18th – 20th May 2010
Travemünde, Germany

The 18th International Conference on Brain Tumor Research and Therapy

Manfred Westphal (Hamburg, Germany)
Oliver Heese (Hamburg Germany)
Rolf Bjerkvig (Bergen, Norway)

Dear Guests of the 2010 Asilomar Conference.

Welcome Letter
Dear Guests of the 2010 Asilomar Conference.

After six years we welcome you back to Europe for the 18th International Conference for Brain Tumor Research and Therapy. To pay tribute to the founder`s idea of a somewhat secluded retreat where we can share our recent findings and ideas for future progress, we chose the old spa of Travemünde, although we would have been proud to have the conference in Hamburg itself. But the city of Hamburg is just too attractive and has so much to offer that it would have been too much of a distraction. Being here we will take the opportunity to visit one of the other major Hanse Cities, Lübeck, - the hometown of Thomas Mann and the place where marzipan was created. Being high up in the North we should be able to enjoy long daylight evenings.

The program is tight, thanks to the very positive response by everybody and there is a number of regulars to this conference who are very sad that other personal commitments kept them from attending this time around. In the tradition that everybody should present we have a program which leaves not much time in the sessions for discussion but ample free time to interact afterwards. For the first time we mixed pediatric and adult neuro-oncology because the fields can rather mutually influence each other than that they are separate.

We hope that you will enjoy the truly international atmosphere of the conference and the location which is one of the major gateways to Scandinavia. We also hope that with the contribution of everybody, we will be able to prove that the Asilomar Conference with its unique format is a vital forum for translational neuro-oncology and will continue to be so in the future when it will move next again to the Americas in two years and then to Asia in four years.

We wish you a pleasant stay in Northern Germany and foremost safe travels,

Manfred Westphal

ORGANISATION

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Venue

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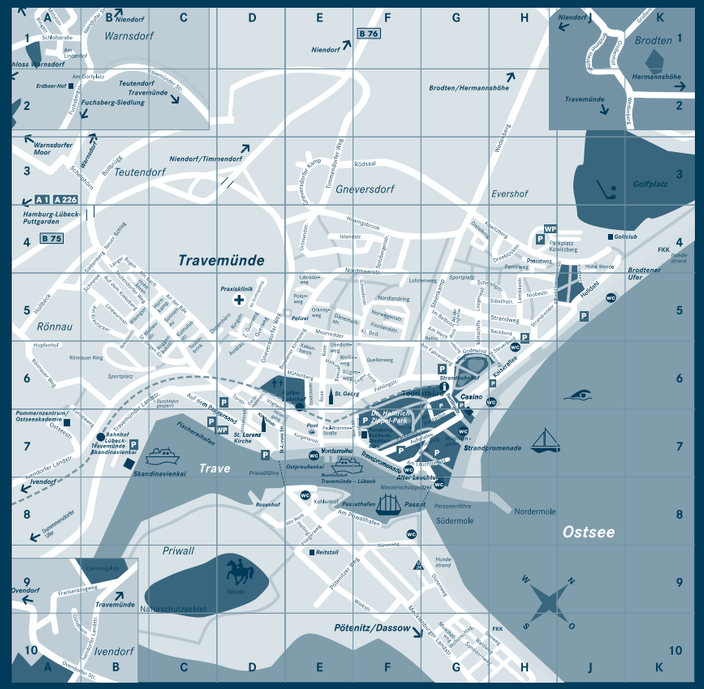
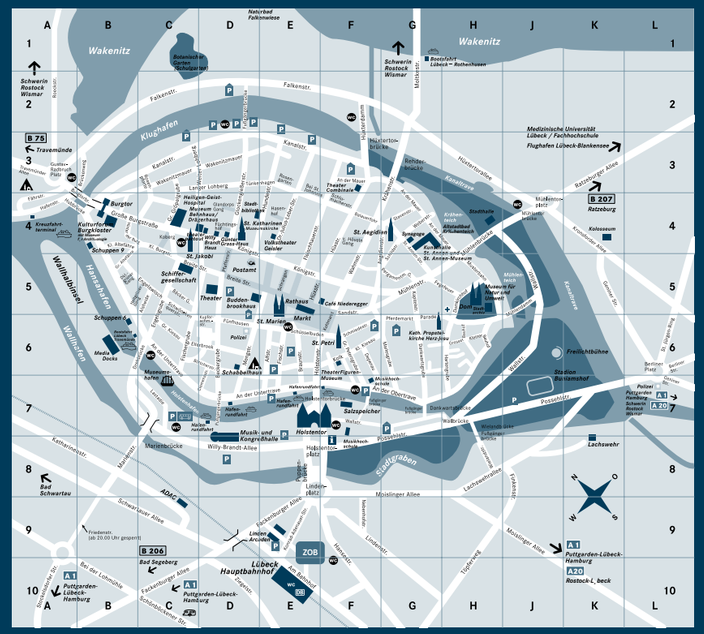


Travemünde



Lübeck

SOCIAL PROGRAM

17th May 2010
Previous day

19:00
Wellcome Reception, „Marktplatz“ Conference Hotel
(19:00 - 22:00)

18th May 2010
1st day

20:00
Barbeque Party, Conference Hotel
(20:00 - 23:00)

19th May 2010
2nd day

16:00
Boat trip to Lübeck, city tour
(16:00 - 22:00)

16:00
Departure Ostpreussenkai, Travemünde (5 min walking distance from hotel, see map)

18:00
Arrival in Lübeck Mediadocks/Behnkai guided tour Lübeck, old town

approx. 19:30
Departure Lübeck Mediadocks/ Behnkai, dinner buffet on the boat

approx. 22:00
Arrival Ostpreussenkai, Travemünde

20th May 2010
3rd day

19:00
Dinner Banquett, Conference Hotel
(19:00 - 22:00)

PROGRAM

17th May 2010

19:00
Welcome Reception

18th May 2010
1st day

8:20
Welcome
Manfred Westphal

8:30
Session I, Basic Science: Stem Cells and Signalling

10:30
Coffee Break

11:00
Session II, Molecular Classification Session I, Profiling

13:00
Lunch Break

14:00
Session III, Molecular Classification II, Clinical Correlates

15:30
Coffee Break

16:00
Session IV, Tumor Innitiating Cells

20:00
Pool Party

19th May 2010
2nd day

8:00
Session V, Basic Science: Angiogenesis/ Invasion

10:30
Coffee Break

11:00
Session VI, New Therapeutic Targets

13:00
Lunch Break

14:00
Session VII, New Therapeutic Approaches

16:00
Excursion to the City of Lübeck by Boat

20th May 2010
3rd day

8:00
Session VIII, Basic Science: Pathways/ Metabolism

9:40
Modelling Brain Tumors, Roundtable

10:30
Coffee Break

12:30
Session IX, Immunology/ Immunotherapy

13:30
Lunch Break

15:30
Session X, Clinical Neuro-Oncology

16:00
Coffee Break

16:30
Session XI
Clinical Trails

17:30
Closing Remarks
Manfred Westphal

19:00
Banquet

Session I, Basic Science: Stem Cells and Signalling

M. Westphal (Hamburg, Germany), H. Kettenmann (Berlin, Germany)

8:30 I-1 **A. L. Vescovi, E. Binda** (Milano, Italy)
Ephrins inhibit tumorigenicity in cancer stem cells of human glioblastomas

8:42 I-2 **K. Yoshikawa, I. Clarke, S. Pollard, M. Suzuki, A. Smith, P. Dirks** (Toronto, Canada)
Novel technique for establishment of glioblastoma stem cell line and pathological verification of distribution and differentiation stage of tumor cells on its xenograft

8:50 I-3 **F. F. Lang, A. Hossain, N. Shinojima** (Houston, USA)
Critical role of TGF-and TFG-receptors in the homing of mesenchymal stem cells to human gliomas

9:00 I-4 **A. Mukasa, J. Wykosky, K. L. Ligon, L. Chin, W. K. Cavenee, F. Furnari** (Boston, USA)
Mutant EGFR signaling is required for maintenance of enhanced in vivo glioblastoma growth and its ablation leads to escape by emergence of receptor-independent mechanisms

