

Section on tumors biennial meeting

April 13–14, 2007; Washington, DC; E-POSTER LISTING

Published online: 22 February 2008
© Springer Science+Business Media, LLC 2008

Impact of brain tumor biopsy and resection on neurocognitive outcomes

Elana Farace, PhD; Zarui Melikyan, PhD;
Jonas M. Sheehan, MD (Hershey, PA)

Introduction: There is potential for surgical resection to increase, induce, or reduce neuropsychological impairment in patients with primary brain tumors. The purpose of this project is to determine the neurocognitive impact of surgery by comparing baseline pre-surgical interventions to post-surgical performance. *Methods:* Seventy-five patients with primary brain tumors who were clinically referred for baseline and post-operative neuropsychological evaluation were given a standard neuropsychological assessment battery measuring a broad range of domains (attention, memory, executive function, visual and verbal memory, psychomotor speed, depression) adjusted for patient demographics using test norms were available. Median age at diagnosis was 39, and tumor types included craniopharyngioma, meningioma, PNET, central neurocytoma, colloid cyst, and all forms of glioma (grades I-IV). *Results:* Eighty-five percent of patients showed improvement of at least one standard deviation, in at least one neurocognitive domain, at 6-weeks post-surgery. No patients declined more than one standard deviation on any neurocognitive domain. Memory showed the biggest capacity for improvement. *Conclusions:* Surgery was found to be, in certain conditions and in certain domains, neutral or even beneficial for patients' neurocognitive function. These findings highlight the safety of surgery as an intervention. Although surgery has the potential to induce deficits through focal damage to surrounding tissue, increased risk

of hemorrhage, etc., it may also improve function, perhaps through resolution of mass effect, relief of intracranial pressure, or even a benefit of debulking.

Sagittal vertebral osteotomy for en bloc resection of malignant tumors involving the lateral spinal column

Frank L. Acosta, Jr., MD; Andrew T. Parsa, MD, PhD;
John Chi, MD, MPH; Christopher P. Ames, MD
(San Francisco, CA)

Introduction: Although total en bloc spondylectomy has proven useful in treating malignant spinal column tumors, it requires extensive instrumentation to achieve postoperative fusion, while its radical exposure of neurovascular structures poses additional risks to adjacent neurovascular structures. More limited en bloc resections may reduce these risks. *Methods:* Retrospective review of the clinical, surgical, and radiographic records of patients diagnosed with malignant tumors involving the unilateral vertebral body without radiographic evidence of extension across the midline of the vertebral body. After en bloc resection via a sagittal vertebral osteotomy, the contralateral vertebral body and posterior elements are preserved. In the cervical spine, the contralateral vertebral artery remains unexposed. *Results:* En bloc resection of lateralized spinal column tumors has been achieved using this technique in a total of six patients. Three patients had a chordoma of the cervical spine; two patients had a leiomyosarcoma of the lumbar spine; and one patient had a chordoma of the lumbar spine. No patient experienced new or worsened neurological

deficits. One patient developed a superficial wound infection postoperatively. No radiographic evidence of local tumor recurrence or hardware failure has been evident in any patient. One patient with a leiomyosarcoma developed metastatic disease at a distant site from surgical resection. *Conclusions:* Sagittal vertebral osteotomy can be an alternative to total en bloc spondylectomy in properly selected patients with lateralized encapsulated malignant spinal column tumors and may reduce the risk of injury to neurovascular structures, as well as allow for fewer motion segments to be immobilized than total en bloc spondylectomy.

Comprehensive biomarkers identification of meningioma progression by mass spectrometry imaging

Nathalie Y. Agar, PhD; Rona S. Carroll, PhD;
Peter M. Black, MD, PhD (Boston, MA)

Introduction: The complexity of cancer imposes further development of diagnostic tools to provide physicians with the ability to individualize treatment. Such an approach requires both increase in the extent of molecular information and expeditious analysis. *Methods:* Our study relies on selection of five progressive meningiomas with samples from both initial and recurring neoplasm. The sample set composition eliminates molecular variables of individual origin, enabling identification of molecular fingerprints for increasing meningiomas malignancy by mass spectrometry imaging (MSI). Briefly, a laser is shot directly onto the tissue section and each laser spot renders a mass spectrum representative of the proteins present at this location, based on accurate molecular mass. The method provides with extensive molecular characterization, and enables molecular dissection of heterogeneous tumors through correlation of protein distribution with histological features. *Results:* We have developed a frozen sample preparation method for MSI in accordance with histology procedures, providing with significant improvements of spatial resolution and execution time. Samples characterization provided high quality mass spectral data, from which signature differences are observed. We are in the process of producing statistical analysis of molecular signatures. *Conclusions:* This study represents the initial phase in efforts undertaken in our laboratory to re-categorize brain tumors at the molecular level by means of MSI and provide more comprehensive diagnostic tools to accommodate needs of personalized medicine. Direct mass spectrometry analysis of tissue affords a wealth of chemical information, and can be completed in terms of minutes, therefore carrying the potential of revolutionizing molecular diagnosis and the hope of improving patient care.

Risk of neurologic deficit following stereotactic biopsy of eloquent brain

Ellen L. Air, MD, PhD; Ronald E. Warnick, MD;
Christopher M. McPherson, MD (Cincinnati, OH)

Introduction: Frameless stereotactic biopsy has been shown in multiple studies to be a safe and effective tool for the diagnosis of brain lesions. However, no study has evaluated whether biopsy of lesions located in eloquent regions is as safe as it is for lesions in non-eloquent locations. We have reviewed our experience with frameless stereotactic biopsy in order to answer this question and determine the risks associated with biopsy of lesions in eloquent regions. *Methods:* Medical records, including imaging studies, were reviewed of all frameless stereotactic biopsies performed by neurosurgeons of the Mayfield Clinic at two institutions from January 1, 2002 to April 30, 2006. Lesion location and biopsy trajectory was classified as eloquent or non-eloquent for each patient. The incidence of all complications, particularly changes in neurologic function, was calculated for each group. *Results:* Over the time period reviewed, 116 biopsies were performed, 53% (62/116) of which predominantly involved eloquent regions of the brain. Five patients (4%) experienced persistent neurologic deficits, three of which were from biopsies of eloquent regions, two from non-eloquent regions ($P = 0.76$). In addition, four patients had transient neurologic symptoms which resolved within 24 h, two from eloquent regions. The diagnostic yield was 94% for eloquent biopsies and 89% for non-eloquent biopsies ($P = 0.37$). *Conclusions:* Frameless stereotactic biopsy of lesions located in eloquent regions is as safe and effective as biopsy of lesions in non-eloquent regions. Therefore, frameless stereotactic biopsy remains a valuable tool for diagnosis of brain lesions whether in eloquent or non-eloquent regions.

Effects of targeted therapy on proliferation and migration in living slices of PDGF driven gliomas

Marcela Assanah, PhD; Jeffrey Bruce, MD;
Peter Canoll, MD, PhD (New York, NY)

Introduction: We have developed a novel glioma model using retroviruses to deliver platelet-derived growth factor (PDGF) to adult white matter progenitors. Highly infiltrative tumors that closely resembled human glioblastomas formed in 100% of the animals by 10 days post injection. The retroviruses also encode GFP allowing identification of the infected cells. In this study we directly monitor migration and proliferation of the PDGF

retrovirus infected cells in living brain tissue using time-lapse microscopy in the absence or presence of small molecule inhibitors of PDGFR signaling. *Methods:* Retroviruses that express PDGF-IRES-GFP or PDGF-IRES-DsRed and pNIT-GFP were stereotactically injected into the SVZ of neonatal rats or subcortical white matter of adult rats. Time-lapse microscopy was performed on 300 μm slices generated from brain tumors at 10 dpi. Gleevec, Wortmannin, and LY 294002 were tested for their effects on tumor cell migration and proliferation. *Results:* By 10 dpi a large mass of GFP+ cells formed at the injection site with numerous GFP+ and DsRed+ cells infiltrating the cortex, striatum and corpus callosum. Time-lapse microscopy revealed that migrating tumor cells frequently stopped and divided en route. The migratory paths of individual GFP+ and DsRed+ cells were tracked (using DIAS), and the migratory speed and frequency of cell division were measured for each condition. Slices treated with each drug showed significant inhibition of proliferation and migration compared to untreated slices. *Conclusions:* The slice culture provides a convenient system to test the effects of chemotherapeutics on tumor cell migration and proliferation in living brain tissue.

A rare case of intracranial thorotrast induced brain neoplasm

G. Balamurali, MBBS, FRCS; Peter Richardson, FRCS; D. du plessis, MD; N. Bryant, PhD (Manchester, United Kingdom)

Introduction: Thorotrast was first used in the 1930s as a contrast medium and in cerebral angiography. It is a suspension of 25% thorium dioxide, with a half life of several hundred years and is mildly radioactive, continuously emitting alpha-particles. In 1947 the first confirmed case of thorotrast induced cancer was reported. It is linked to various cancers but CNS lesions relatively unknown. Extensive literature review shows the high systemic and local risk for over 50 years after exposure. We were able to quantify the dose of exposure by various methods. *Methods:* A 68-year-old male presented with a 3 year history of progressive weakness of the left side. At the age of six he underwent an aspiration of right parietal brain abscess. Thorotrast was injected into the abscess cavity for post op monitoring. CT brain revealed an enhancing right parietal lesion with areas of calcification and edema thought to be a convexity meningioma. *Results:* At operation an aggressive

hemorrhagic tumor with calcifications, edema and large engorged vessels with no definite demarcation as found. A biopsy was taken and procedure abandoned. Histology showed atypical endothelial proliferation consistent with angiosarcoma. Radioactivity of the sample detected alpha and beta emission. Gamma spectrometry showed the typical ^{232}Th decay chain. *Conclusions:* Although once popular as a contrast medium, 'Thorotrast', a radioactive component, has long been discontinued. The cumulative alpha particle dose has local carcinogenic effects with a risk of 97% at 50 years. CNS angiosarcomas are very vascular lesions and surgical option should be well planned.

Relationship of gliomas to the ventricular walls

Kaveh Barami, MD, PhD; Andrew E. Sloan, MD; Amir Mohammadi, MD; Steven Brem, MD (Jacksonville, FL)

Introduction: The role of neural stem cells in gliomagenesis remains controversial. The aim of this study was to determine the anatomic relationship of human gliomas to the lining of the ventricular walls, known as the subventricular zone, an area replete with stem cells. *Methods:* We performed a retrospective radiographic analysis of 100 patients (60 males, 40 females) with gliomas surgically treated at H. Lee Moffitt Cancer Center and sought to determine the relationship of the lesions to the ventricular walls as seen on their MRI's. The lateral ventricular system was divided into four regions: the frontal horn, temporal horn, body and atrium/occipital horn. *Results:* Our results indicate that in 93% of cases the lesions were adjacent to at least one region of the lateral ventricular wall. The body seemed to be the most commonly involved region ($n = 33$). Contiguity of the lesions to the ventricular walls was independent of the glioma size or mass effect. These findings were correlated to cytoarchitectural studies of the human subventricular zone. *Conclusions:* We found that most gliomas were adjacent to the lateral ventricular walls. This finding is consistent with experimental studies, in that there is an intimate association between gliomas and the subventricular zone. Based on our data we were unable to determine if stem cells give rise to gliomas or if gliomas attract stem cells. Future investigations into the biology of the subventricular zone might lead to improved understanding of gliomagenesis as well as development of new therapeutic strategies, ultimately improving the prognosis of patients harboring such tumors.

CYP1B1 expression in glial cell tumors: a potential immunotherapeutic target

Julia Barnett, PharmD (New Orleans, LA); Diana L. Urbauer, MS (Houston, TX); Graeme I. Murray, PhD (Aberdeen, UK); Gregory N. Fuller, MD, PhD; Amy B. Heimberger, MD (Houston, TX)

Introduction: Among central nervous system malignancies, CYP1B1 expression has only been characterized in medulloblastoma. An immunotherapeutic agent targeting this antigen was shown to safely stimulate a good immune response. To evaluate the viability of further research efforts targeting this antigen, we examined the expression of CYP1B1 in glial cell malignancies. **Methods:** We studied the frequency and extent of CYP1B1 expression by immunohistochemical analysis in 269 glial tumors (including all major pathologic types) on a tissue microarray. Results were categorized by percentage of cells stained and intensity of staining within cells. Correlation of CYP1B1 expression with patient prognosis was evaluated by univariate and multivariate analyses. **Results:** Overall, increased CYP1B1 expression in glial tumors was associated with decreased patient survival time (P less than 0.0014 for both percentage and intensity of staining). A significant difference existed in percentage and intensity of staining between astrocytic and oligodendroglial tumors ($P = 0.0002$ and 0.0003 , respectively), between grades of tumors (P less than 0.0001 and 0.0079), and between pathologic types of tumors (P less than 0.0001 and 0.0339). Positive CYP1B1 staining was seen in 81% of glioblastomas, 84% of anaplastic astrocytomas, 61% of oligodendrogliomas, and 67% of anaplastic oligodendrogliomas. Paradoxically, within specific tumor pathologies, there was a trend toward increased survival as CYP1B1 expression increased. However, in a multivariate analysis accounting for other known prognostic variables, CYP1B1 expression appeared prognostically neutral. **Conclusions:** CYP1B1 is frequently expressed in a variety of gliomas and could be used as a target for immunotherapy.

Measuring functional outcome in brain tumor patients following acute rehabilitation

Susan Bell, RN, MS, NP; John McGregor, MD; Robert Vanecko, PT; Sharon McDowell, MD (Ohio)

Introduction: Intracranial neoplasms can be a significant source of disability following surgery. Objective: to measure functional outcomes of patients with brain tumors

following acute inpatient rehabilitation and to determine if any differences between tumor type, functional outcome and discharge disposition exist. **Methods:** Design: Retrospective review of patients admitted to an inpatient rehabilitation center following craniotomy between 2004–2006. Outcome measures: admission and discharge functional independence measure (FIM), FIM subsets (ADL, mobility, cognition), FIM change, LOS, and discharge disposition. **Results:** Forty-one patients were divided into three groups based on tumor type (metastatic (MET) $n = 8$; mean age 64, high grade glioma (HGG) $n = 15$; mean age 58, low grade glioma (LGG) $n = 18$; mean age 52. Mean LOS for acute care and rehab was similar for all three groups. The majority of all patients in all groups discharged to home. There was a statistically significant improvement in mean total FIM from admission to discharge (Admission–Discharge scores: MET 75–103, HGG 68–84, LGG 71–99) for all tumor groups ($P < .01$, $P < .05$, $P < .01$ respectively). There was significant improvement ($P < 0.01$) in all the FIM subcategories (ADL, mobility, cognition) for MET and LGG. Patients in HGG group significantly improved ADL ($P < .05$) and mobility scores ($P < .01$). **Conclusions:** Patients in all tumor groups experienced an overall increase in mean FIM at the time of discharge with no significant difference noted between tumor types. Rehabilitation services may offer a unique opportunity to influence functional outcome in these individuals and influence caregiver burden once discharged to home.

Effects of tumor associated microglia on local immune responses

Courtney Brannan; Andrew T. Parsa, MD, PhD (San Francisco, CA)

Introduction: Microglia are a quiescent CNS resident cell type, capable of evaluating the local environment and alerting cells outside of the CNS to a problem. When activated, they are capable of recruiting peripheral immune cells and initiating local immune responses. Bridging the innate and adaptive responses, microglia can also likely affect tumor cell survival and proliferation. It is unknown how brain tumors affect microglia and how microglia, in turn, can shape the local environment and immune responses. **Methods:** Using cells isolated from the V12 Ras transgenic model, which spontaneously develop tumors similar in phenotype and progression to human gliomas, we evaluated the effects of the tumor environment on microglia. **Results:** We find that microglia undergo drastic phenotypic

and functional changes following exposure to cell lines derived from these animals. Exposure to cell free supernatant is sufficient to induce upregulation of several molecules involved in antigen presentation and increase phagocytosis when compared to microglia isolated from a healthy environment. When challenged with bacterial lipopolysaccharide (LPS) and gamma interferon, microglia upregulate several innate and T cell activating pro-inflammatory molecules, including TNF α , IL-6 and IL-12. These responses are also characterized by robust production of anti-inflammatory molecules at levels far exceeding those produced by microglia from a healthy environment. Tumor associated microglia (TAM) also produce significant levels of RANTES and MCP-1, both of which have been demonstrated to promote tumor growth. **Conclusions:** Together, these data suggest that TAM have the ability to skew innate and adaptive immune responses of local and infiltrating cells while directly impacting tumor survival and growth.

MSH6 is lost in glioblastomas recurrent after temozolomide treatment

Daniel P. Cahill, MD, PhD; Rebecca A. Betensky, PhD; Tracy T. Batchelor, MD; John Iafrate, MD, PhD; David N. Louis, MD (Boston, MA)

Introduction: Glioblastomas are treated with maximal surgical resection followed by adjuvant treatment combining radiotherapy (XRT) and the alkylating chemotherapeutic agent temozolomide (TMZ). Both O-6 methylguanine methyltransferase (MGMT) and the mismatch repair gene MSH6 have been linked to therapeutic resistance in glioblastomas. **Methods:** To comparatively evaluate these resistance pathways in human glioblastomas, we determined the MSH6 coding frame sequence in 47 glioblastomas. In addition, we systematically analyzed a second panel of 42 pre-treatment and post-treatment glioblastoma resection specimens for MGMT and MSH6 protein expression using immunohistochemistry. **Results:** MSH6 mutation was not observed in any pre-treatment glioblastoma (0/39), while 3/14 recurrent cases had somatic mutations, indicating that mutation occurred in association with recurrence ($P = 0.016$). As expected, MGMT status varied amongst pretreatment samples, but no correlation was detected between pre-treatment MGMT and post-XRT + TMZ MSH6 status. MSH6 protein expression was detected in all pre-treatment (15/15) and post-XRT-only-treated (8/8) cases examined. Notably however, a significantly greater proportion of post-XRT + TMZ recurrences had absence of MSH6 (9 of 19, $P = 0.026$). **Conclusions:** Loss of MSH6

occurs in a substantial proportion of glioblastomas recurrent from XRT + TMZ. MSH6 loss does not appear to be merely a proxy for MGMT status, as the two markers were not correlated in our panel. The association of MSH6 mutation and loss with glioblastoma recurrence specifically after TMZ suggests that MSH6 inactivation might mediate TMZ treatment escape. The presence of MSH6 loss only in post-treatment tumors is consistent with the outgrowth of a TMZ-tolerant sub-clone. MSH6 deficiency may therefore function as a clinically-relevant mechanism of TMZ escape.

Treatment of CNS tumors with N-acetyl-D-mannosamine tetrabutryrate

Christopher T. Campbell, BA; Hasan Zaidi, BA; Jie Fu, PhD; Christopher Weier, BA; Srinivasa G. Sampathkumar, PhD; Khan W. Li, MD; Betty M. Tyler, BA; Violette Renard, MD; Mark B. Jones, BA; Justin Hanes, PhD; Kevin Yarema, PhD; Henry Brem, MD, FACS (Baltimore, MD)

Introduction: But4ManNAc (N-acetyl-D-mannosamine-tetrabutryrate) is a novel prodrug that releases butyrate and ManNAc intracellularly thereby combining histone deacetylase inhibition with stimulation of sialic acid production to enhance apoptosis in cancer cell lines. To improve the poor pharmacokinetic properties of But4ManNAc, we demonstrate that this compound can be encapsulated in a polymer delivery system for treatment of intracranial tumors. **Methods:** But4ManNAc was encapsulated in sebacic acid-polyethylene glycol polymers for controlled release over a 7–10 day period. In vitro tests measured activity of liberated compound in cancer cell lines. Then, Balb-C mice injected with EMT-6 cells intracranially were treated with polymer discs loaded with 0 or 30% (w/w) But4ManNAc on day 0 or 5 following tumor injection. **Results:** But4ManNAc produced significant cell death in vitro at = 100 μ M within 3 days for EMT-6 and glioma cells (C6, F98, AL New). Animals treated with the drug formulation, by contrast, showed no overt toxicity. In mice injected with EMT-6 cells, treatment did not significantly prolong median survival. However, treatment with But4ManNAc at day 0 and day 5 increased long-term survival rates from 0% (Controls) to 10 and 30%, respectively. **Conclusions:** This work demonstrates a method for improving the pharmacokinetic properties of carbohydrate based drug candidates. Initial in vivo tests show a potential therapeutic effect of But4ManNAc in a metastatic brain tumor model and warrants further investigation for treatment of intracranial tumors.

