomarker in serum samples of patients with Grade 4 Gliomas. This study aimed to investigate the effect of surgery on cytokine levels perioperatively. MATERIALS AND METHODS: We prospectively collected serum samples immediately preoperatively and within 24 hours after surgery, from 36 patients who underwent biopsy or debulking of tumour. Control serum samples were collected pre and immediately postoperatively from 36 patients with no history of malignancy undergoing surgery for cerebral metastases for benign pathology. We evaluated the levels of 8 cytokines using the Luminex technology. RESULTS: There were significant differences between pre and postoperative levels for three of the eight cytokines IL-6, IL-8, IL-10 (p < 0.05) measured when compared to the control group, the difference was significant (p < 0.05). The median serum cytokine levels perioperatively were not significant (p > 0.05) with regards to the RPA class, the site (supratentorial or infratentorial) and the number of metastases (single or two). CONCLUSIONS: Median survival in this cohort was identical to those in the 2 RCTs that showed survival benefits from surgery. This was significantly longer than that (5.6 months) in the single series demonstrating no benefit. Our results support the previous evidence of improved outcomes with surgery and form a basis for informed discussion with local commissioners.

38. EPENDYMOMA: PREVALENCE, TREATMENT AND MANAGEMENT IN NHS TAYSIDE M Pendleton and HK Lord; NHS Ninewells Hospital, Dundee, DD1 9SY

INTRODUCTION: There is a paucity of published data on treatment and patient outcomes for ependymoma. We present a retrospective audit of prevalence, treatment and outcomes of patients diagnosed with ependymoma in NHS Tayside in 13 year period. METHODS: Cases identified from Scottish Cancer Registry. Age, gender, site of disease, grade of pathology, degree of surgery, post operative radiotherapy, date of progression, second line treatment and survival recorded. RESULTS: Nine patients diagnosed with ependymoma between 1997 and 2010, 7 adults and 2 paediatric cases. The latter were excluded. Age range 19–64, median 38. Three WHO Grade 1, 4 Grade 2 and none Grade 3. Six localised, one metastatic. Two occurred in cervical - thoracic spine; 2 in thoraco-lumbar spine, 1 in frontal lobe distant from lateral ventricle; 1 involved left lateral ventricle and one 4th ventricle with deposits in cervico-thoracic and lumbo-sacral spine. All patients treated with surgery. All received post-operative radiotherapy due to residual disease. None received post operative chemotherapy. Radiotherapy delivered to local tumour site in 6 patients, to cranial spinal axis in 1. 3 patients relapsed, all at site of original disease and in one spread to the third ventricle. All treated with further de-bulking surgery. One received intra-operative radiation. None received chemotherapy. Time to relapse 7 months, 11 months, and 5 years, calculated from date of treatment finishing to date of follow up MRI demonstrating relapse. Survival 100%. CONCLUSION: Low incidence and good survival with surgical resection and post operative radiotherapy, but significant recurrence in small population. Notes gave limited information on patient quality of life (QOL). Larger prospective studies essential. Treatment at relapse to be better defined. Will introduce patient QoL scoring.

39. RADICAL CHEMORADIATION IN ELDERLY PATIENTS WITH GlioBlAtoma: QUALITY OF LIFE (QOL) VERSUS SURVIVAL BENEFIT Mairi Mackinnon, Aoife Williamson, Allan James, Willie Stewart, Brian Clark, and Anthony Chalmers; Beatson West of Scotland Cancer Centre

BACKGROUND: Subgroup analysis in the original Stupp study indicated that patients aged >60 derived least benefit from intensive multimodal therapy. The aim of this study was to investigate the impact of this treatment on QoL. METHODS: Medical records were examined retrospectively. WHO PS was extracted at 3 timepoints: (1) 1st appointment with oncologist, (2) at start of adjuvant chemotherapy and (3) cycle 6 of adjuvant treatment. Survival data were calculated from date of surgery by Kaplan-Meier method using SPSS version 16. RESULTS: Of the 130 patients, 20 were >60 years, 10 patients did not receive adjuvant treatment, 14 completed 1–3 cycles, 9 completed 4–5 cycles and 7 completed adjuvant treatment. Median survival was 12.3 months. One year overall survival (OS) was 55.1%. WHO PS data at these timepoints were available for 40, 40 and 7 patients respectively: 10 pts PS 0, 20 pts PS 1; (2) 10 pts PS 0, 29 pts PS 1, 1 pt PS 2; (3) 2 pts PS 0, 3 pts PS 1, 2 pts PS 2. CONCLUSIONS: The majority of patients’ PS dropped by 1 point during treatment, although we cannot exclude more significant drops in PS causing cessation of therapy. These preliminary data indicate that, with appropriate patient selection and careful on treatment review, radical chemoradiation can be delivered to patients aged 60–70 with acceptable
INTRODUCTION: Medulloblastoma (MB) is the commonest malignant childhood CNS tumour with a high metastatic potential. We have recently demonstrated that during cerebellar development the polycomb group gene Bmi-1 regulates cell adhesion properties through repression of BMP pathway. Bmi-1 is known to inhibit MB growth in mouse models. Here we assess whether overexpression of Bmi-1 in MB may contribute to tumour growth by modulation of cell adhesion. METHODS: 2 MB cell lines DAOY and D458 known to overexpress Bmi-1 were used and D458 was downregulated using Short interfering RNA (SiRNA). Immunolabelling for phosphorylated SMAD proteins (pSMADs) was carried. Trawl migration assays (TW) and time lapse experiments (TL) were performed to assess the invasive properties. The results were compared with the scrambled SiRNA treated cells (controls). Statistical analysis was performed using student t-test. Immunohistochemical staining (IHC) for cell adhesion molecules were performed on cell blocks. RESULTS: We observed an increased labelling index for pSMAD among the Bmi-1 downregulated cells compared to controls – 72.85% vs 57.51% in DAOY and 83.93% vs 75.69% in D458. Interestingly we also found an increased number of multicellular aggregates (average/field) - 2.36 vs 0.55 in DAOY and 4.78 vs 1.61 in D458. Upon concomitant BMP pathway inhibition with Noggin, there was phenotypic rescue noted. The TW assay showed a lower number of cell migration (average/field) – 222 vs 147 in DAOY. The TL experiment demonstrated a decreased motility (average distance in μm) of Bmi-1 downregulated cells – 2.69 vs 2.07 in DAOY and 2.01 vs 1.65 in D458. Finally IHC revealed a differential expression of CD44 and Thrombospondin in Bmi-1 silenced D458 compared to controls. CONCLUSIONS: Our data support the notion that Bmi-1 overexpression contributes to aggressiveness of medulloblastoma probably by repression of BMP pathway.