9:12 I-5 **N. O. Schmidt, K. Hansen, F. Zeigler, K. Lamszus, F. J. Mueller, M. Westphal** (Hamburg, Germany)
Development of a novel three-dimensional extracelluar matrix as a delivery system for the highly efficient intracerebral transplantion of glioma trageting neural stem cells

9:20 I-6 **J. Laterra, Y. Li, C. Eberhart , A. Quinones-Hinojosa** (Baltimore, USA)
C-met signaling supports a stem-like transcriptional program and phenotype in glioblastoma

9:28 I-7 **B. Scheffler** (Bonn, Germany)
Critical assessment of cellular heterogeneity in human glioblastoma

9:36 I-8 **R. Glass, S. R. Chirasani, A. Sternjak, P. Wend, S. Momma, B. Campos, M. Herrmann, C. Herold-Mende, D. Besser, M. Synowitz, H. Kettenmann** (Berlin, Germany)
Bone morphogenetic protein-7 release from endogenous neural precursor cells suppresses the tumorigenicity of glioma stem cells

9:44 I-9 **C. G. Eberhart, T. Pierfelice, K. Schreck, L. Dang, N. Gaiano** (Baltimore, USA)
Differing onocogenic potentials of notch 1, notch 2 and notch 3 in optic gliomas

9:56 I-10 **T. Muraguchi, T. Hoshii, T. Ooshio, K. Naka, A. Hirao** (Kanazawa, Japan)
Roles for ras signaling in neural stem cell homeostasis and malignant glioma progression

10:04 I-11 **R. Fukaya, S. Ohta, Y. Matsuzaki, Y. Kawakami, H. Okano, T. Kawase, M. Toda** (Keio, Japan)
Mif acts as a direct regulator of p53 in glioma cells and is a novel molecular target for brain tumor initiating cells

10:08 I-12 **A. Schulte, L. Krawinkel, H. Günther, S. Zapf, M. Wesphal, K. Lamszus** (Hamburg, Germany)
Modulation of tumour stem cell markers in glioma stem cell-enriched cell cultures by hypoxia

10:12 I-13 **K. M. Joo, J. Jin, B. G. Kang, D. S. Kong, S. J. Lee, Mi. Y. Jo, Y. G. Jin, Y. Kim, J. Muradov, D. H. Nam** (Seoul, Korea)
Neural stem cells prevent irradiation brain function losses by replacing damaged endothelial cells

10:16 I-14 **A. Sato, J. Sunayama, K. Matsuda, K. Tachibana, K. Sakurada, A. Tomiyama, T. Kayama, C. Kitanaka** (Yamagata, Japan)
Regulation of neural stem/progenitor cell maintenance by PI3K and MTOR
(Read only)

Session II, Molecular Classification: Profiling

P. Kleihues (Zurich, Switzerland), R. McLendon (Durham, USA)

11:00 II-1 **R. B. Jenkins, M. Wrensch, P. A. Decker, Y. Xiao, T. M. Kollmeyer, A. L. Rynearson, C. Halder, M. L. Kosel, D. H. LaChance, P. Yang, W. Joseph, W. John** (San Francisco, USA)
Germline polymorphisms in (or near) CDKN2A/B, RETL1, TERT, and CCDC26 are differentially associated with glioma grade and morphologic subtype

11:12 II-2 **K. Aldape, H. Noushmehr, D. J. Weisenberger, K. Diefes, H. S. Phillips, K. Pujara, B. P. Berman, F. Pan, C. E. Pelloski, E. P. Sulman, K. P. Bhat, R. G.W. Verhaak, J. G. Herman, S. B. Baylin, P. W. Laird** (Houston, USA)
Identification of a CPG island methylator phenotype that defines a distinct sub-group of glioma

11:24 II-3 **H. Yan, D. W. Parsons, G. Jin, R. McLendon, B. A. Rasheed, W. Yuan, I. Kos, I. Batinic-Haberle, S. Jones, G. J. Riggins, H. Friedman, A. Friedman, D. Reardon, J. Herndon, K. W. Kinzler, V. E. Velculescu, B. Vogelstein, D. D. Bigner** (Durham, USA)
IDH1 and IDH2 mutations in glioma development

11:36 II-4 **H. Phillips, S. Kharbanda, W. Pope, A. Tran, F. Peale, K. Pujara, R. H. Soriano, W. H. Yong, W. F. Forrest, S. Seshagiri, Z. Modrusan, K. Aldape, C. Escovedo, W. Chen, P. Nghiemphu, M. Prados, K. Lamzsus, M. Westphal, T. Cloughesy, A. Lai** (San Francisco, USA)
IDH1 mutation defines de novo glioblastomas with a distinct constellation of demographic, genetic, epigenetic, and phenotypic features

11:48 II-5 **V. P. Collins, K. Ichimura, L.M. Bäcklund, D. Pearson, D.T.W. Jones** (Cambridge, UK)
BRAF and IDH1/2 alterations in astrocytomas

11:56 II-6 **A. von Deimling** (Heidelberg, Germany)
Impact of IDH1 analysis on brain tumor classification and grading

12:04 II-7 **H. Ohgaki, S. Nobusawa, A. Wierinckx, J. Huang, P. Kleihues, J. Lachuer** (Lyon, France)
Inter-tumoral and intra-tumoral heterogeneities on genomic imbalance in glioblastomas

12:12 II-8 **A. Natsume, Y. Kondo, M. Ito, I. Takeuchi, M. Uesugi, S. U. Kim, J.P.J. Issa, T. Wakabayashi** (Nagoya, Japan)
Epigenetic plasticity regulated by polycomb repressive complex as a mediator of heterogeneity in gbm

12:20 II-9 **B. Radlwimmer, G. Toedt, S. Barbus, M. Wolter, J. Felsberg, B. Tews, F. Blond, M. C. Sabel, S. Hofmann, N. Becker, C. Hartmann, H. Ohgaki, A. von Deimling, O. D. Wiestler, M. Hahn, G. Reifenberger, P. Lichter** (Heidelberg, Germany)
tMolecular signatures classify astrocytic gliomas by IDH1 mutation status and reveal frequent rprm silencing in tp53 wild-type tumors

12:28 II-10 **C. Jones, B. S. Paugh, C. Qu, Z. Liu, M. Adamowicz-Brice, J. Zhang, D. A. Bax, B. Coyle, J. Barrow, D. Hargrave, J. Lowe, A. Gajjar, W. Zhao, A. Broniscer, D. W. Ellison, R. G. Grundy, S. J. Baker** (Surrey, UK)
Integrated molecular genetic profiling of paediatric high-grade gliomas reveals key differences with the adult disease

12:40 II-11 **S. Pfister, M. Remke, A. Benner, W. Werft, M. Ryzhova, H. Witt, W. Scheurlen, S. Rutkowski, A. Kulozik, A. von Deimling, G. Reifenberger, P. Lichter, A. Korshunov** (Heidelberg, Germany)
Medulloblastoma – individualizing therapy based on molecular diagnosis

12:52 II-12 **T. Pietsch** (Bonn, Germany)
Classification of medulloblastomas by histopathology and molecular signatures

Session III, Molecular Classification: Clinical Correlates

V. P. Collins (Cambridge, UK), M. Matsutani (Saitama, Japan)

14:00 III-1 **M. Weller** (Zurich, Switzerland)
Molecular markers predicting natural course and response to therapy in low-grade glioma: an update from the german glioma network

14:10 III-2 **M. J. van den Bent, H. J. Dubbink, A. A. Brandes, R. van Marion, J. M. Kros, W. N. M. Dinjens, P. Wesseling, Y. Marie, A. Idbaih, M. J. B Taphoorn, M. Frenay, D. Lacombe, T. Gorlia, M. Sanson** (Rotterdam, Netherlands)
IDH1 mutations are strongly associated with mgmt promoter gene methylation and are prognostic but not predictive for outcome to pcv chemotherapy in oligodendroglial tumors: a report from the randomized EORTC study 26951

14:20 III-3 **J. C. Tonn** (Munich, Germany)
Glioblastoma recurrence is not associated with changes in the MGMT promoter methylation status – a translational study of the german glioma network