Optimization of adenoviral vector mediated expression in the canine brain in vivo and in canine glioma cells in vitro

Marianela Candolfi, DVM; G. E. Pluhar; Kurt Kroeger; Mariana Puntel; James F. Curtin; Carlos Barcia; AKM G. Muhammad; Elizabeth A. Mc Niel; Andrew Freese; J. R. Olhfest; Peter Moore; Donna Palmer; Philip Ng; Pedro R. Lowenstein; Maria G. Castro, PhD (Los Angeles, CA)

Introduction: Adenoviral delivery of the immune-stimulatory molecule, Flt3L, and the conditional cytotoxic enzyme TK provides long-term survival of glioblastoma (GBM) rodent models. In preparation for a clinical trial in spontaneous GBM in dogs, we tested adenovirus-mediated transgene expression in the dog brain in vivo and in canine J3T GBM cells in vitro. **Methods:** We administered Ads encoding β -Gal driven by the hCMV (Ad-hCMV- β -Gal) or the mCMV promoter (Ad-mCMV- β -Gal) into the cerebral cortex of healthy Beagle dogs and determined transgene expression by immunocytochemistry. We also determined in vitro the transduction efficiency of regulated high capacity adenovirus vectors (HC-Ads) encoding β -Gal under control of the TetON system driven by the mCMV (HC-mTetON- β -Gal) or the hCMV promoter (HC-hTetON- β -Gal) in J3T cells. We also infected J3T cells with therapeutic HC-Ad vectors expressing HSV1-TK or Flt3L. **Results:** Expression of β -galactosidase was higher when driven by the mCMV than the hCMV promoter in the dog brain, without side effects. β -Gal activity in J3T cells was also higher with the HC-mTetON- β -Gal than with the HC-hTetON- β -Gal. Dog glioma cells were efficiently transduced by HC-Ads expressing mCMV-driven HSV1-TK, which induced 90% cell death in the presence of gancyclovir. J3T cells were also transduced with HC-Ad expressing Flt3L under the control of the TetOn system, which expression was stringently inducer-dependent. **Conclusions:** HC-Ads encoding therapeutic transgenes under the control of regulatory sequences driven by the mCMV promoter would constitute excellent vectors for the treatment of GBM in dogs as a prelude to implementing this approach in human GBM patients.

Adenoviral mediated expression of pseudomonas exotoxin fused to IL-13 for glioma therapy

Marianela Candolfi, DVM; Weidong Xiong, PhD; Mariana Puntel, PhD; Chunyan Liu; Kurt M. Kroeger; Sonali Mondkar; Pedro R. Lowenstein, MD, PhD; Maria G. Castro, PhD (Los Angeles, CA)

Introduction: Glioblastomas overexpress IL13a2R, which is absent in the normal brain. We developed an adenovirus

(Ad) expressing the Pseudomonas exotoxin fused to IL13 (IL13-PE) glioblastoma treatment and tested it in vitro in human glioma cells and normal rodent brain in vivo. **Methods:** We constructed an adenovirus, Ad-IL4-TRE-IL13.PE expressing IL-13-PE, and, as a safety feature, a mutated IL-4 that blocks physiological IL13/IL4R. We also constructed a control vector Ad-IL4-TRE-IL13 expressing muIL-4 and IL-13. Transgenes' expression is driven by the bidirectional TRE promoter, which is activated by the transactivator (TetON, expressed within Ad-TetON), in the presence of the inducer, i.e., Dox. **Results:** Successful expression of muIL-4 and IL-13-PE was detected by immunocytochemistry in infected COS-7 and human glioma cells. Cell viability was reduced 70% when the human glioma cells were incubated in the presence of Ad-IL4-TRE-IL13-PE in the "ON" state (Dox+), but remained unaffected in the "OFF" state, indicating that the expression of the chimeric toxin can be tightly regulated. Ad-IL4-TRE-IL13-PE did not affect the viability of COS-7 cells, which do not express the IL13alpha2R. These results suggest that IL-13-PE cytotoxicity is specific to glioma cells. Control vector Ad-IL4-TRE-IL13 did not affect the viability of COS-7 or human glioma cells, neither with or without Dox. Neither the therapeutic nor the control vector elicited toxicity within the normal rodent brain. **Conclusions:** Our results suggest that Ad-mediated intratumoral expression of IL13-PE toxin will lead to effective cytotoxicity in IL-13a2R expressing-GBM cells without side effects to the surrounding non-neoplastic brain.

Combined HSV1-TK/Flt3L immunotherapy induces regression of human glioblastoma xenografts in nude mice

Marianela Candolfi, DVM; AKM G. Muhammad, MD, PhD; James F. Curtin, PhD; Mariana Puntel, PhD; Kurt M. Kroeger; Chunyan Liu; Pedro R. Lowenstein, MD, PhD; Maria G. Castro, PhD (Los Angeles, CA)

Introduction: We previously showed that combined treatment with Ad-Flt3L and Ad-TK dramatically improved survival in large syngeneic rat and mouse models of glioblastoma (GBM). Ad-Flt3L encodes fms-like tyrosine kinase ligand (Flt3L), which recruits and activates dendritic cells into the brain, improving antigen presentation. Ad-TK expresses herpes simplex type 1-thymidine kinase (HSV1-TK), which selectively kills rapidly dividing cells in combination with the prodrug gancyclovir (GCV). We now implemented this combined immunotherapy in athymic mice bearing large intracranial human xenograft GBM. **Methods:** nu/nu Balb/c mice were intracranially implanted with U251 human GBM cells (1.5×10^6) and after 7 days,

they were intracranially injected with Ad-TK (5×10^7 pfu) and Ad-Flt3L (5×10^7 pfu) alone or in combination. Control animals were administered saline solution. After vector/saline administration, mice received intraperitoneal injections of GCV (0.7 mg/100 μ l, once daily for a week). **Results:** Animals bearing U251 tumors that received saline had a median survival of 22 days post-tumor implantation. The administration of Ad-Flt3L doubled the survival when compared to saline treated controls (median survival: 40.5 days; $P = 0.05$), the administration of Ad-TK/GCV alone failed to improve survival when compared to saline treated mice (median survival: 21 days). However, the combination of Ad-TK/GCV and Ad-Flt3L significantly prolonged the survival of tumor-bearing mice, which was 100% after 6 weeks ($P = 0.05$). **Conclusions:** Our results suggest that the combined treatment with Ad-TK and Ad-Flt3L may not only induce an adaptive cellular antitumoral immune response, but would also trigger a strong innate immune response that eliminates the human GBM implanted in athymic mice.

A comparative histopathological analysis of experimental glioblastoma multiforme (GBM) in mice, rats and spontaneous GBM in dogs and humans

Marianela Candolfi, DVM; James F. Curtin, PhD; W. S. Nichols, MD; Akm G. Muhammad, MD, Ph; Gwendalyn D. King, PhD; Carlos Barcia, PhD; G. E. Pluhar, DVM; E. A. Mc Niel, DVM; John R. Ohlfest, DVM; Andrew B. Freese, PhD Peter F. Moore, DVM; Pedro R. Lowenstein, MD, PhD; Maria G. Castro, PhD (Los Angeles, CA)

Introduction: Although rodent glioblastoma (GBM) models have been used for over 30 years, the extent to which they recapitulate the histological features of human GBMs remains controversial. We compared the histopathology of human GBM with spontaneous and experimental GBM models. **Methods:** Paraffin sections from human and spontaneous dog GBM, and intracranial human xenografts GBMs in mice (U251 and U87 in nu/nu Balb/c mice), and syngeneic rodent GBMs (GL26 in C57BL/6 mice, CNS-1 in Lewis rats) were stained with H-E or immunocytochemistry to detect GFAP, vimentin, Von Willebrand factor, CD3 and macrophage immunoreactive cells. **Results:** All GBMs exhibited necroses, neovascularization, pleomorphism, vimentin immunoreactivity, and intense infiltration of macrophages and CD3+ lymphocytes, including human GBM xenografts in athymic mice. Human and dog GBMs, as well as U251 xenografts expressed GFAP, while no GFAP staining was detected in the U87 xenograft and the

syngeneic rodent models. As in human GBM, endothelial proliferation was observed in dog tumors. In all spontaneous and experimental GBMs we found histopathological features compatible with tumor invasion into non-neoplastic brain. **Conclusions:** our data indicate that murine models of GBM appear to recapitulate the human GBM to a greater degree than hitherto recognized, constituting a useful in vivo system for preclinical studies. Additionally, dog GBM may allow testing novel therapies in a spontaneous tumor in the context of a larger brain.

Increased gangliosides in the serum of GBM patients may be responsible for their immunosuppression

Ali Chahlavi, MD (Orlando, FL); Soumika Biswas, BS; Kaushik Biswas, PhD; Sankar Ghosh, PhD; Pat Rayman, MS; Amy Richmond, BS; James Finke, PhD (Cleveland, OH)

Introduction: GBM-lines are known to express gangliosides and death-ligands that can induce apoptosis in T-cells. Immune-competent rats injected intracranially with GBM began expressing increased levels of gangliosides in their serum. This increase correlated with increased peripheral T-lymphocyte apoptosis. We addressed whether GM2 produced by the GBM-lines could be shed to T-cells and promote immune-suppression. **Methods:** Supernatants from three GBM lines that express GM2-synthase and GM2 (U87, CCF4 and CCF52) were incubated with peripheral blood lymphocytes from healthy donors for 72 h. Thereafter, T-cells were tested for apoptosis (staining with annexinV/7AAD) and stained with anti-GM2 antibody to detect expression of GM2 on T-cells. RT-PCR was used to assess the level of GM2 synthase mRNA in T-cells. **Results:** GM2 shed from GBM-lines binds to T-cells from healthy donors which normally do not express any detectable GM2. This GM2 expression on T-cells appears to be the result of GM2 shedding from the tumor line and binding to lymphocytes. This is supported by the demonstration that T cells stained positively for GM2 (anti-GM2 specific antibody) after exposure to supernatants from GBM-lines while T-cells did not contain any detectable GM2 synthase mRNA. Moreover, apoptosis of T cells induced by GBM supernatants was associated predominantly with the GM2 positive T-cells. **Conclusions:** GM2 can be shed from GBM lines, and consequently can incorporate into the T-cells (specifically GM2) inducing them to undergo apoptosis. Blocking the action of ganglioside (GM2) may reduce GBM induced immunosuppression. Studies are planned to test whether plasma from GBM patients will transfer GM2 to T-cells and promote apoptosis.

A new pre-operative grading system for adult hemispheric low grade gliomas

Edward F. Chang, MD; Justin Smith, MD, PhD;
Kathleen Lamborn, PhD; Susan Chang, MD;
Andrew T. Parsa, MD, PhD; Mitchel S. Berger, MD;
Michael McDermott, MD (San Francisco, CA)

Introduction: A variety of prognostic factors for outcomes after surgical resection of low grade gliomas (LGGs) has been described. Patient survival nonetheless ranges broadly from three to 20 years. A pre-operative scoring system that predicts patient outcomes based upon most relevant prognostic variables does not currently exist. **Methods:** A set of demographic and radiographic variables derived from the literature regarding LGGs was used to create a prognostic grading scale. Subjects were assigned one point for each of the following factors: (1) age >50 years, (2) maximum tumor diameter >5 cm, (3) eloquent location, (4) KPS <70, and (5) “diffuse” borders (on T2 weighted MR imaging). A retrospective chart review and follow-up of 203 adult patients with hemispheric LGGs operated at the University of California, San Francisco, were included for analysis. Primary outcomes measures were time to death and tumor progression/recurrence. **Results:** Median follow-up period was 31 months. A statistically significant correlation was found between the pre-operative score and patient outcome, with low inter-observer variability. Three-year patient SURVIVAL was as follows: Grade 0 = 97.3%, Grade 1 = 92.3%, Grade 2 = 80.1%, Grade 3 = 71.5%; Grade 4 = 43.2%. PROGRESSION-free survival at three years was as follows: Grade 0 = 79.9%; Grade 1 = 73.3%; Grade 2 = 65.2%; Grade 3 = 54.3%; Grade 4 = 38.2%. **Conclusions:** This scoring system is a simple and reliable method to predict outcomes after surgery for LGGs. The application of a standardized grading system for LGGs should enable comparison between various clinical series, and should assist in clinical decision-making.

The role of N-myc in glioma formation

Clark C. Chen, MD, PhD (Boston, MA)

Introduction: N-myc over-expression or amplification is observed in a sub-set of malignant gliomas. However, the contribution of such amplification to glial carcinogenesis remains unclear. This issue was addressed using two genetically tractable transformation models. **Methods:** Introduction of specific genetic changes can transform normal cultured human astrocytes into tumorigenic cells in mouse xenograft models. This transformation is achieved by sequential ectopic over-expression of the viral T/t

antigen, hTERT, and H-rasV12G. Alternatively, such transformation can be achieved by ectopic over-expression of the viral E6/E7 antigen, hTERT, and H-rasV12G. We tested whether the over-expression of N-myc functionally substitutes for H-rasV12G in these assays. **Results:** Though N-myc over-expression in the context of T/t antigen + hTERT or E6/E7/hTERT over-expression enhanced cellular proliferation and anchorage independent growth, such over-expression failed induce tumor formation in mice. **Conclusions:** The pro-proliferative and pro-anchorage independent growth properties of N-myc suggest its participation in glioma formation. However, such participation likely occur in the context of cooperative interaction with other tumor suppressor genes or cellular oncogenes.

Complexity underlying glioma resistance to temozolamide and BCNU

Clark C. Chen, MD, PhD; Alan D’Andrea, MD
(Boston, MA)

Introduction: Despite advances in the treatment of malignant gliomas, it remains a devastating disease, causing death in nearly all affected. Since malignant gliomas constitute a disease of infiltrative nature, meaningful treatment plans will require the incorporation of chemotherapy. In randomized trials, local-regional delivery of BCNU (1,3-Bis(2-Chloroethyl)-1-Nitroso-Urea) and concurrent Temozolamide (TMZ)/radiation therapy both conferred survival benefits. However, the benefits were modest due to the development of therapeutic resistance. The study presented here attempts to elucidate the DNA repair pathways underlying this resistance. **Methods:** Protein biochemistry, immunofluorescence microscopy, SiRNA transfections. **Results:** Here we identify a novel repair pathway that is responsible for mediating glioma resistance to TMZ and BCNU. We show that TMZ and BCNU treatment of glioma cell lines led activation of the Fanconi Anemia (FA) DNA repair pathway as evidenced by mono-ubiquitination of the FANCD2 protein as well as FANCD2 nuclear foci formation. Inhibition of FA repair pathway by a small molecule inhibitor (Curcumin) or by Small interference RNA (SiRNA) caused hypersensitivity to TMZ/BCNU in the T98g glioma cell line. This sensitivity is further enhanced by MGMT inhibition using O6-BenzylGuanine, suggesting that the FA repair pathway and MGMT repair pathway cooperate in the repair of TMZ and BCNU induced DNA damage. **Conclusions:** These results presented here suggest that cellular resistance to TMZ and BCNU is mediated by multiple DNA repair pathways, many of which remain currently unidentified. The characterization of these pathways as well as a global understanding of their interactions

are prerequisites to meaningful therapeutic or prognostic models of TMZ and BCNU therapy.

A novel source of tumor specific antigen: polysome selected RNA

John H. Chi, MD; Larry Fong, MD; Andrew T. Parsa, MD, PhD (San Francisco, CA)

Introduction: Cancer immunotherapy is dependent on generating strong and durable antigen specific T cell responses specifically against tumor tissue. Unfortunately, the majority of cancers, including glioma, do not have identified tumor specific antigens. We propose that polysome-selected mRNA represents an enriched pool of transcripts with the potential of containing tumor-unique mRNA which can be used as antigen source. *Methods:* Polysomal RNA was isolated using a sucrose gradient centrifugation technique. Polysomal RNA fractions from glioma cell lines were analyzed for the presence of ARF4L, a putative glioma specific antigen, using Northern analysis. Translational regulation of ARF4L was established via inhibition of Akt pathways with rapamycin. Polysomal RNA from B16-ovalbumin cells were then electroporated into matured C57BL6 bone marrow dendritic cells (DC). After endotoxin stimulation, the ability of transfected DC's to activate ova-specific T cells was assessed by mixing with the MHC I restricted B3Z T-cell hybridoma cells overnight. *Results:* AR4L is elevated in the polysome-selected fraction of mRNA from glioma cells lines compared to normal astrocytes. AR4L is translationally regulated and reduced by rapamycin in immortalized and primary glioma lines with Akt activation. Polysomal RNA transfected BMDC elicited an antigen specific B3Z response while poly-a tailed RNA and total cellular RNA did not. *Conclusions:* ARF4L is translationally regulated and preferentially selectable in the polysomal fraction of mRNA. Pooled polysome selected RNA transfected into DC's can induce an antigen specific T-cell response in vitro. We provide early evidence that suggests tumor specific T-cell responses can be generated using polysome-selected mRNA in the absence of identified tumor antigens.

Silencing of the Wilms' tumor 1 gene decreases the tumorigenicity of human glioblastoma cells

Aaron Clark, BS; Joy L. Ware, PhD; Mike Y. Chen, PhD; Martin Graf, PhD; Timothy E. Van Meter, PhD; Helen Fillmore, PhD; William C. Broaddus, MD, PhD (Richmond, VA)

Introduction: The Wilms' tumor 1 (WT1) gene is expressed by many developing organ systems, including the

central nervous system; however it is not expressed in most normal adult tissues. Our laboratory and others have shown that WT1 is overexpressed in glioblastoma and lower grade gliomas, as well as many other human cancers. However, the function of WT1 in glioblastoma is unknown. *Methods:* U251MG human glioblastoma cells, which express high levels of WT1, were transduced with a sequence coding for short hairpin RNA (shRNA) directed against WT1 using a retroviral vector. After growth in selection media, WT1 expression in pooled transduced cells was detected by Western blotting. Cell growth rates were determined by MTS assay and confirmed by 3H-thymidine incorporation assay. 6×10^6 cells were injected subcutaneously into the flanks of nude mice and tumor growth rates and survival were analyzed. Cell invasiveness was quantified using an in vitro cell invasion assay. *Results:* Viral transduction with WT1 shRNA decreased WT1 protein expression to nearly undetectable levels which persisted for several months. WT1 shRNA transduced cells grew at a significantly lower rate than those transduced with vector alone. The cell proliferation results were replicated in vivo. Furthermore, cells that had downregulated WT1 demonstrated decreased invasion relative to those transduced with vector alone. *Conclusions:* These results are consistent with an oncogenic role for WT1 in glioblastoma, consistent with its prominent overexpression (80%) in these tumors. In addition, the results suggest that WT1 may prove an attractive target for glioma therapy by decreasing proliferation and invasion.

Image-guided radiosurgery boost as an adjunct treatment for high-grade gliomas

Nicholas D. Coppa, MD; Lisa M. Dias, BS; Sean P. Collins, MD, PhD; Walter C. Jean, MD; Brian T. Collins, MD (Washington, DC)

Introduction: With current conventional radiation techniques, it is difficult to deliver "full dose" treatment to gliomas that are close to critical neurological structures without late complications. As chemotherapeutic treatments improve survival, the avoidance of late complications becomes more important. We utilized image-guided radiosurgery as an adjunct to conventional radiotherapy to treat high-grade gliomas, and investigated the safety of this technique. *Methods:* Between January 2002 and July 2006, 17 patients with high-grade gliomas in close proximity to critical structures (i.e., optic nerves, optic chiasm and brainstem) were treated. All patients received conventional radiation therapy after surgery, with a median dose of 50 Gy (range: 45–50 Gy). Subsequently, an additional dose of 10 Gy was delivered in five daily stages utilizing

the CyberKnife® image-guided radiosurgical system. **Results:** During the image-guided radiosurgery treatments, the mean number of radiation beams utilized was 137 and the mean number of verification images was 45. Among the 17 patients, the median treatment volume was 80 cc and the median percent target coverage was 95%. Among the anaplastic glioma cohort, nine patients (90%) were alive at a median follow-up period of 26 months. Among the GBM cohort, four patients (57%) were alive at a median follow-up period of 9 months. There have been no clinical complications directly referable to this radiation regimen in these patients. **Conclusions:** We utilized imaged-guided radiosurgery as an adjunct to radiotherapy to improve the targeting accuracy for treatment of high-grade gliomas. This technique was safe, and allowed for optimal dose-delivery in our patients.