INTRODUCTION: There has been a resurgence in interest in the “Warburg effect”; cellular metabolic adaptations that result in a reliance on accelerated glycolysis in the presence of abundant oxygen, first described in 1924. Recent studies on isocitrate dehydrogenase 1 (IDH1) gene mutations, have highlighted the importance of a balanced redox potential (the balance between oxidized and reduced cellular metabolites) to the development and behaviour of cancers. Nicotinamide adenine dinucleotide (NAD+) and NAD phosphate (NADP+) are involved in numerous cellular redox reactions, the failure of which is associated with the growth, chemoresistance and invasion of malignant cells. In this study, we examine NAD(P)H redox potentials in a number of CNS tumours correlating these with histological diagnosis and grade. DISCUSSION: There are suggestions that cytotoxic NADH imbalance might be a sensitive controller of nuclear transcription. The role of IDH1 mutation on NAD(P)H redox status and the contribution of this to improved prognosis for low and high grade glioma is still not fully understood. The ability to assess NADH in tissues is a crucial step in advancing our understanding of tumour metabolism, offering potential biomarker, intra-operative prognostic information potentially new redox targeted therapies.

INTRODUCTION: QUARTZ addresses a longstanding clinical question: what is the role of whole brain radiotherapy (WBRT) in patients with non-small cell lung cancer (NSCLC) and inoperable brain metastases? All patients receive steroids and optimal supportive care (OSC), and are randomised to WBRT or not. The primary endpoint is quality adjusted life years (QALYS), reflecting the importance of quality of life as well as survival in this very poor prognosis patient group. A non-inferiority margin of 1 week in QALYS was set as clinically important. QUARTZ requires 334 patients and began recruiting in March 2007. Despite widespread support from 77 UK and Australian centres, recruitment was lower than anticipated and the trial was under threat of closure. An issue contributing to the poor recruitment was thought to be the lack of preliminary data regarding the omission of WBRT. METHODS: Permission to release data on the first 151 QUARTZ patients was given by the Trial Steering Committee, who were unaware of the results at the time. Data were analysed and presented to participating centres. RESULTS: 60% of patients were male; the median age was 67 (range 38–85); 50% had good performance status (KPS >70) and 39% had a solitary brain metastasis on CT or MRI scan. The average QALY was 30 days in the OSC alone group, and 31 days in the OSC + WBRT group. Median survival was 7.3 weeks for patients receiving OSC alone, compared to 7.0 weeks for those receiving OSC + WBRT. Treatment with WBRT did not appear to have an obvious effect on QoL. CONCLUSIONS: These data provide preliminary evidence that omitting WBRT is not detrimental to this group of patients and can be used as a base for trial decisions and discussion. They are not definitive results but provide a strong rationale for the trial continuing, to answer this longstanding and important clinical question.
neuropathology and the neuro-oncology MDT; these were combined to form a HBCR. The estimated tumour incidence for the equivalent period was calculated from the National Cancer Registry (NCR) data for the preceding three years (2006–2008). The data derived from these sources were then assessed and compared. RESULTS: 364 cases of primary (79%) and secondary tumours were captured by the HBCR. The 3-year average data published by the NCR estimated 374 new cases of primary tumours versus the 289 in the HBCR. Similar proportions of primary tumours were recorded as high grade (WHO grade III or IV) in the HBCR and NCR. Notification patterns varied according to age at diagnosis, diagnostic group and WHO grade/ICD-10 code. An inverse male-to-female ratio was observed between the HBCR and NCR data (1.12 v. 0.8; respectively). CONCLUSION: Previous studies suggest regional cancer registries may underestimate the incidence of CNS tumours. However, our results suggest a considerable number of new cases are not captured by the data being gathered through existing hospital datasets.

45. FLUORESCENCE GUIDED RESECTION IDENTIFIES DISTINCT COMPARTMENTS OF TUMOUR-INITIATING CELLS IN HUMAN GLOBLASTOMA

Sara GM Piccirillo and Colin Watts; University of Cambridge

INTRODUCTION: Recent evidences in human glioblastoma (GBM) suggest that distinct areas of the same tumour contain “cancer stem cells” (CSCs) endowed with different tumorigenicity. Cells in the tumour margin are supposed to be responsible for the recurrences but the investigation of this area has been carried out based on standard surgical resection. This approach is not objective and is hampered by the infiltrative nature of tumour cells. Aim of this study is how it is possible to interrogate the tumour edges in GBM. METHODS: Primary culture, culture propagation, cell line establishment, multiple immunofluorescence and in vivo experiments were performed as described by Piccirillo et al., 2009 and according to the Cambridge protocol. RESULTS: Here we show that different regions of the same GBM can be objectively identified by the use of 5-aminolevulinic acid. This technique objectively discriminates distinct compartments (mass, necrotic and margin) and does not alter the 90% efficiency of derivation of CSCs from GBM. Of note, only the cells derived from the mass give rise to multipotent long-term expanding CSCs. Margin cells are tumorigenic in vivo but in vitro do not grow under stem cell conditions nor possess the stem cell molecular signature. CONCLUSIONS: Our findings suggest that margin cells are tumorigenic but do not fulfill the criteria of CSCs. Further experiments are ongoing to better characterize the phenotype and genotype of margin cells versus cells derived from the mass and to evaluate response to conventional therapies. Histological analysis will be used to characterize disease in non fluorescent margin.

46. THE ROLE OF SERIAL STEREOTACTIC BIOPSY IN ADULTS WITH BRAINSTEM LESIONS

SRM Qadri, E Pirola, MD Jenkinson, and A Brodbelt; Walton Centre for Neurology and Neurosurgery

INTRODUCTION: Brain stem tumours are rare in adults and are associated with a poor prognosis. Histological diagnosis is required before administration of adjuvant therapy. We investigated the role of stereotactic biopsy in the management of brainstem lesions. METHODS: Retrospective analysis of adult patients with brainstem lesions between 2001–2010. Frame-based serial stereotactic biopsy was used in all cases. Histological diagnosis, adjuvant therapy and clinical outcome were investigated. RESULTS: Twenty-eight patients (13 female; 15 male; age range: 20–76 years) were biopsied. ECOC performance status at presentation was 0–1 (n = 17) and 2 (n = 11). Histopathology diagnoses included: grade II astrocytoma (n = 11), high grade glioma (n = 5), lymphoma (n = 5), pilocytic astrocytoma (n = 2), meningioma (n = 2), non-diagnostic (n = 2), and grade IV astrocytoma (n = 1). Median survival in grade II astrocytoma was 91% at one year, 68% at 2 years and 55% at 5 years. Adjuvant therapy included radioiodine seed implant (n = 3), fractionated radiotherapy (n = 6) and temozolomide (n = 1). Seven patients are still alive. Patients with anaplastic astrocytoma (n = 4) received radiotherapy; there was a 76% median survival at 6 months and 50% at 1 year. In lymphoma cases (n = 5) the median survival was 40% at 1 year following radiotherapy +/- chemotherapy. Median survival for brainstem metastases was 3.5 months. Three patients had non-diagnostic biopsies. Post-surgical outcomes and subsequent developed metastatic breast cancer; two patients showed lesion resolution suggestive of infarct. One patient (3.5%) had worsening deficit following biopsy. CONCLUSIONS: Brainstem lesions in adults are a rare group. Stereotactic biopsy has a low complication rate and is accurate. Histological diagnosis is important for guiding adjuvant therapy and determining prognosis. Low grade brainstem gliomas have an indolent course and a good prognosis following adjuvant radiotherapy.