14:28 III-4 **P. J. French, L. A.M. Gravendeel, M. C.M. Kouwenhoven, O. Gevaert, J. J. de Rooi, A. P. Stubbs, A. Daemen, B. De Moor, P. H.C. Eilers, P. J. van der Spek, J. M. Kros, P. A.E. Sillevis Smitt, M. J. van den Bent** (Leuven, Belgium)
Intrinsic gene expression profiles of gliomas are a better predictor of survival than histology

14:36 III-5 **M. D. Taylor, P. A. Northcott, A. Korshunov, H. Witt, T. Hielscher, C. Eberhart, S. Mack, E. Bouffet, S. C. Clifford, C. Hawkins, P. French, J. T. Rutka, S. Pfister** (Toronto, Canada)
Medulloblastoma comprises four diseases

14:48 III-6 **S. Rutkowski, A. O. von Bueren, S. C. Clifford, D.W. Ellison, R.D. Kortmann, B. Lannering, T. Pietsch** (Hamburg, Germany)
Improved stratification of children and adolescents with medulloblastoma by histological and biological parameters

Tuesday
18th May 2010

14:56 III-7 **P. Metellus, I. Nanni-Metellus, C. Delfino, C. Colin, B. Coulibaly, F. Fina, A. Loundou, M. Barrie, O. Chinot, L. Ouafik, D. Figarella-Branger** (Marseille, France)
Prognostic impact of cd133 mRNA expression in 61 glioblastoma patients treated with concomitant radiochemotherapy: a prospective patient cohort at a single institution.

15:04 III-8 **T. Fujimaki, N. Uemiya, G. Fushihara, H. Neki, K. Fukuoka, T. Suzuki, K. Wakiya, J. Adachi, T. Yanagisawa, K. Mishima, M. Matsutani, R. Nishikawa** (Saitama, Japan)
Soluble IL-2 receptor measure in central nervous system lymphoproliferative diseases

15:08 III-9 **Keisuke Ueki, Yoshifumi Okada, Hadzuki Matsuda, Phyo Kim Department of Neurosurgery, Dokkyo University School of Medicine, 880 Kitakobayashi** (Mibu, Japan)
Fish analysis on oligodendroglial and astrocytic component in oligoastrocytomas indicate 1p loss on top of 19q loss may be a molecular pathway.

15:12 III-10 **J. Adachi, R. Nishikawa, T. Hirose, M. Matsutani** (Saitama, Japan)
Methylation-sensitive high resolution melting analysis :a new quantitative assessment of MGMT promoter methylation in high-grade gliomas.

15:16 III-11 **J. A. Takahashi, M. Shirahata, S. Oba, K. Koizumi-Iwao, S. Saito, N. Ueno, S. Ishii, S. Miyamoto, K. Kato** (Kitano, Japan)
Clinical application of gene expression-based diagnostic system of gliomas

15:20 III-12 **Y. Sonoda, I. Shibahara, R. Saito, M. Kanamori, T. Kumabe, T. Tominaga** (Miyagi, Japan)
Analysis of IDH1 and IDH2 mutations in japanese glioma patients -correlation with other prognostic factors-

15:24 III-13 **H. Okada, K. Sasaki, G. Kohanbash, A. Hoji, R. Ueda, H. A. McDonald, T. A. Reinhart, M. T. Lotze, F. M. Marincola, E. Wang, M. Fujita** (Pittburgh, USA)
MIR-17-92 expression in differentiated t cells – implications for glioma immunotherapy

15:32 III-14 **Y. Muragaki, H. Iseki, T. Maruyama, M. Tanaka, C. Shinohara, T. Suzuki, K. Yoshimitsu, S. Ikuta, M. Hayashi, M. Chernov, T. Hori, Y. Okada, K. Takakura** (Tokyo, Japan)
Information-guided surgical management of gliomas using low-field-strength intraoperative MRI

Session IV, Tumor Innitiating Cells

R. Bjerkvig (Bergen, Norway), M. Weller (Zurich, Switzerland)

16:00 IV-1 **T. S. Jacques, A. Swales, M. J. Brzozowski, N. V. Henriquez, J. M. Linehan, Z. Mirzadeh, C. O'Malley, H. Naumann, A. Alvarez-Buylla, S. Brandner: presenting author** (London, UK)
Brain tumours can arise from stem/progenitor cells in the adult brain through activation of oncogenic pathways: evidence for pathway-specific tumour phenotype

16:12 IV-2 **V. Clément, D. Marino, C. Culdabu, M. F. Hamou, V. Mlynarik, N. den Tribolet, K. Schaller, P. Y. Dietrich, R. Gruetter, M. E. Hegi, I. Radovanovic** (Geneva, Switzerland)
Marker-independent identification of glioma-initiating cells

16:20 IV-3 **K. Lamszus, A. Schulte, H. S. Günther, H. S. Phillips, D. Kemming, M. Westphal** (Hamburg, Germany)
A distinct subset of glioma cell lines with stem cell-like properties reflects the transcriptional phenotype of human glioblastomas and overexpresses cxcr4 as therapeutic target

16:28 IV-4 **D. Zagzag, O. Méndez, J. Zavadil, Y. Lukyanov, D. Santovasi, S. C. Wang, E. W. Newcomb** (New York, USA)
Knock down of HIF-1a in glioma cells reduces migration in vitro and invasion in vivo and impairs their ability to form tumor spheres

16:36 IV-5 **G. Finocchiaro, F. Orzan, S. Pellegatta, V. Caldera, G. Villa, M. Eoli, M. Figus, F. Menghi, P. Tunici, C. Marras, D. Schiffer** (Milano, Italy)
Enhancer of ZESTE 2 (EZH2) is up-regulated in malignant gliomas and its targeting decreases cd133 expression

16:44 IV-6 **A. De Rosa, M. Rossi, S. Pellegatta, P. Tunici, F. Malusa, L. Magnoni, R. Raggiaschi, A. Bakker: presentino authors** (Siena, Italy)
A neural stem cells marker fatty acid binding protein 7 (fabp7) is involved in proliferation and invasion of glioblastoma cells

16:52 IV-7 **G. Zadeh, K. Burrell, A. Guha, R. Hill** (Toronto, Canada)
Imaging and role of bone marrow derived progenitor cells in intracranial tumor neovascularization

17:02 IV-8 **D. H. Nam, K. M. Joo, J. Jin, B. G. Kang, S. J. Lee, M.Y. Jo, Y. Kim, J. Muradov** (Seoul, Korea)
Personalized drug discovery based on cancer stem cell biology

17:10 IV-9 **E. P. Sulman, M. Guerrero, T. Mikkelson, V. Bonato, H. S. Phillips, S. Kharbanda, M. S. Berger, V. P. Collins, L. Petalidis, B. Broom, K. A. Do, V. Baladandayuthapani, K. Aldape** (Houston, USA)
A continuous mesenchymal expression signature is recapitulated in glioma stem cells and correlates with radiation and oncogenic pathway signatures

17:18 IV-10 **M. W. Kieran, S. Mehta, E. Huillard, D. H. Rowitch, A. Saad, C. D. Stiles, K. L. Ligon** (Boston, USA)
Remission and relapse in pediatric low grade astrocytoma: an oppositional relationship between stemness and p53?