Celecoxib inhibits meningioma tumor growth in a mouse xenograft model

William T. Couldwell, MD, PhD; Brian T. Ragel, MD; Randy L. Jensen, MD, PhD; David L. Gillespie, PhD; Stephen M. Prescott, MD (Salt Lake City, UT)

Introduction: Treatments for recurrent meningiomas are limited. We previously demonstrated universal expression of COX-2 in meningiomas and dose-dependent growth inhibition in vitro with celecoxib, a COX-2 inhibitor. We therefore tested the effects of celecoxib on meningioma growth in a mouse xenograft model. **Methods:** Meningioma cell lines (IOMM-Lee, CH157-MN, WHO grade I primary cultured tumor) were transplanted into flanks of nude mice fed mouse chow with celecoxib at varying concentrations (0, 500, 1000, 1500 ppm) ad libitum. Tumors were measured biweekly and processed for MIB-1, Factor VIII, COX-2, and VEGF, and assayed with TUNEL. **Results:** Celecoxib reduced growth of mean tumor volume compared with untreated controls in IOMM-Lee, CH157-MN, and benign tumors. IOMM-Lee tumors removed from celecoxib treatment regained a growth rate similar to the control. Blood vessel density decreased and apoptotic cells increased in treated flank tumors. Diminished COX-2 expression and VEGF were observed in treated IOMM-Lee tumors. **Conclusions:** Celecoxib inhibits meningioma growth in vivo at plasma levels achievable in humans. Celecoxib-treated tumors were less vascular with increased apoptosis. IOMM-Lee tumors treated with celecoxib showed decreased COX-2 and VEGF expression. COX-2 inhibitors may have a role in treatment of recurrent meningiomas.

High-dose proton beam radiation therapy for advanced primary sphenoid sinus malignancies: treatment outcome and prognostic factors

William T. Curry, Jr., MD; Mihn T. Truong, MD; Norbert J. Liebsch, MD, PhD; Derrick T. Lin, MD; Frederick G. Barker, II, MD; Vrishali V. Lopes, MS; Jay S. Loeffler, MD; Annie W. Chan, MD (Boston, MA)

Introduction: We analyzed treatment outcome and prognostic factors in patients with advanced primary sphenoid sinus malignancies treated with proton radiotherapy. **Methods:** Retrospective review of records and imaging of patients with advanced primary sphenoid sinus malignancies who received proton radiotherapy at MGH, 1991–2005. **Results:** Twenty patients fit the criteria. Squamous cell carcinoma was the most common pathology. Ninety-five percent of tumors extended to the clivus, 90% to the cavernous sinus, 65% to the nasopharynx, 15% to the oropharynx, 15% to the optic chiasm, and 25% to the brain. Ten percent had a gross total resection. Median tumor dose was 76 Cobalt-Gray-Equivalents. Median follow-up was 20 months. Two-year local, regional, and distant control rates were 83, 84, and 60% respectively. Disease-free and overall survival rates at 2 years were 35 and 43%. By multivariate analysis, brain invasion ($P = 0.04$) and oropharyngeal involvement ($P = 0.03$) predicted poorer disease-free survival. Brain invasion predicted poorer overall survival ($P = 0.05$). We, therefore, propose a novel staging system for these tumors: Grade A—tumor limited to the sphenoid sinus; Group B—tumor extending beyond the sphenoid sinus, without extension to oropharynx or brain; Group C—tumor invasion of brain or oropharynx. Two-year disease-free and overall survival for group B patients were 58 and 69%. Two year disease-free and overall survival for Group C patients were 0 and 15%. **Conclusions:** High-dose proton radiotherapy can result in good local control for some patients with primary sphenoid sinus malignancies Brain invasion and oropharyngeal involvement are poor prognostic factors, providing the basis for a novel staging system.

Cerebral parenchyma triton tumor with a glial component: case report and literature review

Guipson D. Dhaity, MD; Felix Dominguez, MD; Ignacio Felix, MD (Mexico)

Introduction: The occurrence of a nerve sheath malignant tumor, with a rhabdomyoblastic component (triton tumor) is

very rare, and its location in the cerebral parenchyma is furthermore, extremely rare. Seven cases have been reported in the literature. *Methods:* this a clinical Case report of 64-year-old patient managed in the hospital of Medical nacional Center Sigle XXI of Mexico City. *Results:* This is a 64-year-old man developed monoparesia of the left pelvic limb during the last 4 weeks. A Cranial Computed Tomography (CCT) showed a right frontoparietal lesion which was enhanced, with the contrast medium; the Magnetic resonance Image (MRI) showed a lesion which was enhanced, in an irregular manner, with gadolinio as well as perilesional cerebral edema. A right frontoparietal craniotomy was performed and a total resection of the lesion was carried out. The histological diagnosis was a neurogenic malignant tumor of the peripheral nerve sheath, with a rhabdomyoblastic and scanty glial differentiation. *Conclusions:* There are no previous reports in the literature related to a triton tumor in the cerebral parenchyma with a glial component. This is the case number 8 of a triton tumor intracranially informed in the medical literature. This is the first case reported with a glial component, since it was proved positive to the glial fibrillar acid protein, this glial element does not have a significant prognosis in the evolution and survival of the patient. It has been described more than one hundred cases outside the brain but only seven located intracranially.

Nuclear import of pur-alpha in human astrocytic cells and mouse fibroblasts causes inhibition of cell growth

Keri J. Donaldson, MD; Nune Darbinian, PhD;
Armine Darbinyan, MD; Michael W. Weaver, MD;
Christopher M. Loftus, MD, FACS; Kamel Khalili,
PhD (Philadelphia, PA)

Introduction: Pur-alpha is a single stranded DNA- and RNA-binding protein implicated in a variety of biological events including: transcriptional control, DNA unwinding and replication, cell cycle regulation, DNA damage repair, and interaction with transcription factor E2F-1 and Rb. Pur-alpha contains three basic aromatic class I and two acidic leucine-rich class II repeats in the central region of the protein. Pur-alpha is predominantly cytosolic, but some levels are also found in the nucleus. Here, we demonstrate overexpression in the nucleus suppresses proliferation of tumor cells including human glioblastoma, and mouse fibroblasts. *Methods:* Briefly, we studied whether treatment of cells with Pur-alpha will cause cell growth inhibition. Protein transduction experiments using the bacterially produced and purified GST-Pur-alpha revealed cellular internalization of Pur-alpha protein and a decreased proliferation of glioblastoma cells. Effects of Pur-alpha upon

its nuclear import were demonstrated by utilizing various retroviral vectors and a decrease in Colony Forming Units (CFUs) in a colony formation assay. *Results:* Creation of Pur-alpha knockout mouse fibroblast cell lines stably expressing nuclear Pur-alpha was performed and growth inhibitory effect of Pur-alpha was demonstrated. Cisplatin treatment of mouse fibroblast or Pur-alpha knockout cells demonstrated that Pur-alpha interferes with cisplatin in normal cells and may act as a selective anti-tumor agent, mediating the toxic effects on normal cells during cisplatin therapy. *Conclusions:* Results illustrate efficacy of Pur-alpha in suppressing glioblastoma cell growth, without affecting normal cell growth, and may provide an evidence for the potential use of this protein and its derivative(s) in blocking proliferation of tumor cells.

Interval visual improvement with intermittent drainage of craniopharyngioma cyst via ommaya reservoir: case report

Samer K. Elbabaa, MD; Syndee Givre, MD; Sharon Cush, RN; Matthew G. Ewend, MD (Chapel Hill, NC)

Introduction: Visual loss and visual field deficits are common presenting symptoms of recurrent cystic craniopharyngioma. Management options after repeat craniotomy include radiation as well as other cyst-directed treatment modalities. We report interval resolution of visual symptoms after percutaneous cyst aspiration in one patient with recurrent craniopharyngioma. *Methods:* A 34 year male patient presented with symptoms of progressive visual and behavioral changes. Brain MRI showed a suprasellar enhancing mass with a large cystic component. He underwent craniotomy for apparent gross total resection confirmed by postoperative MRI. Histopathology was consistent with adamantinomatous craniopharyngioma. Postoperatively, the patient had significant recovery of visual acuity and field deficits. Seven weeks later, he returned with recurrent visual symptoms. MRI showed recurrence with large cystic component. He underwent a repeat craniotomy for tumor resection and placement of cyst catheter connected to an Ommaya Reservoir. He again had visual improvement after second surgery. *Results:* The patient presented twice with recurrence of similar visual symptoms at 4 and 5 weeks after the second surgery. Radiation sessions started at the end of fourth week. MRI findings at each presentation were consistent with recurrence of cystic component. The Ommaya reservoir was tapped at each presentation using gentle negative suction. MRI after each tap confirmed significant decrease in size of cyst correlating with improvement in visual symptoms and examination. *Conclusions:* Symptomatic recurrent

craniopharyngioma cysts can be managed with cyst aspiration via implanted catheter rather than repeat surgery while definite therapy such as radiation is being completed. Immediate improvement in visual symptoms may be seen following aspiration.

Effect of HFE polymorphism on outcomes in brain tumor patients

Elana Farace, PhD; Kojan Kainth; Rebecca Slagle-Webb; James R. Connor, PhD; Jonas M. Sheehan, MD (Hershey, PA)

Introduction: There is evidence in our basic and translational science laboratory that a polymorphism in the HFE gene, the most common genetic mutation among Caucasians may be associated with the frequency, progression and prognosis of brain tumors. The purpose of this study was to determine if brain tumor patients with a mutation of the HFE gene have worse outcomes than patients who do not. *Methods:* Seventy-five consecutive patients with mixed tumor types (glioma, meningioma, metastatic disease etc.) were genotyped using cells available from buccal swab and from tumor tissue. Kaplan–Meier survival analysis was used in a univariate test of whether patients with the HFE mutation had a significantly shorter length of survival. *Results:* In all cases in which both specimens were available, the genotyping was concordant, suggesting that this is a true polymorphism. Patients with the HFE mutation lived a shorter number of days (mean = 216) than patients with wildtype (mean = 289) but the difference is not yet statistically significant ($P = .10$). Mean length of follow-up was only 283 days. *Conclusions:* Presence of HFE mutation seems to be associated with shorter length of survival in this preliminary analysis. Longer length of follow-up will be needed for this to reach statistical significance.

Correlating HIF-1a and its downstream molecular markers to survival in adults with glioblastoma multiforme

Jeannette R. Flynn, MD; Karen Salzman, MD; Anita Kinney, RN, PhD; Randy L. Jensen, MD, PhD (Salt Lake City, UT)

Introduction: This study compared HIF-1a, its downstream targets (VEGF, GLUT-1 and CA IX), microvascular density (MVD), and cellular proliferation between glioma tumor grades as well as comparing them to patient survival.

Tumor necrosis on pre-operative imaging was also examined. *Methods:* This study was a retrospective chart review. Clinical data and tissue was collected on 85 patients with GBM, anaplastic astrocytoma, and low grade gliomas. Immunohistochemistry was done to look at the hypoxia related molecules (HRMs). MVD and cellular proliferation indices were established after staining for Factor 8 and MIB-1, respectively. Preoperative images were evaluated for areas of necrosis, tumor and peritumoral edema on 10 patients with GBM. These areas were then correlated to HRMs, both indices and survival. *Results:* No significant correlations between HRMs or the indices and survival in the group of patients with GBM were found. Significant differences were seen between VEGF, GLUT, CA IX, and the MVD index when low grade and medium grade gliomas were compared. A significance was seen with all 4 HRMs as well as both indices when low grade and GBM tumors were compared. Area of necrosis/total tumor area compared to patient survival approached significance. *Conclusions:* HIF-1a appears to be involved in the transformation to GBM but does not seem to predict survival or tumor aggressiveness. This could lead to targets for treatment in the future.

Distribution of brain metastases in relation to the hippocampus

Amol Ghia, MD; Wolfgang A. Tome, PhD; Sayana Thomas, BS; George Cannon, MD; Minesh Mehta, MD; John S. Kuo, MD, PhD (Madison, WI)

Introduction: This study explores the viability of tomotherapy-guided hippocampal sparing conformal radiation therapy by determining brain metastases distribution relative to the hippocampus. *Methods:* The T1-weighted, post-contrast axial MR images of 100 patients with brain metastases from December 2002 to July 2006 at our center were examined. Metastatic lesions and their distance from the hippocampus with 5 mm, 10 mm and 15 mm contoured boundaries were analyzed. *Results:* The 100 patients (52 male, 48 female, median 58.7 years, range 32–84 years) had a total of 272 metastases. Primary tumor histologies were non-small cell lung cancer (47%), breast (13%), melanoma (12%), small cell lung cancer (11%), renal (4%), gynecological (4%) and other (6%). Lesions were distributed in frontal (32%), cerebellar (24%), parietal (17%), temporal (13%), occipital (8%), deep nuclei (4%) and brainstem (3%) locations. Only 3.3% ($n = 9$) were within 5 mm of the hippocampus. About 86.4% ($n = 235$) of metastases were located greater than 15 mm from the hippocampus. *Conclusions:* In an

unselected series of 100 patients with 272 brain metastases, only 3.3% of metastases lay within 5 mm of the hippocampus. Therefore, conformal whole brain radiotherapy that spares the hippocampus with a 5 mm margin may minimize the neurocognitive decline associated with conventional whole brain radiotherapy with minimal impact on tumor coverage. Where indicated, metastases within the hippocampus may be additionally treated with stereotactic radiosurgery.

Immunosuppression: effect of DNA based cytokine secreting vaccine on regulation of the immune response: tregs

Roberta P. Glick, MD; Terry Lichtor, MD, PhD; Insug O-Sullivan, PhD; Amla Chopra, BS; Lisa Feldman, BS; Julian Hardman, BA; Goro Osawa, BA; Edward P. Cohen, MD (Chicago, IL)

Introduction: We have previously reported encouraging results of a novel Immuno-Gene therapy for brain tumors using a cellular vaccine consisting of cytokine secreting fibroblasts transduced with tumor DNA, and expressing tumor antigens. Recently, a unique population of regulatory T cells (Tregs) have been identified that are CD4+ CD25+ and FoxP3+, and suppress T cell mediated immune responses. *Methods:* The objective of the present proposal is understanding mechanisms of immunosuppression by Tregs in brain tumors as a means of targeting this mechanism for increasing the effectiveness of brain tumor vaccine strategies. Naive syngeneic mice were injected with one of the experimental vaccines. Spleens and lymph nodes were removed and the cells were prepared for immunofluorescent staining and cytofluorometric measurements by Facs for the following markers: CD4, CD8, CD25, CD62L, B7-1, B7-2, CTLA-4, and FoxP3. For survival studies, mice were injected with tumor and vaccine intracerebrally (i.c.) and survival and immune responses were determined. *Results:* The results demonstrate that this immunogenic vaccine significantly downregulated the Treg markers CD25 and FoxP3 in the spleen and lymph node, and increased CD4 and CD8 cells in the treated animals. Survival was prolonged in mice with an i.c. tumor treated with i.c. cytokine secreting allogeneic DNA vaccine, associated with an induction of immune responses in brain, spleen, and cervical lymph nodes. *Conclusions:* These data suggest that one mechanisms of increased immunogenicity of this DNA based cytokine secreting allogeneic vaccine may be via the inhibition of Tregs, i.e. via the inhibition of immunosuppression, and suggests a new target for enhancing immunotherapy.

Enhanced delivery of trastuzumab following single fraction radiotherapy in a murine model of intracranial metastatic breast cancer

Peter M. Grossi, MD; Katia Peixoto, BS; Michael R. Zalutsky, PhD; John H. Sampson, MD, PhD (Durham, NC)

Introduction: Despite aggressive treatment including radiation, chemotherapy, and surgery, the prognosis following intracerebral metastatic breast cancer remains poor. Although Trastuzumab, a monoclonal antibody against HER2/neu, is efficacious against systemic breast cancer, it is ineffective against intracranial disease because of exclusion by the blood-brain-barrier (BBB). One mechanism to enhance delivery of larger molecules past the BBB is following radiosurgery. *Methods:* First, intracranial MCF-7/ HER2-18 xenograft tumors were established in athymic balb/c mice. One group received single fraction cranial irradiation (20 Gy). Three days later, all animals received 125I-labeled Trastuzumab. The animals were injected with Evan's blue to localize the tumor and then sacrificed. The tumors, contralateral brain, and other tissues were dissected. The localization of 125I-Trastuzumab in the tumors and other tissues was quantified with a gamma-counter. Second, the efficacy of radiation followed by Trastuzumab was evaluated in a survival model. Intracranial tumors were established in four groups: control, radiation alone, Trastuzumab alone and combination. The radiated animals underwent single fraction radiation (20Gy) after tumor establishment. Three days later, animals received a single dose of Trastuzumab (1 gm/kg) or placebo. *Results:* Localization: Following single fraction radiation, Trastuzumab tumor localization was increased by 62% in radiation treated animals ($P = 0.04$). Survival: The combination of radiation and Trastuzumab significantly increased median survival when compared to placebo (35 vs 18 days), as well as compared to either Trastuzumab (28 days) or radiation alone (20 days), ($P = 0.03$). *Conclusions:* Single fraction radiation enhances delivery of Trastuzumab and increases survival in a murine model of intracranial breast cancer.

Microsurgical treatment of small acoustic neuromas

Bharat Guthikonda, MD; Myles Pensak, MD; Ravi Samy, MD; Philip Theodosopoulos, MD (Cincinnati, OH)

Introduction: Modalities considered for the management of small acoustic neuromas include observation, radiosurgery

and microsurgery. In this retrospective review, we assess microsurgical intervention, specifically affecting facial function and hearing preservation, in patients with small acoustic neuromas. *Methods:* Thirty-five patients underwent microsurgery for acoustic neuroma with less than 1 cm extension into cerebellopontine angle (2003–2006). Of 19 women and 16 men, ages averaged 48.1 years (18–69 years). All patients presented with hearing loss and intact facial function. Surgical approaches included retrosigmoid-transmeatal (71%), translabyrinthine (26%), and middle fossa (3%). Pre- and post-operative facial and hearing functions were reviewed. *Results:* In all patients, the facial nerve was in anatomic continuity at the end of surgery. Gross total resection was performed in 91% of patients. Mean follow up was 6 months (range 1–30 months). At last follow-up, facial function was normal in 83% of patients and good (House-Brackmann 1 or 2) in 92%. Of three patients with delayed facial palsy, two resolved completely. Functional hearing was preserved in 38% of patients who underwent a potential hearing preservation procedure. No operative mortality occurred. Of three patients (8%) with CSF leak, 1 required re-exploration with symptom resolution. Two cases of presumed acoustic neuroma were histologically meningiomas and 1 of presumed meningioma was histologically a schwannoma. *Conclusions:* Microsurgical resection of small acoustic neuromas results in excellent tumor control with preservation of good facial function in most patients and low complication rates. By allowing for definitive histological diagnosis, resection ultimately reduces the need for radiation exposure to nearby eloquent structures.