47. EVALUATING NOVEL POLYMERIC MICROPARTICLE-BASED INJECTABLE MATRICES FOR LOCAL CHEMOTHERAPEUTIC DELIVERY

R Rahman, C Rahman, S Smith, D MacArthur, F Rose, K Shakesheff, and RG Grundy; University of Nottingham

INTRODUCTION: We have investigated a novel formulation based on poly(lactic-co-glycolic acid) (PLGA; FDA-approved) and poly(ethylene glycol) (PEG) blended microparticles for local chemotherapeutic drug release. The biodegradable microparticles inject at room temperature and solidify into a matrix upon delivery into the brain, potentially allowing persistent tissue contact in the protected cavity lining and sustained multiple drug release. METHODS: Viability/growth rate of paediatric brain tumour cells cultured on matrices made from PLGA/PEG microparticles was assessed (Live/dead staining; SEM; alamar blue proliferation assay). UV absorbance was used to determine single/multiple release of etoposide, methotrexate and the histone deacetylase inhibitor trichostatin A (TSA) from matrices. Cytotoxic response from cells cultured on drug-loaded matrices was evaluated using alamar blue assay. RESULTS: Brain tumour cells were biocompatible with PLGA/PEG matrices, with both the apical and basal surface of cells amenable to drug penetration. Sustained TSA and etoposide release over 3 weeks from PLGA/PEG matrices was achieved by diffusion kinetics and followed near zero-order kinetics after an initial burst. Release profiles were similar for matrices loaded with high and low concentrations of drug. CONCLUSIONS: No adverse toxicity from the PLGA/PEG co-polymer alone was observed and matrices permitted sustained drug release in vitro. Cytobineral release of TSA, etoposide and methotrexate from a single formulation will be discussed. In addition, ongoing studies measuring the cytotoxic response of brain tumour cells cultured on drug-loaded matrices will be described. The PLGA/PEG, injectable, self-assembling drug delivery system will be widely applicable for brain tumours for which complete surgical resection is not achievable.

48. PREVALENCE, PREDICTORS AND ASSOCIATIONS OF APATHY IN ADULT SURVIVORS OF AN EARLY CHILDHOOD POSTERIOR FOSSA BRAIN TUMOUR

Claudia Carroll, Peter Watson, Mike Hawkins, Helen Spoudeas, David Walker, Tony Holland, and Howard Ring; University of Cambridge

INTRODUCTION: Apathy is a disorder of diminished motivation defined as a deficiency in behavioural, emotional and cognitive components of goal-directed behaviour. It occurs in several neurological pathologies and is associated with pervasive and disadvantageous effects on daily life. In this study we examined prevalence, predictors and associations of apathy in adult survivors of childhood posterior fossa tumour. METHODS: 118 adult survivors of early childhood (diagnosed before 5 years of age) posterior fossa tumours, and 62 of their siblings, were assessed an average of 32 years after initial tumour treatment, using the Weschler Abbreviated Scale of Intelligence, the Marin apathy evaluation scale and the Composite International Diagnostic Interview. RESULTS: Apathy scores reached or exceeded clinical cut-off in 35% of survivors compared to 18% of the comparison group. In the survivors this was associated with decreased employment, decreased income and lower self-ratings of physical and mental health. Apathy scores at or above cut-off were associated with lower verbal IQ scores and with a current or previous psychiatric diagnosis, but not with age at tumour treatment (all were <5 years), duration of follow-up, history of radiotherapy or tumour type (astrocytoma or medulloblastoma). Clinically significant apathy in the survivors was not associated with a diagnosis of depression or with performance IQ measures. CONCLUSIONS: Clinically significant apathy occurs relatively often in adult survivors of childhood brain tumours and is associated with impaired social functioning and increased psychopathology. Further research should determine whether any tumour or treatment-related variables increase risk of apathy.

49. THE FREQUENCY, CLINICAL ASSOCIATIONS AND LONGITUDINAL COURSE OF MAJOR DEPRESSIVE DISORDER IN ADULTS WITH CEREBRAL GLIOMA

AG Rooney, S McMamara, M Mackinnon, M Fraser, R Ramping, A Carson, and R Grant; Edinburgh Centre for Neuro-Oncology

INTRODUCTION: Currently there is little high-quality evidence as to whether (1) the frequency, (2) independent clinical associations, and (3) the longitudinal course of DSM-IV Major Depressive Disorder (MDD) is associated with primary cerebral glioma. METHODS: This was a twin-centre, prospective, observational cohort study with six-month follow-up. Adults with newly diagnosed cerebral glioma received the Structured Clinical Interview for DSM-IV (SCID) to diagnose MDD. Interviews occurred shortly after the start of radiotherapy (T1), three months later (T2) and six months later (T3). Independent associations between MDD and clinical variables were analysed using logistic regression. RESULTS: At T1 n = 135, at T2 n = 108, and at T3

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50. OLDER PATIENTS WITH GLIOMA HAVE A HIGH FREQUENCY OF A RARE CDKN2A P14/P16 ALLELE

JA Royds, S Al Nadaf, A Ahn, Y-J Chen, A Wiles, D Jellinek, AW Brathwaite, BC Baguley, MR MacFarlane, NA Hung, and TL Slatter; University of Otago School of Medicine

INTRODUCTION: Immortal cells are thought to require a telomere maintenance mechanism (TMM) to prevent critical shortening during repeated cell cycles. However, we have shown that a significant fraction of high-grade gliomas (HGGs) do not have either elevated telomerase or evidence of the Alternate Lengthening of Telomeres (ALT). The TMM in the latter is referred to as “none”. We now wish to determine the underlying genotype for the cyclin-dependent kinase inhibitor 2A (CDKN2A) rs11515 (C500G) polymorphism in HGG, its relation to TMM and how it affects patient outcome. METHODS: CDKN2A 3’ UTR SNP, C/C (500C) polymorphism in HGG, its relation to TMM and how it affects patient outcome. CONCLUSIONS: To date, this is the largest study of MDD in a consecutively presenting cohort of glioma patients. Although most did not become depressed, MDD was a clinically significant complication of glioma, affecting up to one in five patients during primary treatment. Patients with severe functional impairment or previous depression are at higher risk and may benefit from closer follow-up. Depression in glioma can last for months, and clinicians should actively seek and treat it.

51. PREDICTING OUTCOMES OF VOCATIONAL REHABILITATION IN PATIENTS WITH BRAIN TUMOURS: A PILOT STUDY

SL Rusbridge, NC Walmsley, SB Griffiths, PA Wilford, and JH Rees; National Hospital for Neurology and Neurosurgery

INTRODUCTION: The multi-disciplinary Vocational Rehabilitation (VR) Service for patients with brain tumours was funded for one year by Macmillan Cancer Support to provide assistance to patients of working age to return to work, remain in work, or decide to stop work. The impact of VR on work rates and quality of life (QoL) in patients with brain tumours has not been systematically examined to date. The purpose of the current study was to measure the outcome of VR and to create a predictive model of vocational outcomes. METHODS: To date, 39 patients have been seen in the service. Of these, 11 have been discharged with pre- and post-intervention data collected. 8 patients were diagnosed with low grade glioma, 3 with high grade glioma, and 2 with brain metastases. Outcome examinations included work status at entry, hours of work and occupational level. In addition, measures of QoL, fatigue, and mood were assessed. The predictive validity of demographic variables, tumour and treatment details, functional ability, and VR interventions were examined for vocational outcome. RESULTS: Preliminary findings are presented. All 11 patients were in work pre-morbidly and 6 patients were in work at referral (46%). Following intervention, 8 patients were in work (67%) and 3 patients were not working (33%). Paired t-tests revealed no significant differences in QoL, fatigue and mood following intervention. CONCLUSIONS: VR may be useful in assisting patients with brain tumours to return to or remain in work. Due to limited sample size, additional data are being collected to explore further VR and QoL outcomes and to create a predictive model of VR outcome, to be presented at BONS 2011.