17:26 IV-11 **T. Ohnishi, A. Inoue, H. Harada, S. Kohno, S. Ohue** (Ehime, Japan)
Characterization of glioma stem cells and its role in tumor invasion

Session V, Basic Science: Angiogenesis / Invasion

E. G. van Meir (Atlanta, USA), G. Reifenberger (Düsseldorf, Germany)

8:00 V-1 **K. Lu, J. Phillips, H. Liu, G.Bergers: presenting author** (San Francisco, USA)
Mechanisms of evasive resistance to VEGF inhibition in mouse models of glioblastoma

8:12 V-2 **E. G. Van Meir, S. M. Cork, E. M. Sandberg, B. Kaur, H. Mao, Z. Zhang, J. Olson** (Atlanta, USA)
A FURIN/MMP-14 signaling cascade processes BAI1 into a novel secreted anti-angiogenic and anti-tumorigenic factor, vasculostatin-40 (VSTAT40)

8:24 V-3 **K. H. Plate, M. R. Machein, Y. Reiss** (Frankfurt, Germany)
VEGF and angiopoietin signalling pathways regulate angiogenesis and myeloid cell infiltration in gliomas

8:36 V-4 **S. P. Niclou, O. Keunen, M. Johansson, A. Oudin, M. Sanzey, S. A. A. Rahim, T. Taxt, M. Bartos, J. Wang, F. Thorsen, R. Bjerkvig** (Luxembourg, Luxembourg)
Bevacizumab changes blood flow and increases cell invasion in glioblastoma xenografts

8:44 V-5 **W. Wick, P. N. Pfenning, A. L. Thiepold, L. Jestaedt, B. Berger, S. Combs, J. Gronych, L. Dittmann, M. Bendszus, M. Weiler** (Heidelberg, Germany)
Novel anti-invasive and anti-angiogenic mechanisms of MTOR inhibition in glioblastoma

8:52 V-6 **E. Galanis, K. A. Jaeckle, S. K. Anderson, T.J. Kaufmann, J. Uhm, C. Giannini, S. Kumar, D.W. Northfelt, P.J. Flynn, J. C. Buckner** (Rochester, USA)
NCCTG phase II trial of bevacizumab in combination with sorafenib in patients with recurrent glioblastoma multiforme

Wednesday
19th May 2010

9:00 V-7 **M. Czabanka, G.Parmaksiz, A. Palumbo, A. Ullrich, D. Neri, P. Vajkoczy: presenting author** (Berlin, Germany)
Vascular targeting strategies using F8-SIP antibody against the EDA domain of fibronectin in glioma angiogenesis

9:08 V-8 **C. S. Gondi, B. Gorantla, H. Raghu, D. H. Dinh, M. Gujrati, J. S. Rao: presentig author** (Peoria, USA)
Nuclear localization of UPA and its interaction with hoxa5 suppresses p53 expression in glioma cells and activates angiogenic response in endothelial cells

9:20 V-9 **H. Feng, B. Hu, K. Liu, A. Kazlauskas, K. S. Ravichandran, K. Vuori, R. Nishikawa, M. Nagane, S. Y. Cheng: presenting author** (Pittsburgh, USA)
PDGFRA stimulates glioma cell invasion through SRC-dependent tyrosine phosphorylation of a bipartite guanine nucleotide exchange factor dock180

9:28 V-10 **M. Nakada, J. R. Niska, E. Nambu, N. Furuyama, Y. Hayashi, D. Kita, Y. Hayashi, M. E. Berens, J. Hamada** (Kanazawa, Japan)
Ephrin-a2 modulates glioma cell invasion via EPHA2 signaling and correlates with patients survival in glioblastomas

9:36 V-11 **O. Sampetrean, M. Nakanishi, I. Saga, N. Onishi, E. Sugihara, H. Saya** (Tokyo, Japan)
Analysis of invasion patterns in a model of malignant brain tumor

9:44 V-12 **M. Synowitz, D. Markovic, K. Vinnakota, S.R. Chirasani, V. Matyash, S. Lehnardt, B. Kaminska, N. van Rooijen, F. Heppner, R. Glass, H. Kettenmann** (Berlin, Germany)
Glioma associated microglia facilitate tumor invasion due to tumor induced overexpression of mtI-MMP

9:52 V-13 **H. K. Lee, A. Ziv-Av, C. Xiang, S. Cazacu, S. Finniss, N. Giladi, M. Barda-Saad, C. Brodie: presenting author** (Detroit, USA)
RTVP-1 regulates glioma cell migration via interaction with N-WASP

10:00 V-14
S. H. Lee (Seoul, Korea)
Aberrant expression of microRNA-21 triggers diffuse infiltration of human gliomas by disrupting negative feedback regulator, SPRY2

10:08 V-15
F. Thorsen, J. Wang, I. Daphu, P. H. Pedersen, H. Miletic, R. Hovland, S. Mørk, R. Bjerkvig, C. Tiron, E. McCormack, D. Micklem, J. B. Lorens, H. Immervoll (Bergen, Norway)
Novel brain metastases models developed in immuno-deficient animals closely mimics the progression of metastatic brain tumors in patients

10:16 V-16
J. F. de Groot, Y. Piao (Houston, USA)
Macrophages promote glioma angiogenesis and invasion

10:20 V-17
S. Osuka, S. Takano, T. Yamamoto, E. Ishikawa, A. Matsumura (Tsukuba, Japan)
Valproic acid inhibits angiogenesis in vitro and in vivo from glioma cells.

Session VI, New Therapeutic Targets

A. Yung (Houston, USA), J. Sampson (Durham, USA)

11:00 VI-1
J. T. Rutka, O. Moreno, S. Nagai, C. Smith (Toronto, Canada)
The role of the cofiin pathway in human glioma migration and invasion

11:15 VI-2
K. Michaud, D. A. Solomon, E. Oermann, J. S. Kim, M. D. Prados, T. Ozawa, T. Waldman, C. D. James: presenting author (San Francisco, USA)
Pharmacologic inhibition of CDK4/6 suppresses the growth of glioblastoma multiforme in an intracranial xenograft model

11:24 VI-3
G. Reifenberger, F. Liesenberg, B. Malzkorn, M. Zapatka, B. Brors, M. Riemenschneider, M. Wolter (Düsseldorf, Germany)
Role of micro-RNA in glioma pathogenesis

11:36 VI-4
S. Agnihotri, C. Hawkins, W. L. Stanford, Abhijit Guha (Toronto, Canada)
Identifying novel tumor modifier genes involved in gliomagenesis using retroviral gene-trapping mutagenesis screens

11:48 VI-5
R. Jensen, D. Gillepsie, S. Ravichandran, Z. R. (Salt Lake City, USA)
Hypoxia inducible factor inhibition by nanoparticle delivered siRNA inhibits growth and angiogenesis in an intracranial xenograft mouse model

12:06 VI-6
C. Goetz, M. Shveygert, R. Walters, S. Lawson, E. Dobrikova, M. Dobrikov, M. Gromeier: presenting author (Durham, USA)
Targeting MAPK signaling in malignant glioma

12:14 VI-7
T. Joki, T. Arai, J. Fujigasaki, T. Abe (Tokyo, Japan)
MG132 exhibits potent anti-tumor activity in an animal model of malignant glioma when administered via thermoreversible gelation polymers.

12:22 VI-8 **H. Takeshima , T. Fukushima, K. Umezawa, H. Kataoka** (Miyazaki, Japan)
A novel NF-KB inhibitor DHMEQ reduces growth of human glioblastoma cells

12:30 VI-9 **B. Hasselbalch, J. G. Eriksen, H. Broholm, I. J. Christensen, K. Grunnet, M. R. Horsman, H. S. Poulsen, M. T. Stockhausen, U. Lassen** (Copenhagen, Denmark)
Retrospective immunohistochemical evaluation of high-grade glioma patients treated with bevacizumab and irinotecan, with or without cetuximab

12:34 VI-10 **A. J. Chalmers, D. A. Löser, F. A. Dungey, A. Shibata, P. A. Jeggo** (Brighton, UK)
Tumour-specific radiosensitization by inhibition of DNA repair pathways

12:38 VI-11 **A. Guha, S. Agnihotri, C. Hawkins, W. L. Stanford** (Toronto, Canada)
Novel role of neurofibromin in transport of RNA granules

12:42 VI-12 **K. Kurisu, T. Saito, F. Yamasaki, Y. Kajiwara, Y. Watanabe, K. Sugiyama** (Hiroshima, Japan)
Three tesla MRI perfusion image study on differential diagnosis among astrocytic and oligodendroglial tumors

12:46 VI-13 **Y. Hirose** (Toyoake, Japan)
Cyclin-dependent kinase inhibitor enhances temozolomide-induced cytotoxicity in human glioma cells by suppressing dna repair associated with g2 checkpoint

12:50 VI-14 **Authors: TBA**
EPH-a signalling in vitro and in vivo

Wednesday
19th May 2010

Session VII, New Therapeutic Approaches

S. Chang (San Francisco, USA), T. Ohnishi (Ehime, Japan)

14:00 VII-1 **K. S. Aboody** (Duarte, USA)
Neural stem cell-mediated cancer therapy: towards glioma clinical trials

14:12 VII-2 **G. Tabatabai, K. Hasenbach, C. Herrmann, F. Cay, B. Pichler, M. Jucker, C. Lengerke, M. Weller** (Zurich, Switzerland)
Cell-based therapies for malignant gliomas using hematopoietic progenitor cells?