Anatomic study of the chiasmatic sulcus and its surgical implications

Bharat Guthikonda, MD; William Tobler, Jr., BA;
Philip Theodosopoulos, MD; Jeffrey T. Keller, PhD
(Cincinnati, OH)

Introduction: The sphenoid bone is an area of anatomical interest for neurosurgeons as meningiomas often arise from the dura overlying this bone. The surgical relevance of the chiasmatic sulcus is not well described. We describe, in a systematic anatomic fashion, classifications highlighting the variability of this area. We also hypothesize about its clinical relevance, particularly with regards to unexpected tumor remnants. *Methods:* One hundred dry skull bases were analyzed. On each specimen we measured: distance between the two optic struts, distance between the limbus sphenoidale and the tuberculum sellae (sulcal length), length of the planum sphenoidale and the angle of the chiasmatic sulcus. We also noted the presence or absence of a bony

overhang from the planum over the chiasmatic sulcus. *Results:* Four patterns of chiasmatic sulcus region anatomy are described. Type 1 is a short steep sulcus (31%). Type 2 is a short flat sulcus (22%). Type 3 is a long steep sulcus (21%). Type 4 is a long flat sulcus (26%). Eighteen percent of skulls had a bony projection from the planum sphenoidale overlying the chiasmatic sulcus. We name this overhang the chiasmatic ridge. The chiasmatic ridge was mainly present with the Type 1 chiasmatic sulcus. *Conclusions:* The anatomy of the chiasmatic sulcus is variable. Eighteen percent of skulls had a chiasmatic ridge, a bony projection over the chiasmatic sulcus. Since meningiomas can be hidden from view in this area, preoperative CT scanning should be evaluated for the presence of this ridge and tumor resection should extend below the ridge when present.

Co-registration of diffusion tensor tractography and functional MRI data with intraoperative neuronavigation: methods and applications

Bharat Guthikonda, MD; James Leach, MD;
John M. Tew, MD; Philip Theodosopoulos, MD
(Cincinnati, OH)

Introduction: Intraoperative neuronavigation (IGS) during brain tumor surgery is a technique that has become more sophisticated over the last decade. Simultaneously, imaging advances in tractography and functional MRI have made glioma surgery much safer. We present our experience of co-registration of tractography and functional imaging in an attempt to increase the safety of intraoperative neuronavigation in eloquent cortex. Furthermore, we confirmed the accuracy of the imaging data using direct awake cortical stimulation. *Methods:* Preoperative tractography and functional MRI scanning (3T MRI) for both motor and language function were performed in a 51 year old female with a left insular glioma. Areas localized by these studies included Broca's area, Wernicke's area, the arcuate fasciculus and corticospinal tract. Tractography and functional MRI results were co-registered using BrainLab software for intraoperative navigation. The patient underwent a left frontotemporal craniotomy using standard awake techniques. Using direct cortical stimulation, the face motor cortex and areas causing speech arrest, naming difficulty and paraphasias were identified and correlated with IGS predictions. *Results:* Co-registration of tractography and functional MRI data was successful. The representation of crucial language and motor regions by preoperative tractography and functional MRI was confirmed to be highly accurate based on intraoperative awake testing. Safe corridors of approach to this tumor based on

awake cortical stimulation correlated well with the preoperative IGS planning. **Conclusions:** We describe our experience of co-registration of tractography and functional MR imaging for the purpose of intraoperative neuronavigation. This information was confirmed, by direct awake cortical stimulation, to be accurate.

Viral production of malignant brain tumor in rat brain and manner of development and infiltration

Javad Hekmat-Panah, MD (Chicago, IL)

Introduction: The role of virus in production of brain tumors in animals is now clearly demonstrated by various investigators. We have produced over 1,000 brain tumors in rats through intracerebral inoculation of a strain of rat-adapted-murine erythroblastosis virus (KiMSV). The question remains as to how the tumors start, progress, and infiltrate. **Methods:** The brains of 3 litters (nine in each group) of rats were inoculated with the KiMSV virus on the 4th day after birth with 0.005 ml (1.6×10^4 focus-forming units). One animal from each group was killed every other day after inoculation, and the development of the tumors in their brains was studied using light and electron microscope. **Results:** The early evidence of tumor cells appeared 7 days after inoculation, looking like a hypercellular area with lymphoid appearing cells surrounding small vessels; not unlike viral encephalitis. Gradually over the next several days, a small tumor appeared, recognizable with the naked eye. Histological studies revealed a highly vascular tumor consisting of two malignant cell types, mesothelial and glial. Vascular wall contained malignant cells invading the lumen; tumor cells infiltrated the substance of the brain. As tumor cells spread farther from the center of the tumor, they appeared more like glia. **Conclusions:** To understand how a glial-type tumor grows and infiltrates may help to learn how to prevent propagation. This experiment does not give the answer how biologically, but may help understand how histologically; perhaps a step toward learning the former.

Metastatic intracerebral chondrosarcoma

Namath S. Hussain, MD; John Carpenter, MD;
Marco Marsella, MD; L. Philip Carter, MD
(Tucson, AZ)

Introduction: The most common cause of neoplastic intracranial mass lesions is metastatic disease, followed by primary brain tumors. Chondrosarcomatous metastatic

disease to the brain is exceedingly rare and has not been previously reported in the literature. **Methods:** We describe a case of a woman with a known history of primary chondrosarcoma resected from the left lower extremity which metastasized to the left upper and lower pulmonary lobes. Preoperative scanning, intraoperative images, and postoperative pathological slides are reviewed. **Results:** The patient presented with acute onset of right-sided weakness and dysmetria. She was evaluated with an MRI of the brain, revealing a ring-enhancing mass in the left parieto-occipital lobe. Smaller lesions in the left frontal lobe and the right parietal lobe were also noted. The differential diagnosis based on the patient's history and imaging studies included neurocysticercosis (patient had recently traveled to Mexico), metastasis, and primary brain tumor. The patient was taken to the operating room for resection of the largest mass with intraoperative frozen section revealing metastatic chondrosarcoma. Due to the very malignant features seen on intraoperative frozen sections (abundant mitoses, loss of differentiation, and pleomorphism), adjuvant interstitial therapy was considered and Gliadel wafers were placed. The patient's hemiparesis resolved in the immediate postoperative period, and she was discharged to home without complication. **Conclusions:** Chondrosarcomatous brain tumors are rare with few primary cases reported in the literature. We report a case of a metastatic intracranial metastasis from an extracranial source, which has not been previously reported. Our case demonstration should help to clarify adjuvant treatment options.

Inhibiting FasL in gliosarcoma cells decreases tumor volume and enhances the anti-tumor immune response

Timothy Jansen, BA; Sahael Stapleton, BS;
John Laterra, MD, PhD; Alessandro Olivi, MD
(Baltimore, MD)

Introduction: Gliomas are extremely aggressive brain tumors that account for the majority of deaths due to primary brain neoplasms. Such a dismal clinical outcome is attributed to the fact that these tumors proliferate in the brain without significant challenge from the immune system. **Methods:** The immune privilege demonstrated by these tumors is, in part, a result of the expression of immuno-suppressive agents such as the Fas ligand (FasL). Immunotherapy using interleukins, particularly IL-2 and IL-12, is a promising strategy for the treatment of experimental gliomas, but is impeded by T-cell apoptosis. We propose that gliomas impair the T-cell mediated anti-tumor response by expressing FasL, which induces apoptosis of

peritumoral T-cells and decreases tumor cell killing by cytokine-activated T-cells. *Results:* In our study we saw that a panel of glioma cell lines express FasL and that orthotopic tumors are characterized by infiltrating T-cells undergoing apoptosis. When FasL expression is inhibited in 9L rat gliosarcoma cells by siRNA, the loss of protein expression resulted in a 75% decrease in 9L induced T-cell apoptosis in an in vitro co-culture assay. Furthermore, when FasL knock-down cells were implanted into immunocompetent Fisher 344 rats a 40% reduction in tumor volume was observed. *Conclusions:* These data show that 9L derived FasL induces T-cell apoptosis. Our findings also suggest that the Fas counterattack exists in the unique environment of the brain and that inhibiting FasL is a means to increase the anti-tumor immune challenge. We believe that strategies to inhibit brain tumor FasL will be instrumental in enhancing brain tumor immunotherapy and vaccines.

PV-1 and TEM1 in astrocytic brain tumors

Sally Jarvela, MD (Finland); Eleanor Carson-Walter, PhD (Pittsburgh, PA); Timo Jarvela, MD; Hannu Haapasalo, MD (Finland); Kevin Walter, MD (Pittsburgh, PA)

Introduction: Plasmalemmal vesicle associated protein-1, PV-1, and tumor endothelial marker 1, TEM1, are novel microvascular endothelial cell markers of brain tumor angiogenesis. We measured expression of PV-1 and TEM1 in 275 grade II-IV astrocytic brain tumors. *Methods:* Non-radioactive in situ hybridization (ISH) was used to probe 275 microarrayed astrocytic brain tumors. These arrays included 39 grade II, 41 grade III and 195 grade IV astrocytomas. Tumors were assessed for weak, moderate, or strong staining. *Results:* PV-1 was expressed in 84% of the tumors: 42% stained weakly, 32% moderately and 8% strongly. PV-1 staining was inversely associated with tumor grade ($P = 0.027$, Pearson chi-square test). PV-1 expression was also inversely correlated with p53 expression ($P = 0.049$, chi-square test). PV-1 expression seemed to exclude EGFR amplification, since none of the tumors with strong PV-1 staining had EGFR amplification, while 32% of remaining tumors showed EGFR amplification. This correlation did not quite reach statistical significance ($P = 0.058$, chi-square test). A majority (79%) of the tumors expressed TEM1: 43% weakly, 32% moderately and 4% strongly. TEM1 staining also demonstrated an inverse correlation with tumor grade ($P = 0.036$, Pearson chi-square test). TEM1 expression correlated with nitrotyrosine expression ($P = 0.003$, chi-square test). Neither PV-1 nor

TEM1 had any correlation with proliferation index (MIB-1) or apoptotic rate. PV-1 and TEM1 had strong correlation with each other ($P < 0.001$, chi-square test). *Conclusions:* Despite variations in tissue and mRNA integrity, the vast majority of malignant astrocytomas surveyed expressed PV-1 and TEM1. Since these genes are not normally expressed in the adult cerebrovasculature, they represent attractive markers of brain tumor angiogenesis.

Luciferase reporter transfected cells as a mouse model of intracranial meningiomas

Randy L. Jensen, MD, PhD; Brian Ragel, MD; David Gillespie, PhD; Isacc Elam, BS; David Mabey, BS; Jeannette Flynn, MD; William T. Couldwell, MD, PhD (Salt Lake City, UT)

Introduction: Subcutaneous flank meningioma animal models are commonly used because of the ease of tumor growth measurement. An intracranial model would clearly be preferable but tumor measurement is difficult even if mouse MRI capabilities are used. We have developed a number of meningioma cell lines that constitutively express luciferase, making intracranial tumor growth measurement a relatively easily process. *Methods:* Primary and malignant (IOMM-Lee and HB-157) meningioma cell lines were stably transfected with a luciferase reporter plasmid and in vitro luciferase expression verified. These tumor cell lines were injected into the frontal convexity and skull base. Luciferase activity was measured biweekly. Selected animals were also imaged with MR imaging to correlate with the luciferase imaging. After 6 weeks the mouse brains containing the tumors were harvested and analyzed using electron and light microscopy, as well as, immunohistochemistry. *Results:* IOMM and HB-157 cells lines demonstrated vigorous cell growth compared to the primary cell lines. Luciferase activity was correlated with tumor growth over time and MR imaging studies. Intracranial tumors were histologically consistent with meningiomas by light microscopy. Electron microscopy demonstrated desmosomes and tumors were immunohistochemically positive for vimentin and EMA. MIB-1 indices for a given cell line were similar to those calculated when the cell line was grown subcutaneously. *Conclusions:* Meningioma cells can be stably transfected with a constitutively active luciferase reporter. This provides a reliable model for measuring of intracranial meningioma growth. This provides for a relatively easy model of intracranial meningioma that potentially could be used for assessment of various treatment modalities.

Analysis of the in vitro effects of radiation and dexamethasone upon human anaplastic astrocytomas and oligodendrogliomas

Ricky R. Kalra, BA; Michael Zahner, BS;
Ryuji Higashikubo, PhD; Arie Perry, MD;
Michael R. Chicoine, MD (St. Louis, MO)

Introduction: Anaplastic oligodendrogliomas (AO) often have greater responsiveness to radiation than anaplastic astrocytomas (AA) possibly due to loss of chromosomes 1p and 19q. This process is incompletely understood, and how it is effected by dexamethasone has not been investigated. This study assessed effects of radiation and dexamethasone upon AO and AA in vitro. *Methods:* Gliomas (4 AA and 1 AO) were harvested from operative specimens, grown in culture, and assessed with standard histopathology and fluorescence in-situ hybridization (FISH) analysis for 1p and 19q. Cells in culture from all five tumors were divided into four treatment groups (control, radiation, radiation with 4.0 µg/ml dexamethasone, dexamethasone alone). A single radiation dose of 7 Gray was administered on day 1 and cell counts were performed every other day for 10 days with an automated variable cell analyzer with trypan blue staining. *Results:* FISH analysis revealed 1p and 19q deletion in the AO cells in culture. Radiation exposure decreased growth rates for all five cell lines. Growth rates were equivalent across all four AA cell lines, whereas the AO cell line had a slower growth rate and was more susceptible to radiation. For all five cell lines dexamethasone increased growth rates and decreased sensitivity to radiation. *Conclusions:* In this study, the AO with 1p 19q deletions was more sensitive to radiation than the AAs. In vitro dexamethasone reduced effectiveness of radiation and increased growth rates of AA and AO cells. This study provides further methodologies by which to study therapies for malignant gliomas.

Blood–brain barrier disruption and intrarterial chemotherapy for treatment of various brain tumors

Chris S. Karas, MD; Mirza Baig, MD, PhD;
Efthymios Stamoulis, MD; Susan Bell, RN, CNP;
John M. McGregor, MD; Eric Bourekas, MD
(Columbus, OH)

Introduction: Previous trials have shown the efficacy of chemotherapeutic regimens and radiotherapy for the treatment of brain tumors. Chemosensitive tumors such as primary central nervous system lymphoma have shown promising results with blood–brain barrier disruption (BBBD). On the other hand the role of BBBD in gliomas

remains unclear. Our goal was to investigate the effectiveness of the BBBD method and to record the outcomes and complications in patients with various brain tumors for the last 7 years in our institution. *Methods:* Between 1995 and 2002 44 patients were enrolled in the intraarterial chemotherapy with osmotic BBBD trial at the Ohio State University Medical Center. Patients with prior surgery or biopsy and histologically confirmed glioblastoma multiform (GBM), lymphoma, oligodendroglioma, anaplastic astrocytoma or germinoma were eligible. *Results:* Radiologic degree of disruption was noted for each BBBD. Patient outcomes were graded according to survival and Karnofsky Scores (KS) at enrollment and completion of protocol treatment. Tumor response (TR) and progression of disease (PD) were also measured radiographically and clinically. In patients with gliomas, KS and TR were decreased and PD increased post treatment. Patients with germinoma and lymphoma demonstrated improved KS and TR with decreased PD. *Conclusions:* Intraarterial chemotherapy with BBBD is an effective method with minimal complications, with improved outcome and prolonged survival for the treatment of patients with germinoma and primary central nervous system lymphoma. On the other hand, patients with gliomas did not experience noticeable benefits after chemotherapy with BBBD.

Non-clinical safety and biodistribution adenoviral vector containing the herpes simplex virus thymidine kinase gene (Cerepro™)

Gillian Langford, PhD (London, UK);
Seppo Yla-Herttula, PhD, MD (Finland); David
Eckland, MBBS, PhD (London, UK)

Introduction: Cerepro™ is being developed to treat high-grade glioma. Cerepro™ transfects the HSV-tk gene into cells via an adenoviral vector. Following intra-cerebral injection the thymidine kinase phosphorylates ganciclovir (GCV) creating a cytotoxic analogue. *Methods:* Non-clinical safety and biodistribution of Cerepro™ was assessed following a single intravenous (IV) or intracerebral (IC) injection in Crl:WI(GLX/BRL/Han) rats ($n = 198$) at doses of 1.2×10^9 to 1.2×10^{11} viral particles. Selected rats in the IC group also received GCV by intraperitoneal (IP) injection. The animals were followed for 90 days. Biodistribution of Cerepro™ was assessed by QPCR and expression of the transgene was assessed by RT-PCR. *Results:* Following IV or IC injection there was no antibody response to Cerepro™ and no effect on behaviour, body weight, food consumption or haematological parameters. Minor microscopic needle track changes were observed in control and Cerepro™ IC

injection groups. Transient myeloid hyperplasia was observed in only three animals in the IV injection group whilst the group mean spleen weight increased. CereproTM was detected by QPCR at high levels in the brain and at low levels in blood and spleen early following IC injection and decreased with time. Following IV injection high levels were detected in viscera and blood; these decreased with time. High levels of transcription were detected by RT-PCR in brain following IC injection with low levels in spleen; following IV injection, high level transcription was seen in viscera. **Conclusions:** Administration of CereproTM and GCV is well tolerated and biodistribution and transgene expression is as predicted from other non-clinical studies.

Increased immunohistochemical staining for Cox-2 correlates with increased microvascular density in null-cell pituitary adenomas

Janet Lee, MD; Brian T. Ragel, MD;
David G. Rubin, BS; William T. Couldwell, MD, PhD
(Salt Lake City, UT)

Introduction: Cyclooxygenase-2, an inducible inflammatory enzyme, has been shown to be upregulated in numerous cancers (e.g., colon, lung, renal) via pro-proliferative, angiogenic, and anti-apoptotic pathways. We hypothesize that Cox-2 may have a role in the pathogenesis of null-cell pituitary adenomas by increasing proliferative rates and upregulating angiogenesis. **Methods:** Eight null-cell pituitary adenomas were immunohistochemically stained for Cox-2, MIB-1 (proliferative marker), and Factor VIII (microvascular density marker). Cox-2 was analyzed by scoring three random fields (20×) per slide for staining intensity (0–4) and percent of cells stained (0–100%). The Cox-2 weighted index was calculated by multiplying the staining intensity score by the percent of cells stained. MIB-1 was calculated by counting the number of positive cells per 40× high-powered field (hpf). Microvascular density was calculated by counting the number of blood vessels staining for Factor VIII per 20× field. **Results:** Cox-2 immunohistochemical staining fell into low- and high-staining groups, with weighted indices of <2 ($n = 3$) and >2 ($n = 5$), respectively. The number of MIB-1 cells positive per hpf for the low- and high-Cox2 staining groups were 5.6 (+/– 5.1) and 6.8 (+/–4.7), respectively (t -test, $P = 0.55$). The number of blood vessels per 20× field for the low- and high-Cox2 staining groups was 4.3 (+/–2.6) and 9.5 (+/– 5.1), respectively (t -test, $P = 0.01$). **Conclusions:** Increased Cox-2 staining in null-cell pituitary tumors correlates with increased microvascular density. No significant difference in proliferative rates was noted between low- and high-Cox-2 staining tumors. We plan on

correlating pituitary tumor size as well as vascular endothelial growth factor with these results.

Surgical treatment of huge olfactory groove meningioma—interhemispheric approach using bifrontal craniotomy

Sun-il Lee, MD (Republic of Korea)

Introduction: The surgical approach to olfactory groove meningiomas can vary depending in the size and expansion of the tumor. The authors retrospectively analyzed the clinical presentation and the surgical outcome of the patients with huge olfactory groove meningioma and described the advantage of interhemispheric approach using bifrontal craniotomy. **Methods:** Since 1997, a series of 23 consecutive patients with huge size olfactory groove meningioma underwent microsurgical resection using interhemispheric approach via bifrontal craniotomy. The tumor diameter measured 5.5 cm in average. The clinical presentation included headache in 13, visual impairment in 6, mental dysfunction in 4, seizure in 4. Mean duration of the clinical history was 13 months. **Results:** The patient's age ranged from 29 to 52 years old. Seven were in female and six in male. The grade of resection was Simpson II in 18 cases, III in five cases. The majority of the cases presented a typical histology ; 12 meningiothelial, six transitional, five angiomatous. All of patients presented a good recovery at discharge. Three of the cases with small remnant tumor and one with a tumor recurrence after follow-up of 19 months were treated by Gamma knife radiosurgery. **Conclusions:** Our experience with bifrontal interhemispheric approach suggests a great surgical outcome in the treatment of huge size olfactory groove meningioma and there was no postoperative infection and no cosmetic problems. This approach enabled us to minimize frontal lobe retraction, to interrupt the blood supply to the tumor early, and to remove the tumor completely from the frontal base.