52. HUMAN INDUCED PLURIPOTENT STEM (iPS) CELL DERIVED NEURAL STEM CELLS IN MALIGNANT GLIOMA: AN AUTOLOGOUS TROJAN HORSE IN THERAPEUTIC DELIVERY

D Ryan, C Watts, and P Liu; Wellcome Trust Sanger Institute

INTRODUCTION: A theoretical panacea in neuro-oncology would be the ability to selectively target the tumour population and leave the surrounding normal cells unharmed. Normal neural stem cells (NSCs) home to the tumour in a focussed manner, suggesting tumour tropism and not only migrate to the tumour mass, but selectively ‘track down’ microsatellite deposits often quite distant from the primary focus. Using unique induced pluripotent stem cell technology we sought to investigate whether iPSc derived neural stem cells possessed this property, representing a potential autologous therapeutic vector in our armamentarium against malignant glioma. METHODS: Following cellular reprogramming, generated Sanger Human induced Pluripotent Stem cells (SH-iPSCs) were successfully differentiated to neural stem cells using mouse monolayer differentiation protocols. Tumour tropism was assessed using targeted conditioned media from glioma. Using PKH26 fluorescent labelling we sought to investigate whether iPSc derived NSCs would migrate towards the individual tumour cell. RESULTS: Quantitative assessment of iPSc derived NSCs showed superior tumour tropism capabilities relative to the negative control (fibroblasts) at 300% migration efficiency as a percentage of the control (100%). PKH26 fluorescent labeling demonstrated that iPSc NSCs migrated to the level of the individual glioma cell and relative to the current gold standard, fetal neural stem cells, derived NSCs demonstrated a higher efficacy of migration. CONCLUSIONS: Our preliminary proof of concept data has demonstrated that iPSc derived NSCs have inherent tumour tropism and represent a potentially powerful autologous therapeutics vector in malignant glioma.

53. THE METABOLIC AUTOPHAGY PATHWAY REGULATES MIGRATION AND Invasion IN GLIOMA

Sara Galavotti, Maya Shaked-Rabi, Eugene Tulchinsky, Sebastian Brandner, Chris Jones, and Paolo Salomoni; UCL Cancer Institute

INTRODUCTION: High-grade gliomas (HGG) remain among the poorest prognosis primary tumours of adults. One of the factors underlying such dismal prognosis is the ability of glioma cells to invade the brain parenchyma, which makes surgical debunking almost invariably insufficient. Glia is highly heterogeneous and can be classified into molecular subclasses. Among them, the mesenchymal subtype is characterised by mesenchymal/angiogenic markers and is associated with poor survival. The metabolic autophagy pathway has been proposed to contribute to disease progression in aggressive cancers. However, its precise role in glioma has not been fully elucidated. METHODS: We analysed autophagy status in glioma by using publicly available gene expression datasets from over 400 patient samples. Next, we studied autophagy in glioma-initiating cells and determined the impact of its suppression on growth, migration/invasion and bioenergetics. Finally, we investigated the involvement of RAS/MAPK in an upstream regulator of autophagy. RESULTS: We found that a number of autophagy genes, such as DRAM1 and p62 are highly expressed in glioma tumours belonging to the mesenchymal subclass. Patients expressing high levels of the autophagy inducer DRAM1 have poor prognosis. Autophagy appears to be under the control of the RAS/MAPK pathway in glioma. In glioma-initiating cells, suppression of autophagy impairs migration/invasion without overt effects on survival. Autophagy is induced in cells undergoing epithelial-to-mesenchymal transition (EMT) and regulates EMT-triggered migration. Finally, autophagy suppression impinges on mitochondrial respiration. CONCLUSIONS: Taken together, these findings shed new light on the role of autophagy in cancer and propose a new role of this intracellular degradation pathway in the control of migration/invasion.

54. ISOGENIC GLIOMA STEM CELL LINES WITH DIFFERENT EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) AMPLIFICATION STATUS FROM THE SAME TUMOUR

Alexander Schulte, Hauke S Gunther, Svenja Zapf, Sabine Küther, Manfred Wirth, and Katrin Lammerts; University Hospital Manuel Beer, Eppendorf

INTRODUCTION: EGFR amplification is present in almost 50% of all glioblastomas (GBM) and is frequently associated with expression of a truncated, constitutively active variant, EGFRVIII. Experimental limitations to study these alterations exist as both are rapidly lost when GBMs are taken into culture. We developed conditions which facilitate the growth of cell lines that either maintain high-level EGFR amplification (EGFRVIII expression) or not, allowing a clear comparison between cells with a...
heterogeneous EGFR status derived from the same original tumour. METHODS: Fresh tumour material was cultured using glioma stem cell conditions with modifications. The resulting matched pairs of cell lines and original tumours were analysed by RT-PCR, Western Blot, FACS and FISH for different levels of EGFR gene amplification, EGFRvIII expression, EGFR/EGFRvIII, and in vitro tumorigenicity. RESULTS: From 2 freshly resected GBM pairs of cell lines were generated that differed in (i) EGFR gene copy numbers, (ii) expression level of EGFRvIII, and (iii) level of EGFR/EGFRvIII protein. The first pair consisted of one cell line with high-level EGFR amplification and high EGFR protein expression, whereas the isogenic sister-line lacked EGFR amplification. The second pair comprised one line that showed EGFRvIII expression, EGFR amplification and high EGFRvIII/EGFR protein levels, whereas these alterations were absent in the sister-line. Nude mice injected with EGFR-amplified cell lines died significantly earlier than those xenografted with non-amplified lines, and the EGFR/EGFRvIII status was stably maintained in vivo. Xenograft tumours resembled their parental glioblastoma tumours on protein as well as on genomic levels. CONCLUSIONS: Our cell lines provide a model to study the function of EGFR amplification/EGFRvIII expression in glioma cells and assess the impact of intratumoral EGFR status heterogeneity on the response to EGFR-targeting and other agents.