14:20 VII-3 **K. S. Bankiewicz, R. A. LeCouteur, R. J. Higgins, J. R. Bringas, D. Yin, R. F. Larson, M. S. Berger, P. J. Dickinson** (San Francisco, USA)
Optimized convection-enhanced delivery platform: experience in canine spontaneous glioma and non-human primates

14:32 VII-4 **J. N. Bruce, B. Kennedy, C. Yanes, S. Sands, V. Seshan, S. Rosenfeld, A. Sonabend, R. Fine, A. Tannenbaum, K. Lopez, R. DeLaPaz, P. Canoll** (New York, USA)
Convection enhanced delivery of topotecan for gliomas

14:40 VII-5 **M. A. Vogelbaum, J. Palomo, S. Agarwal, J. Ohlfest, W. Elmquist** (Cleveland, USA)
Evaluation of drug delivery to brain tumors: grind and find is not enough

14:48 VII-6 **J. Fueyo, C. Gomez-Manzano, W.K. A. Yung, C. Conrad, F. Lang** (Houston, USA)
Continuous development of delta-24-RGD for the treatment of gliomas

14:56 VII-7 **Y. Ino, M. Takahashi, N. Saito, H. Ikushima, K. Miyazono, T. Todo** (Tokyo, Japan)
Oncolytic HSV-1 (g47Δ) efficiently kills glioblastoma-derived cancer stem-like cells

15:04 VII-8 **T. Todo, Y. Ino** (Tokyo, Japan)
Clinical development of a third-generation recombinant oncolytic HSV-1, g47Δ, for recurrent glioblastoma in japan

15:12 VII-9 **Y.-W. Kim, Ta. J. Liu, G. Powis, D. Koul, W. K. A. Yung** (Houston, Texas)
Identification of novel synergistic targets for rational drug combinations with PI3 kinase inhibitors using sirna synthetic lethality screening

15:20 VII-10 **M. Johansson, J. Utvik, M. Sanzey, J. Verhaagen, R. Bjerkvig, S. P. Niclou** (Luxembourg, Luxembourg)
Experimental glioblastoma growth is inhibited by secreted interstitial delivery of ectodomain neuropilin-1 bodies

15:28 VII-11 **F. Ali-Osman, T. Okamura, S. Singh, H. Friedman, D. Bigner** (Durham, USA)
GSTP1 tyrosine-phosphorylation by egfr mediates a novel mechanism of drug resistance in glioblastoma

15:36 VII-12 **S. Takano, T. Yamashita, R. Mashiko, S. Ohsuga, A. Matsumura, O. Ohneda** (Tsukuba, Japan)
SDF-1 and CXCR7 are key molecules for glioma angiogenesis and invasiveness

15:44 VII-13 **J. N. Sarkaria, M. A. Schroeder, B. L. Carlson, A. C. Mladec, G. J. Kitange** (Rochester, USA)
The influence of temozolomide dose-intensity on treatment efficacy in a panel of gbm xenografts

15:56 VII-14 **K. Mishima, K. Wakiya, T. Suzuki, J. Adachi, S. Ishihara, M. Matsutani, R. Nishikawa** (Saitama, Japan)
Chemo-immunotherapy with osmotic blood-brain barrier opeining for recurrent or refractory primary cns lymphoma

Session VIII, Basic Science: Pathways / Metabolism

K. Aldape (Houston, USA), H. Saya (Kumamoto, Japan)

8:00 VIII-1 **E. Holland** (New York,USA)
Stem-like cells and the perivascular niche

8:12 VIII-2 **F. Furnari, M. del Mar Inda, R. Bonavia, A. Mukasa, Y. Narita, D. W.Y. Sah, S. Vandenberg, C. Brennan, T. G. Johns, R. Bachoo, P. Hadwiger, P. Tan, R. A. DePinho10, W. Cavenee** (San Diego, USA)
Tumor heterogeneity in glioblastoma is an active process driven by a mutant egfr-induced paracrine circuit

8:24 VIII-3 **M. E. Hegi, A. C. Diserens, Y. Kamoshima, M. Kowenhoven, P. Bady, M. Delorenzi, W. L. Lambiv, M. F. Hamou, M. S. Matter, A. Merlo, F. L. Heppner, Y. Yonekawa, K. Frei, L. Mariani, S. Hofer** (Lausanne, Switzerland)
Pathway analysis of glioblastoma tissue after preoperative treatment with the egfr tyrosine kinase inhibitor gefitinib

8:36 VIII-4 **D. W. Fults, V. Coon, T. Laukert, K. Jin Kim, C. A. Pedone** (Salt Lake City, USA)
Molecular therapy targeting sonic hedgehog and hepatocyte growth factor signaling in a mouse model of medulloblastoma

8:44 VIII-5 **K. Latha, V. Chumbalkar, M. Li, Y. Hyeon Hwang, A. Gururaj, R. Maywald, S. Dakeng, L. Diao, K. Baggerly, R. Sawaya, W. Cavenee, F. Furnari, O. Bogler: presenting author** (Houston, USA)
Egfrviii translocates to the nucleus and regulates gene transcription with stat5

8:52 VIII-6 **R. O. Pieper, S. Fang, A. Feletti** (San Francisco, USA)
Reveral of temozolomide resistance by modualtion of a ubiquitin-controlled apoptotic pathway

9:00 VIII-7 **J. P. Steinbach, J. Rieger, D. P. Brucker, M. Weller, C. Nordhammer** (Frankfurt, Germany)
Synthesis of cytochrome c oxidase 2 (sco2): a p53-dependent metabolic regulator that promotes respiratory chain function and protects glioma cells from hypoxia-induced cell death

9:12 VIII-8 **A. M. Wolf, S. Agnihotri: presenting author, D. Munoz, C. Hawkins, A. Guha** (Toronto, Canada)
Developmental profile and regulation of the glycolytic enzyme hexokinase 2 and its association with aerobic glycolysis

9:16 VIII-9 **E. A. Chiocca, J. Godlewski, M. O. Nowicki, A. Bronisz, G. Nuovo, J. Palatini, M. De Lay, J. Van Brocklyn, M. C. Ostrowski, S. E. Lawler** (Colombus, USA)
Microrna-451 regulates lkb1/ampk signaling and allows adaptation to metabolic stress in glioblastoma

9:24 VIII-10 **C. Herold-Mende, B. Campos, F. S. Centner, L. Zeng, K. Dorsch, A. Unterberg** (Heidelberg, Germany)
Impact of differentiation resistance in gliomas

9:32 VIII-11 **A. Svendsen, J. Verhoeff, I.A. Netland, J. Brøgger, H. ImmervollÐ, J. Planagumà, A. Bohne Kjersem, P.Ø. Sakariassen, R. Bjerkvig, J.I. HeggdalÐ, P.Ø. Enger, K.J. Tronstad, W. Van Furth, M. Chekenya** (Bergen, Norway)
NG2/MPG promotes resistance to ionising radiation by elevated peroxiredoxin-1 and dna damage response in glioblastoma multiforme

9:40

Round Table: Modelling Brain Tumors

R. Bjerkvig (Bergen, Norway)

P. Burger (Baltimore, USA)
P. Kleihues (Zurich, Switzerland)
H. Kettenmann (Berlin, Germany)

10:30 IX-1 **V. Chandramohan, C. N. Pegram, S. T. Keir, J. Ayriss, S. E. Szafranski, H. Piao, I. H. Pastan, C.-T. Kuan, D. D. Bigner** (Durham, USA)
Dual-specific immunotoxin D2C7-(SCDSFV)-PE38KDEL for brain tumor therapy

10:42 IX-2 **A. T. Parsa, M. Aghi, B. Ahn, J. Bruce, N. Butkowski, K. Cachola, S. Chang, C. Crane, A. Federoff, S. Han, V. Kivett, M. W. McDermott, M. D. Prados, A. Sloan, T. Tihan, I. Yang, M. S. Berger** (San Francisco, USA)
Autologous Heat Shock Protein Vaccine for Patients with Newly Diagnosed and Recurrent Glioblastoma