Stat-3 inhibitor by liposomal siRNA for the treatment of rodent gliomas

Khan W. Li, MD; I-Mei Siu, PhD; Betty M. Tyler, BA;
James Han; Violette M. Renard, MD; Gustavo Pradilla,
MD; Drew Pardoll, MD, PhD; Henry Brem, MD, FACS
(Baltimore, MD)

Introduction: The role of (Signal transducer and activator of transcription-3) STAT3 in apoptosis, cellular differentiation and modulation of interactions between tumor

cells and the immune system has become increasingly recognized. We have previously shown that STAT3 is upregulated in rodent and human glioma cell lines and that inhibition of STAT3 by small molecule inhibitors prevents glioma proliferation in vitro. We now demonstrate inhibition of STAT3 in rodent glioma cell lines using liposomal siRNAs. **Methods:** In vitro transfections of both C6 and 9L rat glioma cell lines with a pool of STAT3 siRNAs or a negative control pool of scrambled siRNAs were performed using Lipofectamine-2000 or a proprietary lipid. **Results:** Transfection of C6 and 9L with the anti-STAT3-siRNA pool using Lipofectamine-2000, resulted in ~70% knockdown of STAT3 expression. Transfection efficiency of C6 and 9L with a fluorescent labeling gene using the proprietary lipids was greater than 90%. We then tested the efficacy of these lipids in delivering the pool of anti-STAT3 siRNAs. Transfection of C6 and 9L cells using the liposomal siRNA produced a significant reduction (~50%) in STAT3 expression for both C6 and 9L cell lines. **Conclusions:** Liposomal siRNA is a novel in vivo treatment strategy for selective inhibition of cellular gene products. STAT3 is an attractive target for malignant gliomas because of its roles in prevention of apoptosis and modulation of tumor mediated immune response. In these experiments we demonstrate selective STAT3 inhibition using liposomal siRNA. Future experiments will be conducted to determine the effect of liposomal anti-STAT3 siRNA delivered in vivo.

Enhanced immunity to intracerebral breast cancer in mice immunized with fibroblasts transfected with DNA from the breast cancer cells

Terry Lichtor, MD, PhD; Roberta P. Glick, MD; Lisa Feldman, BA; Goro Osawa, BA; Julian Hardman, BA; InSug O-Sullivan, PhD; Edward P. Cohen, MD (Chicago, IL)

Introduction: We have previously shown that a DNA-based vaccine prepared by transfer of genomic DNA from a breast cancer that arose spontaneously in a C3H/He mouse into a mouse fibroblast cell line that secretes IL-2 stimulates a systemic cellular anti-tumor response and prolongs survival in mice with an intracerebral breast cancer. The goal of this study was to determine if enrichment of the vaccine can lead to a stronger anti-tumor immune response. **Methods:** Aliquots of the transfected fibroblasts were divided into pools which were expanded and selected for increased numbers of immunogenic cells. **Results:** In mice with an established

intracerebral breast cancer, the strongest systemic anti-tumor immune response detected in either the spleen cells or cervical lymph nodes by an ELISPOT assay was found in those animals treated with enriched vaccine. This response was mediated predominantly by CD4+ cells although CD8+ and NK/LAK cells also played a role. Furthermore a PCR and FACS analysis of the lymphocytes in the spleen, cervical lymph nodes and brain revealed a decreased expression of Foxp3+ regulatory T cells which are known to be involved in immune suppression. Finally the greatest prolongation of survival was seen in those animals treated with the enriched vaccine. **Conclusions:** Enhanced immunity to the neoplasm was detected in mice with an established intracerebral breast cancer treated solely by immunization with the enriched cell population. The immunity mediated predominantly by CD4+ T cells resulted in a decrease in expression of Foxp3+ regulatory T cells and was sufficient to prolong survival of the mice.

Synergistic potential for treatment of mice with an established intracerebral glioma by combining PPAR- γ thiazolidinedione agonists and IL-2 secreting fibroblasts

Terry Lichtor, MD, PhD; Roberta P. Glick, MD; Alessandra Spagnolo, PhD; Edward P. Cohen, MD; Douglas L. Feinstein, PhD (Chicago, IL)

Introduction: In this study we explored the benefits of treating C57Bl/6 mice with an established intracerebral glioma by combining immunotherapy with IL-2 secreting fibroblasts along with the chemotherapeutic agent pioglitazone, which is an agonist of the peroxisome proliferator activated receptor. **Methods:** The sensitivity of G1261 glioma cells and astrocytes to pioglitazone was determined in vitro. Viability was assessed by measuring lactate dehydrogenase release, and effects on metabolism were determined by measuring superoxide production and levels of superoxide dismutase. **Results:** Pioglitazone was found to induce cell death in G1261 glioma cells grown in vitro while causing only modest damage to astrocytes. Pioglitazone also resulted in a significantly greater induction of cellular superoxide in glioma cells than in astrocytes. Pioglitazone administered intracerebrally ($P < 0.05$) but not orally was found to prolong survival in mice with an intracerebral glioma. Spleen cells from mice injected with IL-2 secreting cells showed a stronger response to glioma cells than controls as measured by an ELISPOT interferon- γ assay. Synergistic effects of combination therapy on prolonging survival were found in mice receiving both pioglitazone and IL-2 secreting

fibroblasts ($P < 0.005$ vs. untreated animals) injected directly into the tumor bed through a unique cannula system. **Conclusions:** Pioglitazone induces metabolic and oxidative stresses that are tolerated by astrocytes but not glioma cells, which could account for increased sensitivity to IL-2 suggesting potential for the use of this FDA-approved drug in the treatment of brain tumors. The data indicates the beneficial effects of combination therapy using pioglitazone and immunotherapy in treatment of gliomas.

Delayed pneumatization of the sphenoid sinus mimicking a skull base mass

Zachary N. Litvack, MD; James K. Liu, MD; Bronwyn E. Hamilton, MD; Julie Falardeau, MD; Daniel J. Guillaume, MD; Nathan R. Selden, MD, PhD; Johnny B. Delashaw, MD (Portland, OR)

Introduction: The MR signal characteristics of the developing sphenoid sinus change over the course of pneumatization, and may be confused with pathologic processes. An intermediate stage of fatty conversion of marrow (the “pre-sphenoid” stage) usually occurs between 7 months and 2 years of age. Complete pneumatization is usually complete by age 10. The authors present a case of delayed sphenoid sinus pneumatization mimicking a skull base mass. **Methods:** The patient is a 15 year old female who presented with progressive headaches and partial right third and sixth nerve palsies. MRI demonstrated a mass in the sphenoid sinus abutting the right cavernous sinus. Twenty-four hours after presentation, the patient suffered a rapid decline in visual acuity. A neuro-ophthalmological exam demonstrated new Grade 2–3 bilateral papilledema with flare hemorrhages. Subsequently, she underwent urgent treatment with corticosteroids, lumbar drainage and a craniotomy for right optic nerve decompression. The sphenoid mass was debulked, and was found to be normal adipose tissue on final pathology. **Results:** Postoperatively, the patient’s visual deficit, cranial nerve deficits, and papilledema resolved. With the knowledge of a negative biopsy, the initial MR findings were interpreted as fatty conversion of bone marrow in the pre-sphenoid stage of pneumatization and a diagnosis of pseudotumor cerebri was assigned. **Conclusions:** Fatty conversion of bone marrow before pneumatization is a normal developmental process that may be misinterpreted as a pathologic condition. Knowledge of developmental pneumatization is important for neurosurgeons. The authors review the stages and neuroimaging of sphenoid sinus development.

Ependymoma of the spinal cord treated with adjuvant or salvage radiotherapy: rethinking dose prescription for residual disease

Stella C. Lymberis, MD; Ioannis Karampelas, MD; Kelvin Chan, BS; Josh Yamada, MD; Ira J. Dunkel, MD; Cameron W. Brennan, MD; Mark K. Rosenblum, MD; Mark M. Souweidane, MD; Philip H. Gutin, MD; Mark Bilsky, MD; Suzanne Wolden, MD (New York, NY)

Introduction: Historically, radiation doses used in the treatment of spinal cord ependymoma are maintained at 45–50.4 Gy in order to minimize risk of radiation myelopathy. We present outcomes of spinal ependymoma treated with conventional radiotherapy treatment outlining patterns of late local failure (LF) and distant neuraxis failure (DF). **Methods:** From 1975 to 2006, 12 patients with spinal ependymoma were identified. Median age of diagnosis was 26.5 years (range 8–48). Six had myxopapillary, five WHO II ependymoma, and one malignant ependymoma. Of the 12 patients: one patient had initial surgery at MSKCC, seven sub-total resection, one biopsy only and three unverifiable. Seven of 12 (58.3%) patients received local adjuvant radiotherapy (45–50.4 Gy) at median 3 months post-operatively; four patients received salvage radiotherapy. **Results:** At median follow-up of 90 months (4–172 months), Overall Survival was 100% at 10-years while Progression Free Survival was 42%, and 25%, at 5- and 10-years, respectively. Overall, disease progression was seen in 83% of patients at median 43 months. Local failure despite adjuvant radiotherapy occurred in 4/7 (57%) patients, median 30 months: LF only (3), DF only (2), LF&DF (1), controlled (1). Disease progression occurred in 4/5 (80%) patients who did not receive adjuvant radiotherapy: LF only (1), DF only (1), LF&DF (2), controlled (1). Paraparesis due to intratumoral hemorrhage occurred in two patients with myxopapillary ependymoma who did not receive adjuvant radiotherapy. **Conclusions:** Conventional radiotherapy in the 45–50.4 Gy dose range is not adequate to achieve local control after subtotal resection. Use of IMRT allows for dose escalation of gross disease within the spinal field to 50.4–55.8 Gy which may improve rates of local control.

Hemorrhagic adult pilocytic astrocytomas: report and literature review of a rare clinical entity

Mark K. Lyons, MD, FACS (Scottsdale, AZ)

Introduction: Pilocytic astrocytomas are found predominantly in children. Reports of these tumors are extremely

rare in the elderly. We report a case and review the literature of adult pilocytic astrocytomas presenting with intracranial hemorrhage. *Methods:* Their histopathologic features do not correlate with an aggressive clinical course or poor prognosis. Intracranial hemorrhage in pilocytic astrocytoma have been reported primarily in the young. Lones and Verity reported a 69-year-old woman with a fatal hemorrhage of a pilocytic astrocytoma in the thalamus. Supratentorial pilocytic astrocytomas appear most commonly as solitary lesions in the temporal lobes. Imaging characteristics of pilocytic astrocytomas are generally well demarcated with variable degrees of enhancement. Some of these tumors infiltrate the surrounding parenchyma. The presence of nuclear pleomorphism, mitotic activity, endothelial proliferation occur in pilocytic astrocytomas, but do not carry the same worrisome prognostic significance. The MIB-1 and p53 labeling indexes are generally negative. Lieu reported review of brain tumors with intracranial hemorrhage finding the highest rate of hemorrhage for the primary brain tumor group occurred in pilocytic astrocytomas. *Results:* Pilocytic astrocytoma in an elderly adult is extremely rare. Temporal lobe pilocytic astrocytomas may arise from nodular heterotopia and may act as a precursor of a pilocytic astrocytomas. *Conclusions:* Pilocytic astrocytomas are almost always benign in their clinical course. Although most commonly found in children, pilocytic astrocytomas can occur in adults. The potential microscopic infiltration of pilocytic astrocytomas into the adjacent parenchyma may influence the likelihood of spontaneous hemorrhage.

Prostate carcinoma presenting as an extraaxial mass lesion mimicking meningioma

Mark K. Lyons, MD, FACS; Joseph F. Draskowski, MD; Kay Miller, MD; Naresh P. Patel, MD; Richard S. Zimmerman, MD; Barry D. Birch, MD (Scottsdale, AZ)

Introduction: Prostate adenocarcinoma rarely invades brain parenchyma, but has significant potential for metastatic involvement for bone and other organ systems. Rarely, elderly males with known prostate carcinoma have been reported to have intracranial extraparenchymal lesions consistent with metastatic disease. We report an unusual case of a young patient with progressive neurological symptoms due to a very large intracranial dura-based prostate metastasis. *Methods:* Case report and review of the literature. *Results:* Fifty-seven-year-old man presented to our emergency department with a several week history of headache, right hemiparesis and new onset partial epilepsy. A radical retropubic prostatectomy for adenocarcinoma was

performed three years before at an outside hospital prior to presentation to our institution. Imaging demonstrated a large enhancing extra-axial left frontal parietal mass measuring 9×3 cm. A left frontal parietal craniotomy and resection of a large dural based extra-axial mass was performed. There was no intraparenchymal involvement of the tumor. The pathology confirmed the diagnosis of metastatic prostate adenocarcinoma. No additional sites of metastatic involvement were identified. The patient underwent external beam radiotherapy to the brain of 3740 cGy and received casodex and lupron therapy. Followup evaluation has demonstrated no evidence of recurrence or disease progression. The literature is reviewed. *Conclusions:* Depending upon the extent of the systemic disease, the prognosis for metastatic prostate adenocarcinoma to the brain can be significantly better than other types of metastatic cancer. This report illustrates the need for the continued awareness that malignant lesions can masquerade as benign lesions, thus emphasizing the need for early and definitive intervention.

Primary and metastatic brain tumors presenting with seizures: evaluation, implications and management

Mark K. Lyons, MD; Laura M. Lynam, MD; Joseph F. Driver-Dunckley, MD; Katherine H. Noe, MD, PhD; Joseph I. Sirven, MD; Richard S. Zimmerman, MD; Naresh P. Patel, MD; Barry D. Birch, MD (Phoenix, AZ)

Introduction: Approximately 10–15% of adult-onset and 0.2–6% of childhood-onset cases of epilepsy are caused by CNS neoplasms. Calculating true frequency of seizures at presentation in the adult population could help guide the evaluation for patients with new-onset seizures. *Methods:* Retrospective analysis done of 147 consecutive brain tumor patients treated from January 2005 through December 2005 at our institution. Characteristics considered included: age, gender, tumor type, tumor location, presence of seizures as presenting symptom or later in the course of disease, antiepileptic drugs used, use of monotherapy versus polytherapy, and EEG findings. *Results:* One hundred forty-seven patients were identified: One hundred and twelve patients with primary CNS tumors and 35 with CNS metastases. Seizure was the presenting symptom leading to diagnosis of the underlying brain tumor in 38% of the primary CNS group and 20% of the metastatic group ($P < 0.05$). Forty-three percent who presented with seizures in the metastatic group had no previous cancer diagnosis. The highest rates of seizure at initial presentation in the primary CNS group were with oligodendroglioma (6/6, 100%), low grade astrocytoma (9/15,

60%), anaplastic astrocytoma (5/10, 50%), meningioma (15/41, 36.6%), and glioblastoma multiforme (6/24, 25%). In the metastatic group, seizures were the presenting symptom in seven patients: non-small cell lung carcinoma (5/15, 33.3%); prostate adenocarcinoma (1/1, 100%); and renal cell carcinoma (1/2, 50%). *Conclusions:* The majority of patients were controlled with a single anticonvulsant which is an encouraging finding with implications for a patient's quality of life. EEG abnormalities in this population may aid in determining which patients are at risk for recurrent seizures.

Idiopathic intracranial hypertension as a manifestation of occult spinal cord astrocytoma: evaluation and management

Mark K. Lyons, MD, FACS (Phoenix, AZ);
Alyx B. Porter-Umphrey, MD (Rochester, MN);
E. Peter Bosch, MD; Naresh P. Patel, MD;
Barry D. Birch, MD; Richard S. Zimmerman, MD
(Phoenix, AZ)

Introduction: Intraspinal tumors rarely cause raised intracranial pressure. Elevated cerebrospinal fluid (CSF) protein is frequently found. The case is unique because of the initial presentation of papilledema, absence of CSF protein elevation, late appearance of myelopathy and development of metastatic high grade astrocytoma to the brain. *Methods:* A 19-year-old male presented with transient visual obscurations. Examination revealed bilateral papilledema. MR of brain was normal. CSF opening pressure of 43 cm H₂O, WBC 1, protein 48 mg/dl and glucose 41 mg/dl. All viral tests, connective tissue markers and heavy metal screens were negative. He was diagnosed with pseudotumor cerebri and underwent LP shunting. Subsequently developed lower extremity weakness. Diagnosed with Devic's syndrome and referred to our institution. Exam: optic disc pallor and afferent pupillary defect, asymmetric spastic paraparesis, hyperreflexia, bilateral Babinski signs, LE vibratory loss and a T5 sensory level. *Results:* MR demonstrated a non-enhancing swollen spinal cord from C7-T4. He underwent spinal cord biopsy and ligation of the LP shunt. Pathology confirmed grade fibrillary astrocytoma. He received 48.6 Gy in 27 fractionated sessions and temozolomide. Persistent headaches responded to VP shunting. Several months later, he developed new onset symptoms and follow-up MR of the brain demonstrated an intraaxial lesion that was resected and pathology confirmed a high grade astrocytoma and subsequent spinal metastases. *Conclusions:* he presentation of a cervico-thoracic malignant spinal astrocytoma as intracranial hypertension and visual loss without elevated CSF protein or myelopathy is rare. The

subsequent development of a metastatic cranial high grade astrocytoma illustrates the poor prognosis of these tumors.

No55 is a potential tumor specific antigen in glioblastoma

Christopher E. Mandigo, MD; Weicker Michael, MD;
Omar N. Syed, MD; Jeffrey N. Bruce, MD
(New York, NY)

Introduction: Glioblastoma remains a lethal disease with a average survival of 12 months despite aggressive therapy. Active Immunotherapy, using vaccination strategies, has proven effective in certain types of systemic cancer. Identification of tumor specific antigens is crucial to the success of active immunotherapy. Immunotherapy of glial tumors is a potentially effective adjunct to standard treatments. The goal of this study was to screen glioblastoma patients for signs of an immune response and to identify the responsible antigens using SEREX (serological identification of tumor antigens by expression cloning.). *Methods:* mRNA was isolated from 3 glioblastoma specimens. The mRNA was used to make a cDNA library representing the entire expression profile of the tumor tissue. This cDNA library was screened with serum from the same patient to identify antibody reactions against all possible tumor-derived proteins. Positive responses were cloned and sequenced to determine the protein identity. *Results:* Three cDNA libraries were screened and one positive result was obtained. Sequencing revealed a 900 kb segment of the 1.3 kb No55 protein. Expression analysis of cDNA derived from 24 patients revealed expression in 22 of 24 patients. Nine normal brain specimens exhibited undetectable amounts of cDNA. No55 is a protein that is involved in meiosis and has a limited expression in non-CNS tissues. *Conclusions:* SEREX analysis has identified No55 as a potential glioblastoma specific antigen. Future testing of patient serum for the presence of No55 antibodies will help evaluate the potential role of No55 in immunotherapy.

Stereotactic radiosurgery as single modality treatment for incidentally-identified non-small cell lung cancer brain metastases

Nicholas F. Marko, MD; John Suh, MD;
Gene Barnett, MD, FACS; Steven Toms, MD, MPH;
Michael A. Vogelbaum, MD, PhD; Sam Chao, MD;
Lilyana Angelov, MD, FRCS(C) (Cleveland, OH)

Introduction: Initial staging workup of non-small cell lung cancer (NSCLC) patients has increasingly led to the

diagnosis of brain metastases in patients who are otherwise neurologically asymptomatic. We present our experience treating these NSCLC metastases with stereotactic radiosurgery (SRS) alone and compare outcomes to those of patients with brain metastases treated at our institution with other strategies. **Methods:** We conducted a retrospective outcomes analysis in patients with incidentally-identified NSCLC brain metastases treated with upfront SRS only. Our radiation oncology and brain tumor databases were reviewed, identifying 832 such patients treated between 1983 and 2006. **Results:** We found 26 patients with incidentally-identified brain metastases (KPS 90–100) treated with SRS alone within 60 days of diagnosis. These patients underwent SRS at a mean of 15.6 days from diagnosis to an average of 1.6 lesions (range:1–7) with a mean lesion volume of 1.83 cm³. The mean prescription was 21.5 Gy delivered to the mean 56.7% isodose line. The mean survival for these patients was 12.3 months. CNS progression (local and/or distant) occurred in six patients (23%, mean 7.6 months). Survival was not statistically different from similar patients treated with whole brain radiotherapy (WBXRT) (*P*-value 0.9776), WBXRT + surgery (*P*-value 0.0666) or WBXRT + SRS (*P*-value 0.6236). **Conclusions:** We demonstrate that patients with incidentally-identified NSCLC brain metastases treated with SRS alone achieved a survival rate comparable to patients managed with other standard therapeutic modalities. Given the more favorable side effect profile and reduced morbidity of SRS, our findings support SRS alone as an excellent therapeutic option for these patients.