55. OUTCOME AND PROGNOSTIC FEATURES IN ADULT PINEOBLASTOMAS: ANALYSIS OF CASES FROM THE SEER DATABASE
Senthil K Selvanathan, Salah Hammouche, Heidj J Salminen, and Michael D Jenkinson; Salford Royal NHS Foundation Trust

INTRODUCTION: Adult pineoblastomas are rare central nervous system tumours. Patient and treatment factors associated with outcome are poorly defined and limited to small retrospective case series and single case reports. Using the Surveillance, Epidemiology, and End Results (SEER) cancer registry, we investigated clinicopathological factors associated with outcome in adult pineoblastomas. METHODS: Adult patients (≥ 16 years old) with pineoblastomas diagnosed between 1990–2007 were identified from the SEER database. Kaplan-Meier survival analysis and Cox models were used to examine the effect of variables on overall survival. The variables analysed included patient’s age at diagnosis, gender, race, tumour location, uni-focal or multi-focal tumour, tumour size, surgical resection and the use of adjuvant radiotherapy. RESULTS: Ninety-five patients were identified, with a median age at diagnosis of 39.2 years. Sixty-one patients (64%) underwent surgery and forty-four patients (44%) received adjuvant radiotherapy. Forty-two patients (44%) had both surgery and radiotherapy. The median overall survival was 176 months. Univariate analysis identified younger age at diagnosis, uni-focal and localised disease as important predictors of overall survival. On multivariate analysis, only age at diagnosis and localised disease emerged as important prognostic factors. CONCLUSIONS: This study represents the largest analysis of adult pineoblastomas to date. Clinically relevant prognostic factors were younger age of diagnosis and localised disease. Surgery and adjuvant radiotherapy did not influence overall survival.

56. GOLD NANOPARTICLE MEDIATED RADIOSENSITIZATION WITH CONCOMITANT TEMOZOLOMIDE FOR Glioblastoma Therapy
Sonali Setua, Colin Watts, and Mark E Welland; Nanoscience centre, University of Cambridge Nanoscience Centre; Cambridge Centre for Brain Repair, University of Cambridge

INTRODUCTION: Glioblastoma is the most common primary malignant brain tumour in adults. Despite recent therapeutic advances, the treatment of glioblastoma remains inadequate. Moreover, present evidences suggest that glioblastoma contains tumour-initiating cancer stem cells. They are resistant to the conventional radiation and chemotherapeutics which results in tumour recurrence. Gold nanoparticle mediated enhanced X radiation therapy can be a viable solution in this regard. Because of its high atomic number, gold preferentially absorbs kV X-rays compared to the soft tissue, resulting in the emission of ionizing Auger electrons. The enhanced free radicals generated by the gold nanoparticle under this condition and temozolomide induced DNA damage can lead to the effective demolition of the malignant tissue. METHODS AND RESULTS: A novel nanocojugate made of human serum albumin and polyethylene glycol were synthesized. The gold nanoparticle capped polyethylene glycol (EPDLS, electron microscopic and UV-Visible spectrophotometric analysis suggest that the nanocojugates are extremely stable at physiological pH. Cell viability assay showed that the nanocojugates are nontoxic. However, the uptake of this nanosystem by glioblastoma tumour-initiating cells yet to be analyzed by EDX spectroscopy and confocal microscopy. CONCLUSION: Preliminary results indicate that the nanocojugates are suitable for biomedical applications. In future we will analyze the potential of this nanosystem for gold nanoparticle mediated radiosensitization in combination with temozolomide induced DNA damage for glioblastoma therapy. Moreover, by functionalizing this nanosystem with appropriate targeting moieties more efficient tumour cell targeted therapy can be developed.

57. RECOMBINANT HUMAN HEAT SHOCK PROTEIN HSP70 AS POSSIBLE ADJUVANT IN TREATMENT OF MALIGNANT BRAIN TUMOURS IN CHILDREN
MA Shevtsov, WA Chakhatryan, AV Kim, KA Samochernych, AV Pozdnuykov, IV Guzhova, IV Romanova, and BA Margulis; Russian Polenov Neurosurgical Institute

INTRODUCTION: Despite progress in the treatment of malignant brain tumours, patient prognosis remains very poor with a median survival of less than 15 months. One of the promising therapeutic strategies can be based on immunomodulatory activity of molecular chaperones, particularly Hsp70. METHODS: Using the C6 glioma xenograft model rats were intratumorally treated with Hsp70 on the 14 day after implantation of C6 cells. Cytotoxic activity of lymphocytes (CTL–test), CD3+, CD4+, CD8+ cell infiltration of animal brains in control and treated groups were assessed. Tumour volume was assessed using magnetic resonance imaging (MRI). All treated rats were followed for survival. In addition, 10 patients with a diagnosis of malignant brain tumour were treated by 5 intratumoural injections of Hsp70. Immunological assays were made both before and after Hsp70 treatment. RESULTS: Intratumoral injection of recombinant Hsp70 increased the survival rate of animals from 20 ± 2 to 28 ± 1 days (P<0.01). This correlated with increased CTL response and massive infiltration with CD3+, CD4+ and CD8+ cells, both in the zone of injection and in tumour itself. Delay of tumour volume growth was detected by MRI in the Hsp70 treated group. Injection of Hsp70 was well tolerated by patients. There were no serious adverse effects. In peripheral blood an increase of markers of T-cell mediated immunity was observed. CONCLUSIONS: Our results suggest that Hsp70 can be developed as an immunostimulant and thus can become a useful therapeutic strategy in treatment of malignant brain tumours.

58. PAEDIATRIC BRAIN TUMOURS DEMONSTRATE VASCULOCENIC MIMICRY IN A THREE DIMENSIONAL CULTURE SYSTEM
SJ Smith, R Rahman, C Rahman, JH Barrow, DC Macarthur, F Rose, and RG Grundy; Children’s Brain Tumour Research Centre

INTRODUCTION: Two-dimensional (2D) monolayer cell cultures represent highly reductionist tumour models due to the loss of physiological extracellular matrix (ECM), making complex three dimensional (3D) processes that contribute to angiogenesis difficult to model. We hypothesised that this could present a novel 3D culture system to create macroscopic tumour aggregates. We describe the formation of a substantial ECM, upregulation of angiogenic pathways, and derivation of vessel like structures from tumour cells (vasculogenic mimicry). METHODS: The Rotary Cell Culture System (RCCS) was used to cultivate ~8mm diameter aggregates of paediatric high grade glioma and medulloblastoma cell lines. Expression of 84 angiogenesis and 84 ECM related genes was assessed by RT-PCR. Immunohistochemistry was performed against vessel related antigens, ECM and angiogenic components. Genome wide gene expression analysis was performed. RESULTS: The tumour aggregates possess a complex structure with distinct proliferating, senescent and necrotic regions, replicating primary tumour, including a similar cell population density. Tubular structures within the aggregates, with expression of antigens such as CD105, represent vasculogenic mimicry by tumour cells. Many key angiogenic and ECM genes are greatly upregulated in 3D compared to 2D culture, including EGFR, IGf1 and TGF beta, (p-values less than 0.05). CONCLUSIONS: The RCCS creates 3D tumour aggregates that recapitulate many key features of paediatric brain tumours, including histology and gene expression. The phenomenon of vasculogenic mimicry will be discussed with respect to our study. Our findings contribute to understanding angiogenesis and possible mechanisms of resistance to current anti-angiogenic therapies.