10:54 IX-3 **A. B. Heimberger, J. Wei, L. Y. Kong, A. Wu, H. Colman, W. Priebe, F. F. Lang, R. Sawaya** (Houston, USA)
Targeting glioma tumorigenesis and immune suppression with p-STAT-3 blockade

11:06 IX-4 **J. H. Sampson, G. E. Archer, R. Schmittling, J. E. Herndon, A. Coan, D. A. Reardon, J. Vredenburgh, A. Desjardins, A. Dechkovskaia, S. McGehee-Norman, D. Lally-Goss, B. A. Perry, A. Friedman, H. S. Friedman, D. D. Bigner, D. A. Mitchell** (Durham, USA)
Interleukin-2 receptor A (IL-2R A/ CD25)-specific antibodies eliminate regulatory t-cells (t_{regs}) and enhances tumor-specific immune responses against cytomegalovirus (CMV) in the context of therapeutic temozolomide (TMZ)-induced lymphopenia in patients with glioblastoma (gbm)

11:18 IX-5 **C. T. Kuan, M. Cai, B. Choi, J. H. Sampson, D. D. Bigner** (Durham, USA)
A bispecific t cell-engaging antibody effectively eradicates EGFRVIII expressing glioblastoma multiforme

11:26 IX-6 **S. Izumoto, N. Hashimoto, T. Yoshimine, A. Tsuboi, Y. Oka, H. Sugiyama, N. Arita** (Osaka, Japan)
Clinical and immunological evaluation of WT1 (Wilms' tumor gene 1) peptide vaccination for patients with recurrent or refractory malignant brain tumors

11:34 IX-7 **G J Pilkington, S Birks, J Danquah, R Hitchman, L King, R. Vlasak, D Gorecki, A M Butt** (Portsmouth, UK)
GD3/GD3A as putative therapeutic targets for glioma

11:46 IX-8 **G. E. Archer, D. A. Mitchell, T. Davis, D. Deadett, J. E. Herndon, A. D. Coan, R. Schmittling, A. Dechkovskaia, D. A. Reardon, J. Vredenburgh, A. Desjardins, H. S. Friedman, A. Friedman, D. Lally-Goss, B. A. Perry, S. McGehee-Norman, D. D. Bigner, J. H. Sampson** (Durham, USA)
Elimination of regulatory t-cells alters the isotype and affinity index of the antigen specific humoral immune response following vaccination with CDX-110 in patients with EGFRVIII expressing gliomas x

11:54 IX-9 **Wang J., Poli A., Thuen M., Thorsen F., Løkka G., Brekke C, McCormack E., Brekken C., Fischer W., Bjerkvig R., Zimmer J., Enger P.Ø., Chekenya** (Bergen, Norway)
Therapeutic targeting of the NG2 proteoglycan with MAB 9.2.27 and adoptively transferred NK cells lyses human glioblastoma multiforme in vivo

12:02 IX-10 **Y. Kato, C. T. Kuan, J. Chang, M. K. Kaneko, R. E. McLendon, C. Pegram: presenting author, P. Fredman, J. E. Månsson, D. D. Bigner** (Durham, USA)
A high-affinity anti-3'-ISOLM1/3',6'-ISOLD1 IGG monoclonal antibody, GMAB-1, raised in lacto-series ganglioside-defective knockout mice

12:14 IX-11 **D. A. Mitchell, G. E. Archer, A. Dechkovskaia, R. Schmittling, H. S. Friedman, D. Lally-Goss, B. Perry, J. E. Herndon, S. McGehee-Norman, R. McLendon, D. D. Bigner,J. H. Sampson** (Durham, USA)
Adoptive cellular therapy targeting cmv antigens in patients with glioblastoma

12:22 IX-12 **U. Bogdahn** (Regensburg, Germany)
Stem cells, tumor stem cells, TGF-SS and treatment concepts

Thursday
20th May 2010

Session X, Clinical Neuro-Oncology

J. T. Rutka (Toronto, Canada), J. C. Tonn (Munich, Germany)

13:30 X-1 **S. Chang, S. Nelson, S. Cha, T. McKnight, J. Phillips, G. Bourne, C. Cloyd, R. Srinivasan, T. Jalbert, A. Elkhaled, K. Lamborn, M. Y. Polley, M. McDermott, A. Parsa, M. Aghi, M. Berger** (San Francisco, USA)
Correlation of physiologic and tissue characteristics for newly diagnosed and recurrent glioma using image guided biopsies

13:42 X-2 **L. Bello, E. Fava, G. Casaceli, F. Portaluri, A. Castellano, A. Falini** (Milano, Italy)
Motor mapping findings and correlation with DTI data during surgical removal of lesions involving motor areas or pathways

13:54 X-3 **Z. Ram** (Tel Aviv, Israel)
Intraoperative evaluation of the optic pathways using subcortical recordings, DTI tractography, and real-time 3-d ultrasound based navigation. a work in progress

14:06 X-4 **D. G. T. Thomas, D. Choi, J. Grieve, S. Brandner, T. Yousry, N Habib** (London, UK)
Development of the habib hexablate 10 radiofrquency ablation device for resection of brain tumours

14:14 X-5 **T. Kumabe, I. Shibahara, M. Kanamori, R. Saito, Y. Sonoda, M. Watanabe, R. Iwata, S. Higano, K. Takanami, Y. Takai, T. Tominaga** (Sendai, Japan)
Imaging of hypoxic lesion in glioma patients by PET with [18f]frp-170, a new 18f-labeled 2-nitroimidazole analog

14:22 X-6 **S.-I. Miyatak, M. Furuse, R. Hiramatsu, M. Fukumoto, N. Nonoguchi, S. Kawabata, T. Kuroiwa, M. Tsuji, M. Fukumoto, K. Ono** (Osaka, Japan)
Possible role of vascular endothelial growth factor in radiation necrosis in the brain-treatment strategy of radiation necrosis

14:30 X-7 **S. Nelson, S. Chang, S. Cha, N. Butowski, M. Prados, K Lamborn, M. Y. Polley, Y. Li, E. Essock-Burns, I. Khayal, L. Jilian, A. Elkhaled, T. Jalbert, C. Williams, M. Berger** (San Francisco, USA)
MR physiological and metabolic imaging for assessing response to therapy

14:38 X-8 **M. Wrensch, R. B. Jenkins, J. S. Chang, R. F. Yeh, Y. Xiao, P. A. Decker, K. V. Ballman, M. Berger, J. C. Buckner, S. Chang, C. Giannini, C. Halder, T. M. Kollmeyer, M. L. Kosel, D. H. LaChance, L. McCoy, B. P. O'Neill, J. Patoka, A. R. Pico, M. Prados, C. Quesenberry, T. Rice, A. L. Rynearson, I. Smirnov, T. Tihan, J. Wiemels, P. Yang, J. K. Wiencke** (San Francisco, USA)
Variants in the CDKN2B and RTEL1 regions are associated with high-grade glioma susceptibility

14:46 X-9 **R. Nishikawa, K. Mishima, J. I. Adachi, M. Matsutani** (Saitama, Japan)
Treatment of central nervous system lymphomas with temozolomide – preliminary results with mgmt promotor methylation status

14:52 X-10 **S. Goldman, J.-S. Lai** (Chicago, USA)
Development of a pediatric perceived cognitive function (pedspcf) instrument for children with cns tumors

15:02 X-11 **E. B. Claus, L. Calvocoressi, M. L. Bondy, J. M. Schildkraut, J. L. Wiemels, P. M. Black, M. Wrensch** (Boston, USA)
Family history and meningioma risk

15:10 X-12 **Y. Narita, Y. Miyakita, M. Ohno, S. Shibui** (Tokyo, Japan)
Clinical manifestation of dissemination in glioblastomas

15:14 X-13 **K. Sugiyama, R. Nishikawa, H. Takeshima, H. Nakamura, T. Aoki, J. A. Takahashi, H. Takahashi, A. Saitoh, Y. Sawamura, K. Kurisu, M. Matsutani** (Hiroshima, Japan)
Safety of radiotherapy plus concomitant and prolonged-term adjuvant temozolomide for glioblastoma in japan – a japanese multicenter phase ii clinical study