17-DMAG inhibits glioma tumor cells and improves survival in a rat brainstem glioma model

Neena Marupudi, BA; Violette M. Renard, MD;
Betty M. Tyler, BA; Khan W. Li, MD; Henry Brem,
MD; George Jallo, MD (Baltimore, MD)

Introduction: Brainstem tumors (BSTs) comprise approximately 15% of CNS tumors in children, and are often refractory to conventional treatments. The discovery of specific neoplastic agents for CNS malignancies could benefit the treatment of this disease. We investigated direct intratumoral delivery 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG), a geldanamycin analog and hsp90 inhibitor, in a rodent brainstem glioma model. **Methods:** Tumor sensitivity was measured by two in vitro assays-clonogenic and MTT- against 9L and F98 glioma lines. In vivo toxicity and efficacy were assessed using our rat BST model. Rats received glioma by cannulated guide screw. Five days later, animals received either no treatment or one of five concentrations of 17-DMAG (0–5.0 µg/ml). Injections were performed every other day

for 6 days and animals observed for neurotoxicity and survival. **Results:** MTT assays showed 50% inhibition of cell proliferation at 0.005 µg/ml and complete cell death at 0.5 µg/ml. Clonogenic assays demonstrated inhibition of tumor cells at doses of 0.1 µg/ml. Dose escalation toxicity established intracranial treatment of 17-DMAG at doses as high as 5.0 µg/ml. In the efficacy study, median survival for the control group was 15 days. While no significant efficacy was observed in the low dose groups of 0.01–1.0 µg/ml 17-DMAG (median survival of 17 days for all groups), significantly improved survival was observed with 5.0 µg/ml 17-DMAG (median survival 21 days, *P* = 0.0009). **Conclusions:** 17-DMAG is cytotoxic to rat glioma cell lines in vitro, and local delivery of 17-DMAG appears to prolong survival of tumor-lesioned rats. 17-DMAG is a potential chemotherapeutic agent for the treatment of BSTs.

Maltose-tri-O-methyl nordihydroguaric acid (maltose-M3N) is a potential chemotherapeutic agent in the treatment of gliomas

Neena Marupudi, BA; Violette M. Renard, MD;
Betty M. Tyler, BA; Khan W. Li, MD; Ru Chih Huang,
PhD; Henry Brem, MD; George Jallo, MD (Baltimore, MD)

Introduction: Maltose-tri-O-methyl nordihydroguaric acid (maltose-M3N) is a non-toxic nordihydroguaric acid derivative from the plant *Larrea tridentata*. Maltose-M3N has demonstrated anti-tumor properties in several cell lines including murine and human melanomas and colon cancer. Based on these previous studies, we explored the possibility of using this transcription inhibitor in the treatment of brain tumors. **Methods:** 9L and F98 gliosarcoma cells were treated with various concentrations of maltose M3N or I-maltose-M3N (range 0–500 µM) for 48–72 h followed by MTT assays for cell viability. **Results:** At 48 h, cell viability was significantly decreased starting at 100 µM concentrations of drug, and complete loss of cell viability was achieved at concentration of 250 µM and higher. At 72 h, loss of cell viability showed a more dose responsive curve, with loss of cell viability starting at as low as 2.5 µM and increasing as drug concentration was increased. Less than 10% of the cells were viable at 100 µM concentration treated for 72 h and no cells were viable at concentrations greater than 250 µM. **Conclusions:** These results suggest that maltose-M3N is a stable drug under in vitro conditions that has cytotoxic activity against brain tumor cells. Longer exposure of the cells to the drug results in an increase in its anti-tumorigenic capabilities, most likely because maltose-M3N is a cell cycle dependent chemotherapeutic agent.

Imaging optical properties of brain tumors, grey matter, and white matter in near-infrared light

Marlon S. Mathews, MD (Orange, CA); David Abookasis, PhD (Irvine, CA); Henry Hirschberg, MD, PhD (Orange, CA); Bruce J. Tromberg, PhD (Irvine, CA); Mark E. Linskey, MD (Orange, CA)

Introduction: The authors report the results of using Modulated Imaging (MI) technology that utilizes spatially modulated near-infrared (NIR) light, to determine the optical spectroscopic properties of brain tumors, gray matter (GM) and white matter (WM). Compared to other optical methods, MI has the unique capability of quantitatively imaging tissue chromophore concentration. **Methods:** The MI-device consists of a Quantum-Tungsten-Halogen light source, spatially modulated into a sine-wave configuration, and projected from a commercially available projector into tissue. Reflected light is captured on a camera and processed. The brains of six BDIX rats (three rats with BT4C tumor in the right parietal lobe, and three control rats) were imaged *ex vivo* using MI. Spectroscopic information was obtained in the NIR-region (660 nm to 980 nm, in steps of 10 nm yielding 33 points to fit each spectral curve) and analyzed in regions within each brain—five each from the brain tumor, GM, and WM, yielding a total of 75 measurements. **Results:** Absorption and scattering of NIR light was found to be significantly different ($P = 0.002$; $P = 1.5 \times 10^{-24}$ respectively; ANOVA) in tumor, GM, and WM. In addition MI detected significant differences in the GM-absorption, WM-absorption, and WM-scattering ($P = 0.001$, 0.0002, 0.005 respectively; *t*-test) between brains with implanted tumors and control brains. **Conclusions:** In the near-infrared range the optical properties of brain tumor, GM, and WM are distinguishable using MI. MI may be sensitive to changes in the optical properties of GM and WM in brains harboring malignant tumors. MI technology may be utilized in intraoperative devices to enhance tumor margin resection.

Klippel Trenaunay Syndrome and cerebral hemangiopericytoma: a newly recognized association

Marlon S. Mathews, MD; Mark E. Linskey, MD; Ronald C. Kim, MD; Gregory Y. Chang, MD (Orange, CA)

Introduction: Klippel Trenaunay Syndrome (KTS) is a rare mesodermal phakomatosis consisting of capillary malformations, varicose veins, and limb hypertrophy.

Concomitant congenital anomalies include syndactyly, spina bifida, atresia of the ear canal, congenital dislocation of the hip, metatarsal and phalangeal agenesis. KTS has been associated with several vascular malformations and tumors. We present the first report of malignant cerebral hemangiopericytoma in a patient with KTS. **Methods:** A 33-year-old male presented with a 4 month history of worsening headache, blurred and decreasing vision, nausea, and vomiting. Physical examination showed a large port-wine stain on his left leg, left hip, and left trunk. The rash blanched with finger pressure and showed immediate refill when pressure was relieved. He had hypertrophy of his left lower extremity when compared to the right and partial syndactyly of the 2nd and 3rd digits in all four extremities. CT and MR scans showed a large 9×6 cm mass overlying his planum sphenoidal and the lesser wing of his sphenoid bone with infiltration or compressing the right optic nerve and encasement of the supraclinoid internal carotid and middle cerebral arteries. Following embolization of branches of the MCA supplying the tumor, it was surgically resected. **Results:** Histopathology confirmed Hemangiopericytoma. **Conclusions:** Although KTS is benign condition known to be associated with benign vascular malformations and tumors, the possibility of malignant vascular tumors like hemangiopericytomas should be considered. There has been only one previous report of KTS associated with a pulmonary hemangiopericytoma, and our case represents the first reported case of a possible association with intracranial hemangiopericytoma.

Screening for motility-associated genes in malignant astrocytoma cell lines

Kyung-Sub Moon, MD; Shin Jung, MD, Prof; Hyang-Hwa Ryu, MS; Tae-Young Jung, MD; In-Young Kim, MD; Jin-Shu Guang, MD; Jin-Yong Hao, MD; Young-Il Jeong, PhD; Sam-Suk Kang, MD, Prof (Republic of Korea)

Introduction: The identification of invasion mechanism in the malignant astrocytoma could indicate therapeutic new strategies to reduce further spreading and/or to treat the invading cells as more specific target. Local invasion *in vivo* is related to motility of cells *in vitro*. The purpose of our study was focused on detection of possible genetic changes as the determining factors for motile ability in the malignant astrocytoma. **Methods:** we selected malignant astrocytoma cell lines with different motilities by using simple scratch test. For gene profiling, we applied RNA

differential display using Genefishing™ DEG 101, 102 kit, and then validated them by RT-PCR and northern blot. **Results:** We confirmed four of 39 gene that showed different expressions in RT-PCR. Galectin-1, YWHAB, Calpain and metallothionein were highly expressed in motile cell lines and FLJ22905 was reverse result. The genes of Galectin and calpain have reported as possible genes related to tumor invasion in the malignant astrocytoma but the others haven't. **Conclusions:** Our study show that these genes may act as possible determining factors of high motility in astrocytoma cell lines and can give us more explanations about the molecular invasion mechanism in astrocytomas.

Anaplastic astrocytoma subtypes with different imaging and prognostic qualities

Hugh D. Moulding, MD, PhD; Song Lai, PhD;
David W. Andrews, MD (Philadelphia, PA)

Introduction: Objective Using MRI and specifically diffusion tensor imaging (DTI) we assessed to what degree anaplastic astrocytoma (AA) subtypes (enhancing or non-enhancing, expansive growth pattern or mixed/infiltrative patterns) infiltrate surrounding brain parenchyma. We show these data in the context of our recent findings that these different AA subtypes having very different prognoses. **Methods:** We retrospectively identified patients with pathologically confirmed WHO grade III astrocytoma who underwent craniotomy at our institution from 1994 through 2006. Patients were subgrouped by radiographic features such as enhancement (EAA) vs. non-enhancement (NEAA), as well as MRI growth patterns, such as expansive vs. mixed/infiltrative. DTI was used to evaluate how fiber tracks relate to the AA in patients from these different AA subgroups. **Results:** We retrospectively identified 61 patients who met eligibility criteria, 35 were EAA vs. 26 were NEAA, 28 were expansive vs. 31 were mixed or infiltrative. Both tumor groups manifested the same histologic characteristics with no significant difference in the Ki-67 labeling index. DTI analysis of patients within the expansive AA subgroup showed that fiber tracts are pushed out and away from the developing tumor, as opposed to those in the mixed/infiltrative group. Neighboring fiber tracts in patients with mixed/infiltrative tumors were shown on DTI to be engulfed by tumor. **Conclusions:** Expansive AA do not invade surrounding brain parenchyma and should be aggressively resected, ideally with DTI guidance. The lack of tumor infiltration documented by DTI could in part explain the better functional status and more favorable prognosis of this patient subgroup.

Proton therapy for high-grade gliomas

Yoko Nakasu, MD; Hiroshi Fuji, MD; Yuji Ishida, MD; Koichi Mitsuya, MD; Satoshi Horiguchi, MD; Hideyuki Harada, MD; Hirofumi Asakura, MD; Tetsuo Nishimura, MD; Shigeyuki Murayama, MD (Japan)

Introduction: Accelerated protons have been used in an attempt to limit treatment-related morbidity in the central nervous system by reducing the integral dose to adjacent normal tissues. **Methods:** Sixteen patients underwent proton therapy for high-grade gliomas since February 2005. Patients were excluded if target coverage exceeded 10 cm in diameter. The mean age was 50.6 years. Thirteen patients underwent surgery: eight patients with grade-4 astrocytomas, four grade-3 astrocytomas, and one grade-3 oligoastrocytoma. Other three children with brain-stem glioma did not undergo surgery. Supratentorial gliomas were treated with a dose of 70 GyE (Gray equivalent) protons, or 36 Gy photon plus 34 GyE protons. Brain-stem gliomas were treated with a dose of 55.8 GyE protons. Adjunctive chemotherapy was given to all patients. Performance status was analyzed with Karnofsky performance status and Barthel Index. Magnetic resonance images and Methionine-PET were correlated with clinical findings. **Results:** Follow-up ranged from 2.8 to 21.8 (median 8.3) months. Two patients showed deterioration of neurological and performance status during the course of adjuvant therapy, due to worsening of brain edema. Other 14 patients improved or remained stable during and immediately after the therapy. Seven instances of treatment-related minor morbidity were identified. Eight patients had radiographic evidence of local failure. Three of these patients have died of disease. The median survival has not been reached. **Conclusions:** Protons provided superior target dose coverage and sparing of normal structures. As dose-volume parameters correlate with acute and late toxicity, proton therapy should receive serious consideration for the treatment of selected gliomas.

WHO grade II & grade III supratentorial ependymomas in adults: a standardized treatment algorithm

Toba Niazi, MD; Randy L. Jensen, MD, PhD
(Salt Lake City, UT)

Introduction: Supratentorial cortical ependymomas and their anaplastic variants are relatively uncommon central nervous system neoplasms that afflict pediatric populations and to a lesser extent adult patients. Whereas the treatment algorithm in the pediatric population is well established, that in the adult population is less well recognized.

Methods: We describe four adult patients with extraventricular supratentorial ependymomas, two with WHO grade III (anaplastic variant) tumors and two with grade II tumors. We describe clinical presentation, imaging studies, and pathology of these patients and review the literature on management of supratentorial and anaplastic ependymoma. **Results:** In all patients, gross total resection was achieved. In the grade III subset, additional radiation therapy was administered. One patient had progression of his disease 5 months postoperatively and began chemotherapy. His disease remains stable 6 months later. The second patient has no recurrence after 10 months, and no adjuvant chemotherapy has been given. In the grade II subset, no radiation therapy was required. Mean follow-up duration of 14.5 months yielded no recurrence of tumor. **Conclusions:** The treatment algorithm for adults with supratentorial ependymomas has been somewhat undefined in the literature. On the basis of our limited experience, it appears that only close observation is required after gross total resection of WHO grade II tumors. In the case of completely resected grade III variants, adjuvant radiation therapy is recommended with chemotherapy used as a salvage therapy as necessary. With further observation of more patients, more definitive recommendation can be made in the future.

Magnetic resonance perfusion imaging predicts oligodendroglial cytogenetic subtypes and determines profiles of tumor angiogenesis

Donald M. O'Rourke, MD; Robert G. Whitmore, MD; Jaroslaw Krejza, MD, PhD; Gurpreet K. Kapoor, PhD; John Woo, MD; Jason Huse, PhD, MD; Stephanie Bloom, MSN, RN; Kevin Judy, MD, FACS; Ronald Wolf, MD, PhD; Myrna R. Rosenfeld, MD, PhD; Jaclyn A. Biegel, PhD; Elias R. Melhem, MD (Philadelphia, PA)

Introduction: Chemosensitivity of oligodendroglial neoplasms and prolonged patient survival can be predicted based upon the LOH of chromosomes 1p and 19q. Although the genes involved with oligodendroglial pathogenesis on chromosome 1p and 19q have not been identified, noninvasive characterization of these tumors through advanced imaging techniques may direct the search for candidate genes. **Methods:** This study was performed to determine whether there existed differences in relative tumor blood volume (rTBV) as determined by magnetic resonance (MR) perfusion-weighted imaging between oligodendroglial tumors with 1p or 1p/19q LOH (group 1) versus those with 19q LOH or intact alleles (group 2). **Results:** Thirty patients with oligodendroglial neoplasms were studied retrospectively. In WHO grade II neoplasms, the rTBV was significantly greater ($P < 0.05$) in

group 1 compared to group 2. In grade III neoplasms, the differences between group 1 (2.83) and group 2 (2.88) were not significant. The rTBV was significantly greater ($P < 0.05$) in grade III neoplasms (2.88) compared to grade II neoplasms (1.99). The threshold value of 2.08 had a predictive accuracy of 86% (sensitivity 100%, specificity 86%, positive predictive value 88%, and negative predictive value of 100%). Notably, there was increased expression of VEGF and VEGF-induced angiogenic proteins in 1p/19q-deleted tumors, suggesting that the genes on chromosomes 1p and 19q may include negative regulators of angiogenesis and tumor vascular invasion. **Conclusions:** Collectively, our data demonstrate the utility of advanced MR imaging in predicting molecular correlates of tumor malignancy and angiogenesis in distinct cytogenetic subsets of oligodendroglial tumors.

Emergent craniotomy in the management of petroclival meningiomas

Ryusuke Ogawa, MD; Toshihiko Kuroiwa, MD; Shin-ichi Miyatake, MD; Atsushi Aoki, MD; Yoshihito Miki, MD; Atsushi Doi, MD; Hiroyuki Ohnishi, MD (Japan)

Introduction: Petroclival meningiomas are typically slow-growing tumors, and indications for emergent craniotomy are limited. We report a rare case of petroclival meningioma, presenting massive intratumoral hemorrhage, and discuss surgical strategies of these rare conditions. **Methods:** A 63-year-old woman suddenly developed deep coma and quadriplegia. She had a history of craniotomy for partial removal of a right petroclival meningioma 4 years ago. Although she had received stereotactic radiation afterward, the residual tumor had been growing gradually. Her CT scans upon deterioration revealed massive intratumoral hemorrhage and marked compression of the brainstem. She underwent emergent craniotomy. In order to archive rapid decompression of the brainstem, we first attempted evacuation of the hematoma and tumor debulking through an epidural subtemporal route. Without time-consuming bone dissection, this was done through an expanded Meckel's cave, filling soft tumor. **Results:** After completion of an extradural middle fossa approach, Meckel's cave was opened at its lateral margin along with the trigeminal nerve complex, and the tumor within the Meckel's cave was removed. The main mass in the posterior fossa was then accessed through the porous trigeminus. After evacuation of the intratumoral hematoma, we then proceeded to the standard anterior petrosectomy to obtain a wider operative corridor, and removed the posterior fossa tumor as usual. **Conclusions:** In this emergent situation, access to the posterior fossa was first accomplished through a Meckel's cave, not through an anterior transpetrosal window.

This is a modification of the standard anterior petrosal approach, and may be applicable to other cases of emergency.

Harvest and culture of neural stem cells from adult normal human brain and malignant glial tumors

Michael J. Petr, MD, PhD; Ronald E. Warnick, MD;
Masato Nakafuku, MD, PhD (Cincinnati, OH)

Introduction: To develop a technique to harvest and culture adult human normal and malignant neural progenitor cells for comparative analysis by investigating the role of progenitor cells in primary brain malignancy. **Methods:** Tissue samples from patients after glial tumor resection and epilepsy temporal lobectomy were collected and cultured under conditions conducive to stem cell survival. Sampling was collected without consideration of patient sex, race, or glioma grade. Cultures underwent at least three passages. Growth curves were recorded. Final pass neurospheres were placed in differentiation cultures and underwent immunocytochemistry for known stem-cell markers. Immunocytochemistry was performed for comparison with matching immunohistochemistry specimen to demonstrate changes in expression from in vivo to in vitro and multilineage capability of precursor cells. **Results:** Adult human brain contains cells positive for stem cell markers most prominent in the subventricular zone. These same markers are abundant in glial origin tumors. Differentiation cultures of progenitor cells from both normal and neoplastic brain tissue show expression of neuronal, astrocytic, and oligodendritic markers. **Conclusions:** Human adult neoplastic and non-neoplastic brain contains neural progenitor cells, positive for stem cell markers and express neuronal, astrocytic, and oligodendritic markers in differentiation culture. These progenitors are found within glial origin tumors and non-neoplastic subventricular zones and can be maintained in culture. Morphology, sphere-forming units, and growth rates of neoplastic neuronal progenitor cells differ from non-neoplastic progenitor cells. Isolation of cultured progenitor cells from normal human adult brain and primary brain tumors provides new means of investigation.