59. A WALK IN THE PARP?: THE POTENTIAL TO TARGET DNA REPAIR IN PAEDIATRIC HIGH GRADE GLIOMA
SJ Smith, A Long, JH Barrow, DC Macarthur, B Coyle, and RG Grundy; Children’s Brain Tumour Research Centre

INTRODUCTION: Paediatric high grade gliomas (pHGG) remain tumours with a poor prognosis for which novel therapeutic strategies are needed. Poly(ADP-ribose) polymerase (PARP) is known to have multiple
functions in tumours including single strand DNA repair and induction of caspase independent apoptosis. It has been suggested as a therapeutic target in adult malignancies and this study examines whether it could be a potential target for glioblastoma. Microarrays comparisons of formalin fixed pFFGG were examined by immunohistochemistry for levels of PARP and apoptosis inducing factor (AIF) expression. Full retrospective clinical data were also available for this cohort and statistical analysis was performed to assess for the effect of PARP status on prognosis. Copy number of 1q was assessed by array comparative genomic hybridisation and fluorescent in situ hybridisation. RESULTS: Level of PARP immunopositivity had a statistically significant positive correlation with survival in supratentorial paediatric high grade glioma. AIF staining was notable for its expression in the majority of tumours but with higher levels of expression in non-neoplastic brain. Extent of surgical resection and administration of chemotherapy or radiotherapy were the other significant factors on multivariate analysis. No correlation was found between gain of 1q and immunopositivity for PARP. CONCLUSIONS: This study shows that PARP is expressed at significantly higher levels in many pFGG and has a significant correlation with prognosis, though this increased expression is not DNA copy number driven. In these tumours the ability of PARP to activate AIF appears to have been lost. PARP may therefore represent a promising therapeutic target for these lesions.

60. MEASURING ELASTICITY OF HIGH-GRADE HUMAN BRAIN TUMOUR CELLS
Zaynah Matherally, James R Smith, Luke Dickson, and Geoffrey J Pilkington; University of Portsmouth

INTRODUCTION: CD44 is over-expressed on cancer cells and plays a key role in adhesion, migration and invasion. The increased action of CD44 facilitates the structural scaffold of cancer cells. METHODS: We have investigated the mechanical properties of high-grade human gloma cells (SNB-19) using atomic force microscopy. Normal human astrocytes (CC-2565) were used as a control. We also examined siRNA CD44 knock-down SNB-19 cells. An AFM probe was pressed into cell surfaces (on and away from nuclei; n = 2 x 50) and Young’s Moduli (E) determined. Fluorescence images, using F-actin and GFAP staining, were also acquired. RESULTS: Nuclei regions of all cell types had low E-values (SNB-19 and CC-2565 cells, 0.67 ± 0.05 kPa and 0.64 ± 0.06 kPa, respectively; siRNA CD44 knock-down, 0.28 ± 0.03 kPa), attributed to ease of nucleus displacement. Away from nuclei, SNB-19 cells were more rigid than CC-2565 cells (2.34 ± 0.58 kPa and 1.09 ± 0.13 kPa, respectively), whilst siRNA CD44 cells were less compliant (0.61 ± 0.08 kPa) than SNB-19 and CC-2565 cells. DISCUSSION: Greater rigidity of SNB-19 cells may be explained by the probe sensing the underlying hard glass surface, since malignant cells with active CD44 are thinner due to extension of invadopodia. Results are discussed with reference to cell cytoskeleton and AFM literature. AFM nanotodentation could serve as a potential tool for testing anti-cancer drug efficacy and understanding tumour invasion.

61. TOWARDS ESTABLISHING THE EFFECTS AND MECHANISM OF ACTION OF A SERIES OF INDOLES IN AN IN VITRO CHEMOSENSITIVITY SYSTEM FOR GLIOMA TREATMENT
Saurabhi Prabhu, Frederick Harris, Robert Lea, and Timothy J Snape; UCLan

INTRODUCTION: Substituted indoles and related structures have been shown to exhibit potent anticancer activity against breast cancer cell lines. Functional effects of structurally similar substituted indoles against the human glioblastoma cell lines, 1321N1 and U87MG, have been investigated by comparing the effects of these compounds to conventional anti-cancer drugs. METHODS: Cell viability in the presence of the test compounds was measured using an MTS assay and corroborated by an ATP cell proliferation assay as well as a Trypan blue exclusion test. The significance of reaction oxygen species (ROS) in the process was determined using an Image-iT LIVE ROS kit from Invitrogen. RESULTS: Both cell lines were treated with the 1321N1 and U87MG. The idea that the mechanism of action of these compounds may be due to enhancement of the generation of ROS was investigated and found to be confirmed over a similar time course using a suitable fluorogenic marker. Moreover, it was shown that the addition of an antioxidant (ascorbic acid) abolished the potency of the most active compound. CONCLUSION: Here, it has been demonstrated that certain substituted indoles are able to have a rapid, deleterious effect on the viability of two glioma cell lines and indicated that ROS generation may be an important factor in cell death.

65. AN AUDIT OF REFERRAL PATHWAYS FOR CNS IMAGING OF COMMON NEUROLOGICAL CONDITIONS IN CHILDREN (≤16 YEARS) AGAINST ROYAL COLLEGE OF PEDIATRICS AND CHILD HEALTH (RCPCH) BRAIN TUMOUR REFERRAL GUIDELINES
Maya Sussman, Sophie Wilne, William Whitehouse, Gabby Chow, Jo-Fen Liu, and David Walker; University of Nottingham

INTRODUCTION: Brain tumours constitute a differential diagnosis of many childhood neurological presentations; CNS imaging is essential for diagnosis or exclusion of tumour pathology. METHODS: Handoff maps were used to visualise pathways to CNS imaging. RESULTS: Data was collected from 41 paediatric neurology clinic patients and 11 newly diagnosed patients with brain tumour using a structured interview tool combined with case-note review. Compliance with referral and CNS imaging guidelines conditional on headache, vomiting, seizure and motor symptoms was measured. Hospital appointment and CNS imaging waiting times were calculated. Handoff maps showed involved in tortuous routes to CNS imaging, causing avoidable cumulative delay. CONCLUSIONS: The results support the need for a national awareness campaign linked to the RCPCH guidelines. The “HeadSmart - be brain tumour aware” campaign will be launched in June 2011 in the UK in response. The structured interview tool and handoff maps will be used as part of the Headsmart campaign’s evaluation and educational outreach programme (http://www.rcpch.ac.uk/hpp).

66. EXPLOITING CONFORMATIONALLY RESTRICTED UREAS AS BIOLOGICALLY ACTIVE C-G DOUBLE BOND ANALOGUES AGAINST GBM CELLS IN VITRO
TJ Snape, A Karakoula, F Rowther, and TJ Warr; University of Wolverhampton

INTRODUCTION: The combretastatin family of biologically active stilbenes possess promising antimitotic activity through interaction with the colchicine binding site on tubulin. We have synthesised 5 novel combretastatin analogues which exploit conformationally restricted bond rotations and thus mimic the cis-geometry of combretastatin. We compared the in vitro activity of these analogues to the combretastatin phosphate salt, CA4P. METHODS: Established synthetic and analytical techniques were employed to prepare the compounds and analyse their structures and conformations. A tubulin polymerisation assay was used to assess in vitro microtubule interaction activity. The cytotoxic and anti-proliferative effects of all compounds were evaluated in 3 GBM short-term cell cultures and the established glioma cell line U251. RESULTS: CA4P had higher activity than the 5 analogues in all in vitro assays. It was the most efficient inhibitor of tubulin polymerisation with a Vmax of 0.43 at 10 μM compared to the analogues (range 0.64–0.76). CA4P also induced the highest levels of cytotoxicity, apoptosis and mitotic arrest. Of the analogues, the two most similar in shape and functionality to CA4P were the best tubulin inhibitors, and better than colchicine. Conversely, the analogue least like CA4P was inactive in all cell cultures and a poor inhibitor of tubulin. Gratifyingly, the analogue most like inactive trans-combretastatin was the worst tubulin inhibitor. CONCLUSIONS: Although the biological activity of the analogues was lower than CA4P, the structure-activity relationship of these novel compounds enhances our understanding for future drug development. We have demonstrated an excellent correlation between conformation and functionality and validated this new class of combretastatin analogue.