Thursday
20th May 2010

15:18 X-14 **S. Kohno, S. Ohue, Y. Kumon, T. Ohnishi** (Ehime, Japan)
Usefulness of methyl-[11c]-l-methionine pet in the extensive tumor resection in glioma

15:22 X-15 **H. Sasaki, Y. Hirose, M. Katayama, K. Yoshida, T. Ohira, T. Kawase** (Tokyo, Japan)
Neoadjuvant approach for gliomas

Session XI, Clinical Trials

M. van den Bent (Rotterdam, Netherlands), W. Wick (Heidelberg, Germany)

16:00 XI-1 **M. Prados, K. Lamborn, T. Cloughesy, P. Wen, W.K.A. Yung, S. Chang, I. Mellinghoff, J. Kuhn, C. Stiles, T. Batchelor, K. Ligon** (San Francisco, USA)
An early phase clinical trials consortium

16:12 XI-2 **M. R. Gilbert, M. Wang, K. Aldape, A. G. Sorensen, T. Mikkelsen, F. Bokstein, S. Y. Woo, S. J. Chmura, A. K. Choucair, M. Mehta** (Houston, Texas)
RTOG 0625: a randomized phase ii trial of bevacizumab with either irinotecan (CPT) or dose-dense temozolomide (TMZ) in recurrent glioblastoma (GBM)

16:24 XI-3 **D. A. Reardon, S. Sathornsumetee, A. Desjardins, J. J. Vredenburgh, R. E. McLendon, J. Marcello, J. E. Herndon II, A. Mathe, M. Hamilton, J. Norfleet, S. Gururangan, A. H. Friedman, H. S. Friedman** (Durham, USA)
Phase II trial of bevacizumab and erlotinib in patients with recurrent malignant gliomas

16:36 XI-4 **P. H. Gutin, L. E. Abrey, K. Beal, A. Lassman, A. M. Omuro** (New York, USA)
Safety and efficacy of bevacizumab with hypo-fractionated radiotherapy in malignant gliomas-a sensitizer and a protector?

Thursday
20th May 2010

16:44 XI-5 **O. Heese, C. Senft, C. Braun, T. Pietsch, M. Warmuth, F. Bach, M. Westphal** (Hamburg, Germany)
Phase III anti-EGF-receptor antibody (OSAG-101) for newly diagnosed glioblastoma: safety and current status

16:52 XI-6 **M Westphal, Z Ram, P Warnke, P Menei, S Yla-Herttuala** (Hamburg, Germany)
Efficacy and safety results from the aspect study : gene therapy for operable high-grade glioma with herpes simplex virus-thymidine kinase (ADV.HSV-TK) gene therapy and ganciclovir; a phase III, randomized trial of 250 patients

17:00 XI-7 **L. Gore, P. G. Fisher, R. Hayashi** (Baltimore, USA)
Phase I trial of arsenic trioxide chemoradiotherapy in the treatment of infiltrating astrocytomas of childhood

17:08 XI-8 **T. Aoki, T. Mizutani, K. Nojima, T. Takagi, R. Okumura, Y. Yuba, T. Ueba, J. A. Takahashi, S.-I. Miyatake, K. Nozaki, W. Taki, M. Matsutani** (Osaka, Japan)
Phase II study of ifosfamide, carboplatin and etoposide for patients with glioblastoma at first relapse

17:12 XI-9 **Asai, H. Oshige, T. Uesaka, S. Yanagisawa, J. I. Takeda, K. Yoshimura, K. Kawamoto** (Osaka, Japan)
Clinical trial with temozolomide and interferon-beta in an alternating weekly regimen against recurrent malignant gliomas-a preliminary report

17:16 XI-10 **M. Nagane, K. Kobayashi, Y. Shiokawa** (Kyorin, Japan)
Adjuvant chemotherapy with distinct modes of action for temozolomide-refractory high grade glioma

ABSTRACTS

18th May 2010
1st day

19th May 2010
2nd day

20th May 2010
3rd day

I-1 Ephrins inhibit tumorigenicity in cancer stem cells of human glioblastomas

Angelo L. Vescovi and Elena Binda
Università degli Studi Bicocca-Millan, Milano, Italy

Brain tumors, particularly glioblastomas, embody cells endowed with tumor-initiating capacity and the defining functional features of neural stem cells. These are tentatively called cancer stem cells and, upon orthotopic transplantation into immunosuppressed animals, establish faithful phenocopies of the original human lesion. We have found that key regulatory systems, which play pivotal roles in normal neural stem cell physiology – namely bone morphogenetic proteins – also regulate the tumorigenic ability of cancer stem cells from human glioblastomas (GBSCs). Hence, the study of regulatory mechanisms of normal neurogenesis may lead to the identification of novel inhibitors of GBSCs and may result in the development of novel and more specific therapeutic strategies for brain cancers. The Eph family of receptor tyrosine kinases and their ligands, called ephrins, regulate a wide array of physiological processes, including neurogenesis within the adult brain stem cell niche. Notably, deregulated activity in this system has been linked to the development of many types of human cancers. We have now found that the mRNA for receptors and ligands of the ephrin pathway are found in primary GBM tissues and in the GBSCs therein. Both, by RT-PCR, western blotting and FACS analysis, we observed that some specific Eph-receptors and their cognate ligands were systematically over-expressed in 5 out of the 5 GBM specimens (both primary and GBSCs) investigated. Stimulation by ligands in vitro led to receptor down-regulation, activation of the appropriate intracellular transduction pathways, loss of both proliferation, clonogenicity and self-renewal capacity and to thedepletion of the GBSCs pool. Increased apoptosis and differentiation were documented to underpin this phenomenon which, in turn, resulted in the loss of tumorigenic ability of GBSCs. In fact, transient treatment of GBSCs with ephrin ligands, significantly hindered the ability of these cells to initiate tumours in vivo. Treating pre-etablished tumors with ephrin ligands produced a major drop of their growth in vivo. This confirms that some key regulatory systems of adult neurogenesis are retrieved in GBSCs and that their modulation can be used to reduce tumorigenic potential. in a specific fashion, thus paving the way to development of new candidate approaches for the cure of human gliomas.

I-2 Novel technique for establishment of glioblastoma stem cell line and pathological verification of distribution and differentiation stage of tumor cells on its xenograft.

Koichi Yoshikawa[1,2], Ian Clarke[2], Steven Pollard[3], Michiyasu Suzuki[1], Austin Smith[3], Peter Dirks[2] [1] Department of Neurosurgery, Yamaguchi University School of Medicine, [2] Arthur and Sonia Labatt Brain Tumor Research Center, Program in Developmental and Stem Cell Biology, The Hospital for Sick Children, University of Toronto, Canada, [3] Wellcome Trust Centre for Stem Cell Research Centre and Department of Biochemistry, University of Cambridge, UK

Introduction: We successfully apply the monolayer culture method to establish glioma stem cell lines from surgical samples(Cell Stem Cell 4(6):568-80,2009). We present our novel technique for establishment of GBM stem cell line and some particular feature of its xenografts.
Materials and Methods : We used 3 glioblastoma surgical samples. We cultured cells from each sample on monolayer methods with EGF/FGF without serum. After conformation every cell lines fulfill the criteria of glioma stem cell, we inject cells into NOD/SCID mouse brain and observed the pattern of invasion and differentiation of tumor cells pathologically on its xenograft.
Results :We established 3 GBM stem cell lines from 3 samples successfully. Every lines fulfill the criteria of glioma stem cell. They express stem cell markers such as Sox2, Nestin and have ability of multilineage differentiation. All lines have tumor initiating ability in vivo. Although ratio of CD44 expression is almost 100% in all lines, interestingly ratio of CD133 expression varied as follows; 96.5%, 1.4%, 43.6%. Xenograft have glioblastoma specific feature such as necrosis/pseudopallisading. The undifferentiated GFAP(-)Nestin(+) tumor cell showed strong invasive capacity, invaded contralaterally even in the early stage. Undifferentiated GFAP(-)Nestin(+) tumor cell favored invasion to subventricular zone (stem cell niche). In all xenogarft, invasive front is formed by undifferentiated GFAP(-)Nestin(+) tumor cell. The higher CD133 expressed line has the stronger invasive capacity. Lower CD133 line formed somewhat well margined mass. Differentiated GFAP(+) tumor cell remained injection site and expanded and formed tumor bulk.
Conclusions: The novel monolayer culture method is useful to establish glioma stem cell line and provides enough chances to study glioma stem cell from surgical sample. The xenografts of GBM stem cell lines recaptured the pathological feature of original sample patient. Undifferentiated stem-like tumor cell have strong invasion ability and formed invasive front of tumor.