Resection of tumors near eloquent brain using continuous electrophysiological monitoring: consecutive series of 31 patients

Sujit S. Prabhu, MD, FRCS; Sudhakar Tummala, MD;
Sami F. Elestwani, ST (Houston, TX)

Introduction: Invasive electrophysiological monitoring remains the “gold standard” for safe resection of tumors

in eloquent brain tissue. **Methods:** A total of 33 procedures in 31 patients were performed using continuous motor evoked potentials (MEP's) and somatosensory evoked potentials (SSEP's) using a paddle grid sutured to the dura at surgery. **Results:** Gliomas (54%) and metastases (39%) formed the major tumor groups. Upper extremity MEP's were obtained in 90% of the cases while in only five patients reproducible lower extremity MEP's were obtained. SSEP's were obtained in 100% of procedures. Neurological deficits immediately after surgery was seen in 39% of the cases, though no significant changes were seen in the MEP recordings. However, at one month 12% had a new residual deficit. Twenty six patients (78%) had a gross total resection of the tumor. One patient had an intraoperative seizure. **Conclusions:** Continuous MEP's recording is a safe technique to maximize tumor resection near eloquent brain areas. Though MEP's and SSEP's did not predict a postoperative neurological deficit a significant number of patients recovered at one month possibly indicating reversible factors like brain retraction and edema may not directly interfere with the MEP monitoring.

Focused therapy in lieu of whole brain radiation for metastatic disease

Matthew R. Quigley, MD; Russell Fuhrer, MD;
Stephen Karlovits, MD (Pittsburgh, PA)

Introduction: As whole brain radiation therapy (WBXRT) is associated with cognitive decline, especially in long-term cancer survivors, we have offered our patients a focused therapeutic approach reserving WBXRT only for failure. **Methods:** Retrospective review of all patients treated at our institution with newly diagnosed metastatic brain disease (four or fewer lesions) who had not received WBXRT previously. **Results:** There were 63 patients, 28 males, mean age 58.5. Pathology was 35 lung, eight renal, eight breast, three melanoma, three colon, two unknown and one each of assorted others. Nineteen patients had multiple lesions. Thirty-four underwent resection as initial treatment (22 solitary), and seven had delayed resections for six SRS in-field and one out-of-field recurrences. Seven patients had additional SRS for two in-field and five out-of-field recurrences. A total of 11 patients (11/63 = 17.5%) received WBXRT, one for a 6 cm post-operative bed, two for a local surgical recurrence and eight with progressive diffuse new lesions. Survival for the entire cohort was 14.0 months. post-initial treatment. Using Cox regression model survival related to any resection ($P = .0001$) and RPA = 1 ($P = .05$). Among patients with solitary metastases, survival was

significantly better in the group for which surgery was used initially (19.6 vs. 7 months, $P = .02$) despite six patients receiving rescue resections following SRS failure. **Conclusions:** In a cohort of patients referred to neurosurgery with newly diagnosed metastatic brain disease, WBXRT may be withheld in the vast majority without compromising survival. RPA = 1 patients who were resected fared the best.

Aquaporin-4 expression in the normal human fetal and adult brain

Alfredo Quinones-Hinojosa, MD; Grettel Zamora-Berridi, BA; Bryant McIver, BS; Shaan M. Raza, MD; Joaquin Camara-Quintana, BS; George Jallo, MD; Fred Lenz, MD, PhD (Baltimore, MD); Geoffrey Manley, MD, PhD (San Francisco, CA)

Introduction: Aquaporins are a class of water channel proteins found to regulate membrane water permeability in several organ systems. These proteins are purported to play a role in vasogenic edema, and tumor cell migration. According to animal studies, aquaporin-4 (AQP4) is predominantly found on ependymal cells and astrocytes contacting blood vessels. Yet, there is a paucity of studies analyzing the topographic expression in humans. **Methods:** The study focused on mapping AQP4 expression in non-cancer human brain samples including: subventricular zone (SVZ), hippocampus and cortex specimens from post-mortem fetal (27-week gestational age, $n = 5$) and adult (age-range 18–80 years, $n = 20$) tissue. Intra-operative specimens obtained during temporal lobectomies and endoscopic cranial procedures were also analyzed ($n = 4$). The morphology and localization of AQP4-positive cells were examined using immunohistochemistry (IHC), immunocytochemistry (ICC) and Western blots (WB). **Results:** This study demonstrates the expression of AQP4 in non-cancer human fetal and adult brains in immunostaining, and WB. The results reveal the presence of this protein not only on SVZ astrocytes, but also on the ependymal layer of the lateral ventricles. In particular, strongest expression was found in the temporal horn of the fetal and adult SVZ contrasted with minimal cortical signal. **Conclusions:** This thorough characterization of AQP-4 in non-cancer human fetal and adult brains is the first step towards elucidating AQP-4 mechanisms of action that may play a role in several pathophysiological phenomena: vasogenic edema, and tumor cell migration. A better understanding of the function of aquaporin-4 could allow for novel therapeutic strategies to modulate these tumor associated events.

Assessment of the grade of resection in large skull base meningiomas

Alejandra T. Rabadan, MD; Diego A. Hernandez, MD; Martin Kitroser, MD; Patricia Maggiora, MD; Cristina Besada, MD(Argentina)

Introduction: To assess the grade of resection in large skull base meningiomas. **Methods:** A cohort of 24 patients with skull base meningiomas of large size (major diameter 3–7 cm) was retrospectively evaluated. Grade of resection was assessed by the Simpson Scale. Follow up was performed with Gadolinium-MRI yearly. **Results:** There were: sphenoidal plane 4 cases, sphenoid ridge 4 cases, temporal fossa 1 case, petrous 5 cases, petroclival 5 cases, posterior incisura 3 cases, and foramen magnum meningiomas 2 cases. Surgical approaches were related to the localization. Gross tumor resection (Simpson 2) was obtained in 15 patients (62.5%); and subtotal (Simpson 3–4) in nine (37.5%). The main surgical difficulties were: complete resection of dural attachment (24 cases); compression of cranial nerves and/or displacement of brain stem (23); tumoral encasement of cranial nerves or cranial arteries (9); the sinus or venous variants hampering the procedure (2); and hydrocephalus (7). The median follow-up period was 3 years. In the gross tumor resection group, one patient underwent reoperation due to recurrent tumor. In the subtotal resection group, three patients required treatment because of progression of the tumor remnant (reoperation, two cases; radiosurgery in one case). **Conclusions:** Simpson 2 was the best grade of resection achieved due to the complexity of resection of the dural attachment. The subtotal resections were related to vascular and/or cranial nerve encasement by the meningioma. Postoperative management requires a long term follow-up.

Assessment of the postoperative functional status of malignant tumors in eloquent areas of the brain

Alejandra T. Rabadan, MD, PhD; Diego A. Hernandez, MD; Patricia Maggiora, MD; Marcelo Pietrani, MD; Maria F. Seclen, MD, (Argentina)

Introduction: Many centers in our region currently rule out surgery when tumors involve eloquent areas due to the lack of adequate levels of evidence regarding the postoperative neurological status. We assessed the functional results of patients with tumors in eloquent or paraloquent brain areas. **Methods:** A cohort of 46 cases of malignant brain tumors (31 gliomas, 15 metastases), was retrospectively analyzed. Median age: 55 years-old. Eloquence was

classified according to Sawaya. Preoperatively all patients had Gd-MRI; some cases, also had functional MRI or tractography. Postoperatively, Gd-MRI was early performed. Inclusion criteria for resective surgery were the Karnofsky score (KPS) and the anesthetic risk (ASA). Microsurgery and guidance with neuronavigation or stereotaxy were performed. When low-grade gliomas in language or motor areas were presumed, intraoperative neurophysiological monitoring was used. KPS was evaluated at 30-days after surgery. **Results:** Preoperatively all patients had KPS = 60 and ASA = 3. Twenty-eight tumors were in eloquent and 18 in paraloquent areas. Gross total resection was obtained in 67.3% and subtotal in 32.6%. At 30-days after surgery, improvement or stability of KPS occurred in 43 cases (93.4%); and KPS declining in three cases (6.5%) not related to the surgical manipulation (hematoma, myocardial infarction and meningitis). **Conclusions:** In our experience, the eloquence should not be considered an isolated variable for the surgical indication because the modern neurosurgical techniques offer a framework of protection for the critical brain areas. Our results could contribute with the construction of necessary evidence-based criteria for surgical indications in neuro-oncology.

Peri-operative and long term outcomes in the management of parasagittal meningiomas invading the superior sagittal sinus

Shaan Raza, MD; Gary Gallia, MD, PhD; Henry Brem, MD; Jon Weingart, MD; Donlin Long, MD, PhD; Alessandro Olivi, MD (Baltimore, MD)

Introduction: Parasagittal meningiomas invading the superior sagittal sinus (SSS) pose formidable challenges to surgical management. Invasion is often considered a contraindication to surgery due to associated morbidity, such as cerebral venous thrombosis. We report our most recent experience with the resection of parasagittal meningiomas invading the SSS. **Methods:** Between 1992 and 2004, 110 patients with parasagittal meningiomas underwent surgery at the Johns Hopkins Medical Institutions. Clinical charts, radiological studies, pathological features and operative notes were retrospectively analyzed; only those patients with minimum 12 months follow-up ($n = 61$) were further studied. **Results:** Tumor distribution by location along the SSS was: 21% anterior, 62% middle, 17% posterior. All patients were managed with initial surgical resection with radiosurgery for residual/recurrent disease if indicated (19.6%). Pathologic examination revealed 83% grade I meningiomas, 12% grade II meningiomas, 5% grade III

meningiomas. Simpson grade I/II resection was achieved in 81% of patients. Major operative complications included: venous thrombosis/infarct (7%), intra-operative air embolism (1.5%), death (1.5%); long-term outcomes assessed included recurrence (11%), improvement in Karnofsky Performance Score (85%). **Conclusions:** On the basis of our study, the incidence of intra-operative complications is 7% in the setting of a recurrence rate of 11% with a mean follow-up of 41 months. In comparison to the published literature, the data corroborates the rationale for our treatment paradigm: lesions invading the sinus can initially be resected to the greatest extent possible without excessive manipulation of vascular structures while residual/recurrent disease is observed and often managed with radiosurgery.

Favorable characteristics for long term survival in high grade gliomas

Scott C. Robertson, MD (Midwest City, OK)

Introduction: High grade gliomas have a high mortality rate. Despite aggressive surgical resection, XRT, and recent chemotherapy advances the percentage of patients surviving at 2 years is less than 10%, and long-term survival rare. During the course of our treatment we have identified several characteristics, which long term survivors have in common. **Methods:** We studied 29 patients diagnosed with high grade gliomas (type IV) from a single practice from 1998 to 2006. We looked at age, location, size and shape of tumors, amount of edema, type of surgery, adjuvant treatment, and pathological findings. Twelve patients had only biopsies of the tumor, the remaining 17 patients had a gross total resection. Gliadel (BCNU) wafers were placed in the resection cavity of all 17 patients. Post operatively all patients received adjuvant radiation and chemotherapy. All of biopsied patients died with a mean survival 4.9 months. Nine out of 17 (52.9%) of our patients who had surgical resections are still alive past 24 months. **Results:** Favorable characteristics among the surviving patients included younger age, frontal lobe location, smooth cystic tumors with ring enhancement and very little irregularity, surgical margins were smooth with limited local invasion observed during resection, very little necrosis observed during resection, tumor size less 4.5 cm at its greatest diameter, aggressive surgical resection with Gliadel wafers placement in the surgical bed followed by post-op XRT and Temodar chemotherapy. **Conclusions:** High grade glioma patients with the favorable characteristics identified above carry a better prognosis for survival. Aggressive surgical management should be consider in these cases.

MGMT expression and temozolomide response in a patient with a malignant spinal cord astrocytoma

Richard A. Rovin, MD; Robert Winn, PhD
(Marquette, MI)

Introduction: Malignant astrocytomas of the spinal cord are unusual lesions. Consequently, there is no clear standard treatment regimen. We report on a patient whose spinal cord astrocytoma progressed despite surgery, radiation therapy and a second surgery. The tumor and neurologic deficit responded favorably to temozolomide. This dramatic response prompted a retrospective evaluation of the tumor's MGMT expression. MGMT antagonizes the effect of temozolomide and its expression in cerebral GBM is associated with a worse prognosis. MGMT expression and response to temozolomide in a malignant spinal cord astrocytoma has not been reported previously. **Methods:** Temozolomide was administered in standard fashion: 200 mg/m² for the first 5 days of a 28 day cycle. RNA was extracted from the formalin fixed paraffin embedded surgical tissue. MGMT expression was assessed using RT-PCR. **Results:** After six cycles of temozolomide MRI showed no evidence of tumor. This tumor showed no MGMT expression. **Conclusions:** This is the first report correlating the response of a malignant spinal cord astrocytoma to temozolomide with MGMT expression. Our result is consistent with reports showing tumors with low MGMT expression are responsive to temozolomide and are associated with longer time to progression and median survival. The role of genetic profiling in guiding clinical treatment and in the design of clinical trials will be discussed.

ZBTB7 expression in malignant glioma

Richard A. Rovin, MD; Robert Winn, PhD
(Marquette, MI)

Introduction: The genetic changes that distinguish malignant from normal glial tissue principally affect cellular pathways involved in cell cycle control and DNA repair. The p14ARF-MDM2-p53 pathway is altered in the majority of gliomas. p14ARF loss is an integral feature in gliomagenesis. A recently described oncogene, ZBTB7, specifically represses transcription of p14ARF. The ZBTB7 gene is located on chromosome 19p13.3. This locus is overrepresented in many GBMs. ZBTB7 is expressed in a number of malignancies, though its expression has not yet been described in glioma. This is the first report regarding

ZBTB7 expression in glioma. **Methods:** Sixteen gliomas (15 GBM, 1 pilocytic astrocytoma) were evaluated for ZBTB7 expression using a RT-PCR protocol. We designed a PCR primer set and also used a primer set described in the literature. RNA was extracted from operative tissue as well as from formalin fixed paraffin embedded archived specimens. **Results:** We found ZBTB7 expression in 14 of 15 GBM and in the pilocytic astrocytoma. **Conclusions:** Loss of p14ARF functionality is important in glioma formation. One factor decreasing p14ARF expression is the oncogene, ZBTB7 whose gene product is a specific repressor of p14ARF transcription. This study confirms that ZBTB7 is overexpressed in glioma. Identifying genetic abnormalities in glioma opens avenues for novel, targeted therapies. Our lab is now developing methods to decrease ZBTB7 expression that ideally will translate into a clinically relevant treatment for malignant glioma.

Cushing's disease: the effect of inferior petrosal sinus sampling on outcome in transsphenoidal surgery

Jonathan H. Sherman, MD (Charlottesville, VA);
Daniel Prevedello, MD (Pittsburgh, PA);
Nader Pouratian, MD, PhD; John A. J, Jr., MD;
Mary Lee Vance, MD; Edward R. Laws, Jr., MD
(Charlottesville, VA)

Introduction: Inferior petrosal sinus sampling (IPSS) can assist the surgeon confirming the central source of Cushing's disease in patients with equivocal MRI. The effect of IPSS on outcome in patients with Cushing's disease undergoing transsphenoidal resection is evaluated. **Methods:** Using our clinical database from 1992 until 2005, we collected adult patients with Cushing's disease who had no prior interventions, an equivocal MRI and underwent transsphenoidal surgery at the University of Virginia. Presence or absence of IPSS, age at diagnosis, gender, intra-operative findings, rate of remission, rate of recurrence and time of follow-up were analyzed. **Results:** Between 1992 and 2005, 108 patients were identified. There were 31 men and 77 women. Mean age at diagnosis was 42.4 years (range 18–78). Eighty-seven patients had pre-operative IPSS with or without a high dose dexamethasone suppression test (DST) while 21 patients had a DST alone. In the IPSS-group, 62.1% had an adenectomy and 12.6% had a hemihypophysectomy. In the DST-alone-group, 71.4% had an adenectomy while no patients had a hemihypophysectomy. Surgical remission was 82.7% in the IPSS-group and 76.2% in the

DST-alone-group. Rate of recurrence was 5.8% in the IPSS-group and 33.3% in the DST-alone-group ($P < 0.05$). Mean follow-up was 22.8 months for the IPSS-group and 44.8 months for the DST-alone-group. **Conclusions:** A positive IPSS can give confidence to the surgeon allowing for more aggressive surgery. The use of IPSS in patients with Cushing's disease and equivocal MRI displayed an improved rate of remission and recurrence after transsphenoidal surgery. However, longer follow-up is necessary to confirm these findings.

A model for studying vasogenic brain edema using intra-arterial mannitol infusion

Anshu Shukla, MS; Zhi-Jian Chen, MD, PhD; William C. Broaddus, MD, PhD; Panos P. Fatouros, PhD; Frank D. Corwin, MS; Peter J. Haar, MD, PhD (Richmond, VA)

Introduction: Convection-enhanced delivery (CED) is a proven method for targeted drug delivery to the brain that circumvents the blood-brain barrier (BBB). Little study has been conducted in understanding CED in pathological brain states. This is of importance when dealing with CED in brain tumors and other pathologies, where vasogenic edema (VE) exists. The current study aims to characterize a model of VE suitable for studying CED by using intra-arterial mannitol infusion to disrupt the BBB and mimic the increased cerebral capillary permeability characteristic of VE. **Methods:** Edema was produced in the right hemisphere of the rat brain using multiple infusions of 25% mannitol (0.25 ml/kg/s over 30 s) delivered through the right internal carotid artery. Evan's Blue dye was delivered through the right femoral vein to verify BBB disruption. Before and after mannitol delivery, animals were scanned using magnetic resonance imaging. **Results:** T2-weighted magnetic resonance imaging revealed consistent edema formation. Water maps post processed from T1-weighted images revealed statistically significant higher water levels in the ipsilateral gray and white matter within an hour of the first infusion. Apparent diffusion coefficient (ADC) values and histological examination (light and electron microscopy) suggested a significant element of cytotoxic edema, in addition to findings consistent with vasogenic edema. **Conclusions:** This model provides a reproducible technique for generating a large area of edema for CED study. The observed edema seems to include both vasogenic and cytotoxic components. Further studies while titrating to changes in ADC and fractional water content may result in a model with a greater component of VE.

A phase II study utilizing focal radiation in patients with 1-3 brain metastases

Allen K. Sills, MD (Memphis, TN); Stephen B. Tatter, MD (Winston-Salem, NC); Kostas N. Fountas, MD (Macon, GA); Robert W. Fraser, MD (Charlotte, NC); Anthony L. Asher, MD (Charlotte, NC); Michael A. Vogelbaum, MD (Cleveland, OH); Kevin D. Judy, MD (Philadelphia, PA); Gregory W. Canute, MD (Syracuse, NY); Randy L. Jensen, MD (Salt Lake City, UT); Allen Redmond, RN, BSN (Memphis, TN)

Introduction: Addition of radiation following brain metastases resection has shown reduction in local failure rates. Deferring whole brain radiation may reduce the risk of neurological deficits related to radiation toxicity. This study evaluates the GliaSite[®] RTS in combination with stereotactic radiosurgery (SRS) in patients with 1-3 brain metastases. **Methods:** The GliaSite[®] RTS is implanted at surgery and filled within 21 days with Iotrex[®], a 125I solution. A total dose of 60 Gy to 5 mm is delivered to the tumor bed. Remaining lesions may be treated with SRS. Primary objectives include six-month and one-year local control. **Results:** Forty-one patients have been treated. Median age is 60 years (range 37–78). Pathology includes: Lung (23), Breast (5), Melanoma (4), Unknown (3), Renal (2), Uterine (1), Ovarian (1), Liver (1) and Squamous-skin (1). Local tumor recurrence is documented in one case at 9 months. Distant recurrence is documented in four cases at 1 month, six cases at 3 months, three cases at 6 months and one case at 9 months. Local and distant recurrence are documented in one case at 3 months. One case of biopsy proven radiation necrosis is documented at 12 months and one case of PET positive radiation necrosis is documented at 15 months. Quality of life measures remain stable in patients reaching 12-month follow up. Grade 3 or greater toxicity attributed to treatment includes: radiation necrosis (2), cerebral abscess (1) and tumor cavity hemorrhage (1). **Conclusions:** Enrollment will continue for a total of 50 patients. Preliminary results seem encouraging regarding safety and efficacy.