67. TO INVESTIGATE WHETHER THERE IS EVIDENCE OF A CORRELATION BETWEEN THE PTV AND THROMBOCYTOPENIA DURING CONCOMITANT TREATMENT IN PATIENTS WITH NEWLY DIAGNOSED GBM
Aosta Williamson and Mairi Mackinnon; Beatson West of Scotland Cancer Centre

INTRODUCTION: Subgroup analysis in the original Stupp study indicated that patients aged >60 derived less benefit from intensive multimodal
therapy. The impact of radical concomitant treatment on quality of life (QoL) of these patients has not been studied. The aim of this study was to investigate the QoL at 3 separate time points (using WHO performance status (PS) as a surrogate of QoL). METHODS: Medical records were examined retrospectively. WHO PS was extracted at 3 timepoints: (1) 1st appoint- ment with oncologist, (2) at start of adjuvant chemotherapy and (3) cycle 6 of adjuvant treatment. Survival data were calculated from date of surgery by Kaplan Meier method and survival differ- entiation was calculated using log rank test. RESULTS: Of the 40 patients, 7 were lost during follow-up and 14 patients died during the study period. Median survival duration was 12 months. One year overall survival (OS) was 55.1% and 12 months OS data at the timepoints were available for 40, 40 and 7 patients respectively: (1) 20 pts PS 0, 20 pts PS 1; (2) 10 pts PS 0, 29 pts PS 1, 1 pt PS 2; (3) 2 pts PS 0, 3 pts PS 1, 2 pts PS 2. CONCLUSIONS: The majority of patients’ PS dropped by 1 point during treatment, although we cannot exclude more significant drops in PS causing cessation of therapy. These preliminary data indicate that, with appropriate selection and careful on treatment review, radical chemoradiation can be delivered to patients aged 60–70 with acceptable deterioration in PS. Future studies will prospectively assess QoL and duration of stable/responding disease after completion of adjuvant treatment.

68. MALIGNANT CNS PRIMARY LYMPHOMAS: A CHALLENGE OF MANAGING. EXPERIENCE OF A 20 YEARS FOLLOW UP
AK Zasak, V Varsos, A Panteli, O Karypidou, and AM Zapentheans; Red Cross Hospital Athens

INTRODUCTION: Primary central nervous system lymphoma (PCNSL) is a rare form of the non-Hodgkin’s lymphoma arising within and confined to the CNS. PCNSL is of a particular interest for several reasons. First this tumour has increased in incidence over the past several decades. Therefore, although it remains relatively rare, the differential diagnosis remains very important from other intracranial or spinal mass lesions. Second unlike many primary brain tumours, PCNSL is well responsive to treatment and aggressive management may lead to prolonged remission or cure. Finally, the long term consequence of aggressive therapy was result in significant neurologic dysfunction. METHODS AND RESULTS: In our study within the period 1990–2010, 25 patients have been diagnosed with PCNSL. 17 out of them were cerebral PCNSL and 8 were spinal PCNSL. From the 17 cerebral cases, 16 were high grade diffuse large B-cell lymphomas and 1 was characterised as low grade lymphoma. All the patients underwent surgic- al operation for a biopsy or gross total excision and treated with corticoster- oids and radiotherapy (50 Gy). Chemotherapy (methotrexate) applied in 20 of the patients. Patients were monitored with monthly MRI scans after initiation of methotrexate chemotherapy. CONCLUSIONS: The optimal treatment for PCNSL has not been established. Radiation therapy alone produces a median survival duration of only 18 months. Combination che- motherapy and radiation therapy more than doubled survival time (up to 50 months) but such success was achieved at the price of a greater than 50% incidence of dementia in those who survived more than 18 months of these regimens.

69. Y-BOX BINDING PROTEIN-1 (YB-1) INHIBITION TRIGGERS DIFFERENTIATION OF NORMAL AND CANCER STEM CELLS FROM THE BRAIN
Abbas Fotovat, Samah Abu-Ali, Pei-Shan Wang, Loic Deleyrolle, Cathy Lee, Joanna Triscotto, James Y Chen, Sonia Franciscio, Yasuhiro Nakamura, Yasuo Sugita, Takeshi Uchiumi, Michiko Kuwano, Blair R Levent, Sheila K Singh, Alex Jury, Chris Jones, Hiroaki Wakimoto, Brent A Reynolds, Catherine J Pallen, and Sandra E Duna; University of British Columbia, Vancouver, Canada; University of Florida, Gainesville, Usa; St. Mary’s Hospital, Kurume, Japan; Kurume University, Kurume, Japan; Kyushu University, Fukuoka, Japan; McMaster University, Hamilton ON; The Institute for Cancer Research, Royal Marsden Hospital, Surrey, England; Massachusetts General Hospital, Boston Massachusetts

INTRODUCTION: The Y-box-binding protein-1 (YB-1) is up-regulated in many human malignancies including glioblastoma (GBM). It is also essen- tial for normal brain development, suggesting that YB-1 is part of a neural stem cell (NSC) network. MATERIALS AND METHODS: The techniques used to investigate this project included the use of transgenic mice, isolation of primary NSCs from mice, differentiation of normal human neural stem cells (NSCs) under differentiation conditions using bone morphogenetic protein-4 (BMP-4) and triggered differentiation via coordinate loss of GSK3-b. Further, differentiation of CSCs with 1% serum or bone morphogenetic protein-4 (BMP-4), suppressed YB-1 protein expression. Likewise, YB-1 expression was lost during differentiation of normal human NSCs. Consistent with these observations, YB-1 was preferentially expressed in highly undifferen- tiated primary gliomas based on the analysis of WHO grade IV tumors (n = 49 cases). YB-1 was also co-expressed with Bmi-1 (Spearman’s 0.80, p < 0.001) and Sox-2 (Spearman’s 0.66, p < 0.001) based on the analysis of 282 cases of high-grade gliomas. These CSC markers were also highly expressed in 10 (15%) of GBM patients that subsequently relapsed.

CONCLUSIONS: YB-1 is a key feature of stem cells where it functions to suppress differentiation.