Age at diagnosis, treatment combinations and survival among elderly patients with AA and glioblastoma multiforme

Jill Barnholtz-Sloan, PhD; Vonetta L. Williams, BA; John L. Maldonado, BA; Heather G. Stockwell, PhD; Marc C. Chamberlain, MD; Andrew E. Sloan, MD (Tampa, FL)

Introduction: Treatment of elderly glioma patients is controversial. **Methods:** Retrospective cohort study using

SEER Program-Medicare linked data. 1,753 patients with primary GBM and 205 with primary AA were identified in matched SEER-Medicare database as 66 years old or greater 1991–1999. Odds of various treatments and risk of death by treatment were calculated by histology, race, income, co-morbidities, marital status, geographic region, tumor location, and gender. **Results:** Age differences existed for all treatment combinations for GBM but not for AA. For GBM, odds of having singular treatments increased with age, as did odds of having radiation and biopsy. Odds of treatment combinations decreased with increasing age. GBM patients 75+ years had 2.5-fold increased odds of having biopsy only (OR = 2.53, 95% CI (1.78,3.59)), 1.5-fold increased odds of having surgery only (OR = 1.47, 95% CI (1.15,1.87)), 31% decreased odds of having radiation and surgery (OR = 0.69, 95% CI (0.57,0.84)), and 65% decreased odds of having chemotherapy, radiation, and surgery (OR = 0.35, 95% CI (0.23,0.53)) compared to individuals 66–74. Compared to individuals receiving radiation and surgery, AA who had biopsy only (HR = 3.67, 95% CI (2.05,6.58)) had an increased risk of death. GBM who had biopsy only (HR = 5.62, 95% CI (4.61,6.85)), surgery only (HR = 3.48, 95% CI (3.01,4.04)), or radiation and biopsy (HR = 1.66, 95% CI (1.44,1.93)) had increased risk of death while those receiving chemotherapy, radiation and surgery had decreased risk of death (HR = 0.75, 95% CI (0.63,0.90)). **Conclusions:** Elderly GBM patients were more likely to be treated with single modalities with increased risk of death. These differences were less for AA.

The resectability of recurrent craniopharyngiomas

Juraj Steno, MD, PhD; Ivan Bizik, MD (Slovakia)

Introduction: Radical removal of recurrent craniopharyngioma (RC) has been reported to be difficult with high risk. The aim of the study was to analyze these aspects of our management of RC. **Methods:** Seventeen patients with RC of 90 operated on by the authors during a 15-year period (1991–2005) and eight others initially operated on elsewhere were analyzed for outcome. Resection was considered radical based on the surgeons opinion and post operative MRI. **Results:** Radical removal for first recurrence was possible in 14/25 patients. Radical resection was achieved in 10/11 tumors regrowing in the third ventricle but only in 4/14 suprasellar extraventricular or intrasellar/suprasellar RCs because it was easier to dissect the tumor from gliotic scar than from extensive fibrous adhesions. The 25 patients in the series had a total of 35

operations for recurrence (seven for the second, two for the third and one for the 4th RC); 20 of which were considered radical resections (57%). There was no surgical mortality; three patients had permanent unilateral worsening of vision and two had progression of obesity. Three patients died 7, 17, 22 months after the last operation. Twenty-two patients (88%) are alive. There has been no radiological recurrence in 11 of 14 patients after radical removal of the first recurrence. Eleven patients have an asymptomatic remnant of tumor. **Conclusions:** (1) Safe radical removal is possible in more than half of patients with RC. (2) RC in the 3rd ventricle is easier to resect.

The long-term impact of whole brain irradiation on the stem cell and oligodendrocyte precursor compartments

Viviane S. Tabar, MD (New York, NY)

Introduction: The cellular basis of long term radiation damage in the brain is not fully understood. We administered 25 Gy to adult rat brains while shielding the olfactory bulbs. **Methods:** Quantitative stereological analyses were serially performed over a period of 15 months. **Results:** Our data reveal immediate and permanent suppression of subventricular zone (SVZ) proliferation and neurogenesis that persisted for the entire 15 months. The olfactory bulb demonstrates a transient but remarkable SVZ-independent ability for compensation and maintenance of the interneuron population. The oligodendrocyte compartment exhibits a complex pattern of limited proliferation of progenitors but steady loss of the oligodendroglial antigen O4. As of 9 months post radiation, diffuse loss of myelin starts in all irradiated brains. Counts of capillary segment and length demonstrate significant loss one day post radiation but swift and persistent recovery up to 15 months post XRT. MRI imaging confirms loss of volume of the corpus callosum and early signs of demyelination at 12 months. Areas of focal necrosis appear beyond 15 months and are clearly preceded by widespread demyelination. Human white matter specimens obtained following radiation confirm early loss of oligodendrocyte progenitors and delayed demyelination with preserved capillaries. **Conclusions:** This is the first study to quantitatively document the fate of individual cell populations over an extended period post-radiation. Radiation injury is associated with irreversible damage to the neural stem-cell compartment and loss of oligodendrocyte precursor cells in both rodent and human brain. The rat model detailed here can serve as an excellent tool for cell replacement or other therapeutic strategies.

Quantum dots are phagocytized by macrophages and co-localize with experimental glioma

Heather Jackson, BS (Cleveland, OH); Osman Muhammad, BS (Cleveland, OH); Hamid Daneshvar, MS (Cleveland, OH); Jennifer Nelms, PhD (Cleveland, OH); Alexandra Popescu, MD (Cleveland, OH); Michael A. Vogelbaum, MD, PhD (Cleveland, OH); Marcel Bruchez, PhD (Pittsburgh, PA); Steven A. Toms, MD, FACS (Cleveland, OH)

Introduction: The identification of neoplastic tissue within normal brain during biopsy and tumor resection remains a problem in the operative management of gliomas. Nanoparticles phagocytized by macrophages in vivo may allow optical nanoparticles to co-localize with brain tumors and serve as an optical aid in the surgical resection or biopsy of brain tumors. **Methods:** Fisher male rats were implanted intracranially with C6 gliosarcoma cell lines. Two weeks after implantation of tumors, 705 nanometer emission Qdot ITK Amino(PEG) Quantum Dots were injected at doses of 3–17 nanomoles. Twenty-four hours post quantum dot injection, the animals were sacrificed and their tissues examined. **Results:** Quantum dots are avidly phagocytized by macrophages and are taken up by liver, spleen and lymph nodes. A dose response relationship was noted. At low doses, the majority of the quantum dots are sequestered in the liver, spleen and lymph nodes. At higher doses, increasing quantities of quantum dots are noted within the experimental brain tumors. Macrophages and microglia co-localize with glioma cells, carrying the quantum dot, thereby optically outlining the tumor. Excitation with blue or UV wavelengths excites the quantum dots, giving off a deep red fluorescence detectable with charge coupled device (CCD) cameras, optical spectroscopy units, and in dark field fluorescence microscopy. **Conclusions:** Quantum dots are optical nanoparticles which, when delivered in nanomole doses, are phagocytized by macrophages and microglia infiltrating experimental gliomas. The optical signal may be detected, allowing for improved identification and visualization of tumor, potentially augmenting brain tumor biopsy and resection.

Progressive dementia as a rare presentation for pituitary adenoma

Andrew J. Tsung, MD (Peoria, IL); Huan Wang, MD (Boston, MA); William Olivero, MD (Champaign, IL)

Introduction: The majority of patients with symptomatic pituitary adenomas typically present with endocrinopathy and/or visual loss. We report a case of giant cystic pituitary

adenoma in a patient initially presenting with progressive dementia. **Methods:** A 60-year-old man presented with progressive dementia to his primary care physician. As part of a standard dementia evaluation, MRI of the brain demonstrated moderate obstructive hydrocephalus and a giant cystic mass extending from the sella to the level of the lateral ventricles, and posteriorly to the thalamus. The suprasellar and the intra-sellar component demonstrated contrast enhancement. **Results:** An endocrine workup was performed revealing normal hormonal profiles. The patient was taken to the operating suite for cyst decompression and removal of the suprasellar component of the tumor through a bifrontal craniotomy. Pathology revealed the presence of red blood cell breakdown products within the cystic fluid while the suprasellar solid components were characteristic of non-functioning pituitary adenoma. The patient had complete resolution of his dementia post-operatively. **Conclusions:** We present the unique case of a giant cystic pituitary adenoma presenting with dementia. Although mass lesions are known to cause focal deficits, confusion, and behavioral disorders, dementia is not commonly seen. Secondary adrenocortical insufficiency has been reported to cause dementia, however this patient had normal laboratory profiles. We believe that in this patient the dementia may have resulted from a combination of a very large mass in the frontal lobes and hydrocephalus. Why this patient developed such a large cyst in association with a pituitary tumor is unclear.

20Gy radiotherapy with locally delivered OncoGel® (6.0-mg/ml Paclitaxel) significantly prolongs survival in an experimental rodent glioma model

Betty Tyler, BA; Violette Renard, MD; Sameer Kaiser, BS (Baltimore, MD); Kirk Fowers, PhD (Sandy, UT); Henry Brem, MD (Baltimore, MD)

Introduction: Paclitaxel, a cellular proliferation inhibitor, is effective against gliomas but has poor penetration into the CNS with dose-limiting toxicities when administered systemically. To enhance efficacy and limit toxicity, paclitaxel has been incorporated into a thermo-sensitive polymer depot delivery system by MacroMed, Inc. In this experiment, we demonstrate the safety and potential efficacious synergistic effects of OncoGel® given with radiotherapy (XRT) in rats challenged with intracranial 9L glioma. **Methods:** Sixty animals were intracranially implanted with 9L gliosarcoma and divided into groups receiving placebo (ReGel), XRT, OncoGel 6.0 mg/ml, or OncoGel with single dose 20 Gy XRT. Treatments were given either simultaneously or 5 days after tumor implant. **Results:** Animals receiving ReGel showed no difference from Controls

(median survival of 13 days): Day 0 or Day 5 ReGel had a median survival of 14 and 17 days, respectively. Animals receiving OncoGel on Day 5 had a median survival of 17 days. The groups receiving XRT alone, OncoGel Day 0, or OncoGel Day 5 plus XRT had a median survival of 26 ($P = 0.0001$), 31 ($P = 0.0001$), and 32 ($P = 0.0255$) days, respectively. As of Day 67, animals receiving Day 0 OncoGel 6.0 + XRT had not yet reached median survival ($P = 0.0001$). **Conclusions:** These results indicate that OncoGel 6.0 mg/ml is safe for intracranial injection in rats. Groups receiving XRT alone, OncoGel Day 0, or OncoGel Day 5 plus XRT had significantly improved survival. Animals receiving Day 0 OncoGel + XRT had a statistically significant increase in survival with potential long-term survivors and significance compared to XRT alone ($P = 0.0182$).

Glioma specific toxicity of gene-silencing double-stranded RNA molecules

Jose E. Valerio, MD; Joao Marques, PhD;
Bryan Williams, PhD; Michael Vogelbaum, MD, PhD
(Cleveland, OH)

Introduction: GBMs are sensitive to activation of the interferon (IFN) system. Certain forms of small interfering RNA (siRNA) can non-specifically activate the IFN system in a tumor specific manner. We examined the effects of transfection of modified siRNAs on GBM cells compared to normal human astrocytes. **Methods:** T98G, U251, D54, U87, LN229 glioma cell lines, CCF88 (primary GBM culture) and normal human astrocytes (NHA) were used. Subclones of U87, LN229 and NHA overexpressed eGFP. Chemically synthesized siRNAs of varying size (21–27 base pairs, (bp)) and configuration (blunt ended or with 2 bp overhangs) were used to target eGFP. **Results:** Transfection of neoplastic cells with siRNAs of varying size (21–27 bp) and configuration (0 = blunt, 2 = 2 bp overhangs) (21 + 2, 25 + 0, 27 + 0, 27 + 2) produced dose dependent toxicity with the largest siRNAs producing the most cell loss ($P < 0.05$). Efficient knockdown of eGFP expression (>95%) by all targeted siRNA constructs, but not by scrambled control siRNAs, was observed in LN229 and U87 cells independent of cell loss. Knockdown of eGFP but no significant toxicity was observed in NHA. Apoptosis was observed in LN229 and U251 cells but not in U87. **Conclusions:** Treatment with modified siRNA constructs produces both targeted gene knockdown and toxicity in neoplastic cells but no toxicity in normal astrocytes. Further development of this novel and apparently specific

anti-tumor therapeutic approach will involve further evaluation of its tumor specific mechanisms, evaluation of potential synergistic targets for knockdown as well as development of appropriate delivery methods for these small molecules.

Local delivery of epirubicin for the treatment of an experimental rodent glioma

Ditty Vick; Samuel Hertig, BA; Betty M. Tyler, BA;
Khan W. Li, MD; Violette M. Renard, MD;
Thomas Koztowski, BS (Baltimore, MD); Federico G.
Legnani, MD (Italy); Henry Brem, MD; Alessandro
Olivi, MD (Baltimore, MD)

Introduction: Epirubicin, a chemotherapeutic agent used for treatment of metastatic breast cancer, has significant cytotoxic activity making it a candidate for the treatment of malignant gliomas. However, poor CNS penetration and systemic toxicity at high doses limit its clinical use. To minimize toxicity and increase local concentrations, epirubicin was incorporated into a biodegradable polymer delivery system. The pharmacokinetics of this formulation was assessed in vitro, CNS toxicity was established in vivo, and efficacy was tested against an experimental gliosarcoma model. **Methods:** Epirubicin was incorporated into (poly[1,3-bis(carboxyphenoxy)propane-co-sebacic-acid] polymers (pCPP:SA)) at various loading doses. Absorbance was established and release was analyzed. Intracranial dose escalation toxicity was determined in F344 rats. Efficacy studies included 27 rats implanted with 9L gliosarcoma. Animals received either no treatment ($n = 12$) or 50% epirubicin:pCPP:SA ($n = 17$) 5 days following tumor implant. Kaplan–Meier curves were generated for survival and brains were processed for histological evaluation. **Results:** Fifty percent epirubicin:pCPP:SA released $63 \pm 9\%$ within 48 h. Dose escalation of intracranially implanted polymers showed no toxicity study up to 40 days after polymer implantation. Efficacy studies against established tumor demonstrated that the group receiving 50% epirubicin polymer had a statistically significant increase in survival as compared to controls (median survival not reached vs. median survival of 12.5 days; $P < 0.0001$) with 66% animals resulting in long term survival (LTS). **Conclusions:** Epirubicin:CPP:SA polymers show sustained release and are safe for intracranial implantation. Epirubicin:CPP:SA polymers were shown to be effective in the treatment of an experimental rat glioma model with 66% LTS. These studies warrant further investigation of this drug for the treatment of malignant glioma.

Leiomyosarcoma metastasis to the skull

Franklin D. Westhout, MD; Marlon Mathews, MBBS, BSc; Mark Linskey, MD (Orange, CA)

Introduction: Uterine leiomyosarcoma infrequently metastasizes to the brain, and even less frequently to the skull bone. We present a rare case of leiomyosarcoma metastasis to the skull. **Methods:** A 44-year-old right handed woman with a 3 year known history of a metastatic leiomyosarcoma presented with an enlarging lump over her right occiput. She had multiple abdominal metastases, for which she received multiple surgeries and systemic therapy with docetaxel and gemcitabine. She had been on a chemotherapy holiday over the summer and near the end of September noticed a “lump” in her right occiput. She also started to develop headaches, but no other neurologic symptoms. MRI revealed a large enhancing mass in the right occipital and sub-occipital region extending inward over the transverse sinus near its junction with the torcula as well as extending outward elevating her scalp, with an epicenter in the right occipital skull bone. **Results:** On surgical exploration the mass was found to be an internally exophytic firm tumor that was adherent to dura. The outer dural layer was resected with the mass in an en bloc fashion, followed by methylmethacrylate and good soft tissue closure. Histopathology identified a metastatic leiomyosarcoma. Twenty days following surgery the tumor bed was treated with Gamma knife Radiosurgery using 16 gray/45% isodose; to a total of 35.6 gray. **Conclusions:** Leiomyosarcoma metastases to the brain are rare, and less common metastasize to the skull. Brain metastatic lesions of leiomyosarcoma respond well to Gamma Knife Radiosurgery, preceded by debulking surgery depending on the size of the lesion.

PAX6 increases glioma cell susceptibility to detachment and oxidative stress

Yi-Hong Zhou, PhD (Orange, CA); Jason Y. Chang, PhD (Little Rock, AR); Yuanjie Hu, MSc (Orange, CA); Eric Siegel, MSc; Latarsha Stanley (Little Rock, AR)

Introduction: Our previous research revealed that PAX6 plays a role in suppression of GBM cell growth and tumorigenicity, and that low PAX6 expression is a poor prognostic factor for GBM. An understanding of how a decrease of PAX6 expression confers selective advantages on tumor cells may extend our understanding of the mechanism of GBM development. In this study, we investigated the effect of PAX6 on cell growth and

survival in a stressful environment including detachment-induced stress. **Method:** After either adenoviral-mediated transient over-expression or transfection-mediated stable over-expression of PAX6 in the glioma cell line U251HF, we studied detachment induced alterations of cellular ROS levels (by H2DCF-DA staining) and viability (by the MTT assay). **Results:** ROS levels increased following cell detachment but PAX6 over-expressing cells retained higher level of ROS than parental control cells. PAX6 over-expression attenuated GBM cell recovery of growth after detachment-induced stress. Addition of antioxidant improved the viability of PAX6 over-expressing cells, but did not restore proliferative ability. Addition of serum can restore proliferation. **Conclusions:** These results suggest that PAX6 over-expression makes glioma cell more susceptible to cellular and environmental stresses. If the same occurs in vivo, then glioma cells with reduced PAX6 expression may have a real selection advantage over the other cell type to survive in and migrate out of a stressful intratumoral environment, such as necrotic zone. We will further delineate this function of PAX6 using a glioma model that is representative of clinical GBM.

Characterization of PAX6 regulation of VEGF in glioblastoma

Yi-Hong Zhou, PhD (Orange, CA); Debra A. Mayes, PhD (Little Rock, AR); Yuanjie Hu, MSc (Orange, CA)

Introduction: High expression of vascular endothelial growth factor (VEGF) is a hallmark of GBM. **Methods:** We discovered a novel function for the transcription factor PAX6 (which is involved in the development of the central nervous system, eye and pancreas) in suppression development of GBM. We revealed that a low level of PAX6 in astrocytic gliomas is a poor prognostic factor for patients. In addition, PAX6 suppressed cell invasiveness in vitro, partially via suppressing the expression of the pro-invasive gene encoding matrix metalloproteinase-2 (MMP2). Our preliminary data showed that PAX6 also suppressed the expression of VEGF in GBM cell lines exposed to normoxic conditions after overexpression via adenoviral-mediated (Ad-PAX6) or stable transfection of PAX6. In this study, we investigated whether PAX6 affected the Akt-signaling pathway, through which the tumor suppressor PTEN was reported to suppress VEGF expression. **Method:** After infection of GBM cell lines (U251HF and U87) with Ad-PAX6, western blot assays were performed to monitor the phosphorylation status of Akt and GSK-3 β . **Results:** PAX6 did not alter the phosphorylation status of AKT or its downstream target GSK-3 β in U87 and

U251HF cells. This is in striking contrast to the effect shown after infection with Ad-PTEN, which dramatically suppressed phosphorylation of AKT and consequently abolished phosphorylation of GSK- β . *Conclusions*: PAX6-mediated suppression of VEGF in GBM cells does not

occur through the same mechanism as that of PTEN-mediated suppression. Moreover, co-overexpression of PAX6 and PTEN caused an additive suppression of VEGF. The mechanism(s) through which PAX6 suppresses VEGF in GBM has yet to be identified.