70. TAILORING DECISION NAVIGATION (DN) TO HIGH GRADE GLIOMA PATIENT’S NEEDS
SC Shepherd, SE Scott, D Bowyer, LM Wallace, and B Hacking; Coventry University

INTRODUCTION: In 2008–10 Decision Navigation (question-listing, audio-recording, note-taking) was piloted with Prostate cancer patients. Qualitative evaluation shows DN helped patients participate in treatment decisions; quantitative measures report navigated patient’s confidence in decision making was significantly enhanced. The National Institute for Health and Clinical Excellence2 (NICE) recommends brain tumour patients should be involved in making decisions about healthcare. This study aims to develop and tailor DN to the needs of patients with high grade glioma (HGG). METHODS: Two consecutive focus groups were conducted with brain tumour patients and relatives (n = 7). Seven HGG patients took part in in-depth interviews post-surgery prior to their initial oncology appoint- ment. Data was analysed using framework analysis3. RESULTS: Focus groups reported a “nagging” worry about a diagnosis they were not pre- pared for. Retaining information was difficult, while thinking of and asking questions was frightening. Patients described feeling like a statistic and being part of the medical process without being consulted. Pre-diagnostic interviewees reported: honest and open information from sur- geon’s pre-surgery allowed patients to feel more in control, although waiting to discuss pathology was an uncertain and anxious period, not helped by a lack of clear procedural information from post surgery to diagnosis. CONCLUSION: The period of waiting for diagnosis is fraught with uncer- tainty. The navigation intervention should be implemented at this early stage as a form of support and dialogue. Facilitation to think ahead about relevant questions, encouragement to voice questions, and the provision of personalised information from the consultation would be greatly beneficial for both patients and carers.

71. NORMALIZED CEREBRAL BLOOD VOLUME IN THE PERI-TUMOURAL REGION HELPS IDENTIFY INFILTRATION
LA Molsen, R Jena, JH Gillard, and SJ Price; University of Cambridge

INTRODUCTION: The undetected invasion of glioblastomas is a leading cause for failure of current treatments and poor prognosis. Post-mortem studies showed that glioma cells may extend for less than 1 cm from the tumour edge in 25% of patients and for more than 3 cm in further 20%. As GBMs invade, they need to develop blood supply. In a previous study, we showed that it is possible to detect the invasive margin of GBM by com- paring the isotropic (p-map) and anisotropic diffusion (q-map) abnor- malities. AIM: In this study, we aim to see if angiogenesis can be detected in invasive regions by comparing the rCBV in this infiltrative region and other peri-tumoural regions. MATERIALS AND METHODS: 20 GBM patients (mean age 61), were imaged pre-operatively. All patients had stand- ard anatomical MRI sequences and standard DTI and DSCI perfusion MRI. DTI series were analyzed by FSL (Oxford, UK), while DSCI series were analyzed by Nordic Ice (NordicNeuroLab, Norway). The p and q maps were calculated as previously described2. The p and q abnormalities were assessed on their respective maps from mice, differentiating RNAs against YB-1, 10% FBS, BMP-4 or BNDI, immunofluorescence, flow cytometry, immunoblotting, neurosphere growth assays and the analysis of stem cell markers by immunostaining tumour tissue microarrays. RESULTS: Here we show that YB-1 was highly expressed in the subventricu- lar (SVZ) of most fetal brain cultures but not in terminally differentiated primary astrocytes. Conversely, YB-1 knock-out mice had reduced Sox-2, nestin and musashi-1 expression in the SVZ. While primary murine neurospheres were rich in YB-1, its expression was lost during differentiation. Furthermore, YB-1 expression was preferentially expressed in highly undifferen- tiated primary gliomas based on the analysis of WHO grade IV tumors (n = 49 cases). YB-1 was also co-expressed with Bmi-1 (Spearman’s 0.80, p < 0.001) and Sox-2 (Spearman’s 0.66, p < 0.001) based on the analysis of 282 cases of high-grade gliomas. These CSC markers were also highly expressed in 10 (15%) of GBM patients that subsequently relapsed.

CONCLUSIONS: YB-1 is a key feature of stem cells where it functions to suppress differentiation.
of cortical vessels or choroid plexus (falsely elevated rCBV). The results from all patients were compared using a paired t-test. RESULTS: Normalized rCBV values recorded from the proposed infiltrative region ranged had a range of 2.1–8.2, while the values recorded from peri-tumoural region outside the isotropic abnormality ranged from 1.2–3.8. Comparing these values revealed significant difference between the presumed infiltrative region and other peri-tumoural regions (p = 0.00015). CONCLUSION: The current results add strong evidence to the ability of the DTI to outline the infiltrative regions around the GBM, proving that there is significant difference of rCBV between what is presumed to be the infiltrative margin of the GBM and other peri-tumoural regions. This still needs more validation by biopsy.

72. TARGETING POLO-LIKE KINASE (PLK) FOR THE TREATMENT OF BRAIN TUMOURS AND ITS UNIQUE CAPACITY TO ELIMINATE CANCER STEM CELLS THROUGH SOX-2 INHIBITION

C Lee, A Fotovati, M Verraault, H Wakimoto, B Reynolds, C Dunham, M Rally, J Hukin, S Singhal, S Singh, and SE Dunn; University of British Columbia

INTRODUCTION: In a genome-wide siRNA library screen of 691 kinases, we previously reported that polo-like kinase 1 (PLK1) inhibition suppressed the growth of paediatric glioblastoma cells in vitro. PLK1 was a particularly attractive molecular target for paediatric oncology because BI2636, a PLK small molecule inhibitor, has been evaluated in phase I and II clinical trials for adult cancers. In this study, we examined PLK inhibitors for the treatment of gliomas and specifically addressed the impact on brain tumour-initiating cells (BTICs) given that they are often resistant to current therapies and as such are believed to facilitate relapse. METHODS: PLK was inhibited with small interfering RNAs and BI2536 to assess their impact on tumour cell growth and viability. Primary BTICs were isolated from patients and the explanted cells were challenged with PLK inhibitors in a dose-dependent manner. Neurosphere and monolayer growth was assessed over time. The consequence of PLK inhibition on Sox-2, musashi and BMI-1 was assessed by immunoblotting and qRT-PCR. Further, the ability of PLK inhibitors to suppress tumour growth was assessed orthotopically in mice. RESULTS: PLK inhibition with siRNA or BI2536 significantly blocked the growth, caused G2/M arrest and induced apoptosis of GBM and medulloblastoma cell lines (IC50 = 2–3 nM). However, PLK1 inhibition exerted very modest effects on the growth of immortalized human astrocytes. Of note, PLK1 inhibitors were cytotoxic to the GBM cell line SF188 that is TMZ-resistant. PLK1 was readily detectable in primary patient-derived BTICs (n = 8–10 samples) and moreover inhibiting it suppressed the stem cell marker Sox-2, neurosphere growth and induced cell death. Further, BI2536 crosses the blood brain barrier and prolonged survival of mice with GBM. CONCLUSIONS: We have demonstrated for the first time that primary BTICs are exquisitely sensitive to PLK inhibition and that BI2536 has in vivo activity using an orthotopic model of GBM. Similarly, primary medulloblastoma BTIC cells are dependent upon PLK for survival and as such further pre-clinical development is justified. To conclude, inhibition of PLK1 may help overcome drug resistance and prevent disease relapse, which are two major challenges for long-term survival of patients.