I-3 Critical role of TGF-B and TFG-B-receptors in the homing of mesenchy mal stem cells to human gliomas

Frederick F. Lang, Anwar Hossain, Naoki Shinojima
Department of Neurosurgery and Brain Tumor Center, The University of Texas MD Anderson Cancer Center.

Introduction: Human mesenchymal stem cells (hMSCs) are capable of targeting and delivering therapeutic agents to gliomas after systemic administration. Endogenous MSCs also have a tropism for brain tumors and may contribute to glioma angiogenesis. Despite the increasing importance of hMSCs in glioma biology and therapy, the mechanism underlying the tropism of hMSCs for gliomas remains unclear. TGF-b is a factor expressed by gliomas that has multiple tumor-related functions. We hypothesized that TGF-b mediates the attraction of hMSCs for gliomas.
Methods and Results: Elisa assays demonstrated that U87 and U251 gliomas secrete high levels (0.7+0.04ng/ml) of TGF-b, whereas LN229 secrete low levels (0.1+0.01ng/ml). In vitro Matrigel invasion assays showed that significantly more hMSCs migrated toward conditioned media (CM) of U87 cells (42+1 hMSCs/10hpf) compared with LN229 (26+2 hMSCs/10hpf) ($P<0.01$). Treatment of CM from U87 or U251 cells with anti-TGF-b-neutralizing antibody attenuated hMSC migration ($p<0.01$). Pretreatment of hMSCs with TGFß-Receptor kinase inhibitor or knockdown of TGFb Receptor II using shRNA significantly reduced hMSC migration compared with control-treated hMSCs. To demonstrate that role of TGF-b in vivo, TGF-b was stably knocked-down in U87 cells using lenti-shRNA technology (generating U87-TGF-neg). Intracranial xenografts of U87-control (N=3) or U87-TGF-neg (N=3) were established in the frontal lobes of nude mice. After seven days, tumor-bearing mice were injected with gfp-labeled-hMSCs into the carotid artery and sacrificed 3 days later. The average number of hMSCs in U87-TGF-neg tumors (7.7±1.7 cell/mm2) was significantly less than that of the U87-controls (26.1±5.7 cell/mm2, $P<0.0002$). To assess the role of TGFb-receptors, gfp-labeled-hMSCs were stably transduced with lentivirus containing shRNA against TGFbRII (N=6), with control lentivirus (N=4), or with media (N=4) and injected into animals harboring U87-xenografts. hMSCs with knock-down of TGFß RII showed significantly less homing to U87 xenografts (3.8±0.6 cells/mm2) compared with control-treated hMSCs (22.1±3.5 cells/mm2, $P<0.01$). Likewise, pretreatment of hMSCs with TGFß-receptor kinase inhibitor (N=3) reduced MSC tropism by 50% compared with untreated MSCs (N=3).
Conclusion: Tumor-derived TGF-b mediates the homing of hMSCs for gliomas in vitro and in vivo by activating TGFb-receptors on hMSCs. These findings can be exploited for advancing hMSC-based treatments.

I-4 Mutant EGFR signaling is required for maintenance of enhanced in vivo glioblastoma growth and its ablation leads to escape by emergence of receptor-independent mechanisms

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Introduction: Epidermal growth factor receptor (EGFR) gene amplification occurs in approx. 40 % of all glioblastomas. A truncated extracellular domain mutant form, DEGFR (also known as EGFR*, de2-7EGFR or EGFRvIII), which is found in approximately 20-30 % of human glioblastomas, is the most commonly occurring mutant form and is shown to confer enhanced tumorigenecity. DEGFR has been used as a tumor-specific drug target in kinase inhibitor and antibody-based therapies, however, it has not been established as being required for tumor maintenance despite its importance for such therapeutic approaches. Moreover, recent experience with kinase inhibitor therapy indicates that tumors will eventually grow through the therapy, either because of desensitizing mutations in the kinase itself or through the activation of alternate oncogenic pathways.
Material and Methods: To address these issues, we developed a tet-regulatable system, in which DEGFR expression levels in tumor cells inoculated subcutaneously into nude mice are controlled by doxycycline-containing drinking water administered to mice. The cell line chosen for this approach is the human glioblastoma cell line, U373MG, which we have previously shown requires DEGFR for xenograft establishment.
Results: In this model, we have shown that suppression of DEGFR attenuates tumor growth and thereby is important for tumor maintenance. Similar to the clinical experience with kinase inhibitors, after a period of stasis, tumors eventually regain aggressive growth without re-expression of ΔEGFR. In order to determine how the tumors acquired this ability, we used gene expression microarrays and found that a novel gene, referred to as SDE (Substitute for DEGFR Expression)-1 is highly expressed in tumors in which DEGFR had been suppressed and thus have escaped the need for DEGFR to maintain tumor growth. SDE-1 is also expressed in human GBMs and knockdown of this gene's expression in our derived DEGFR -independent tumors suppressed tumor growth.
Conclusions: Taken together, we conclude that ΔEGFR is required for both tumor establishment and maintenance, and that gliomas undergo selective pressure in vivo to employ alternative compensatory pathways to acquire aggressiveness. Such alternative pathways function as substitutes for ΔEGFR signaling in vivo and should therefore be considered as potential targets for additional therapy.

I-5 Development of a novel three-dimensional extracelluar matrix as a delivery system for the highly efficient intracerebral transplantion of glioma trageting neural stem cells

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Introduction: Neural stem cells (NSC) display inherent pathotropic properties that can be exploited for targeted delivery of therapeutic genes to invasive malignancies in the central nervous system. Optimizing transplantation efficiency will be essential for developing relevant cell-based brain tumor therapies. To date, the real-world issue of handling and affixing NSC in the context of the neurosurgical resection cavity has not been addressed. Stem cell transplantation using biocompatible devices is a promising approach to counteract poor NSC graft survival and integration in various types of neurological disorders.
Materials and Methods: Here, we report the development of a three-dimensional substrate which is based on extracellular matrix purified from tissue-engineered skin cultures (3DECM).
Results: 3DECM enables the expansion of embedded NSC in vitro while retaining their uncommited differentiation status. When implanted in intracerebral glioma models NSC were able to migrate out of the 3DECM to targeted glioma growing in the contralateral hemisphere and this was more efficient than the delivery of NSC by intracerebral injection of cell suspensions. Direct application of a 3DECM implant into a tumor resection cavity led to a marked NSC infiltration of recurrent glioma. The semisolid consistency of the 3DECM implants allowed simple handling during the surgical procedure of intracerebral and intracavitary application, and ensured continuous contact with the surrounding brain parenchyma.
Conclusion: Here, we demonstrate proof-of-concept of a matrix-supported transplantation of tumor-targeting NSC. The semisolid 3DECM as a delivery system for NSC has the potential to increase transplantation efficiency by reducing metabolic stress and providing mechanical support especially when administered to the surgical resection cavity after brain tumor removal.

I-6 C-MET signaling supports a stem-like transcriptional program and phenotype in glioblastoma

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Introduction: Glioblastoma and other solid malignancies contain neoplastic stem-like cells (GBM-SCs) that resist treatment and efficiently propagate tumor growth. The development of therapies that target the small but important pool of GBM-SCs requires a more complete understanding of the molecular mechanisms that regulate the GBM-SC phenotype. The receptor tyrosine kinase c-Met and its ligand HGF support the formation, malignant progression, dissemination, and therapeutic resistance of numerous cancers including GBM. This current work identifies a role for c-Met signaling in maintaining the neoplastic stem-like cell phenotype.
Material and Methods: Human glioblastoma-derived neurospheres were established from human surgical specimens and from human GBM xenograft lines and maintained in neural stem cell medium lacking serum and containing EGF and FGF. C-Met expression was determined by flow cytometry, qRT-PCR and immunoblot analyses. Nestin, CD133, SoX2, KLF4, and c-Myc expression was determined by qRT-PCR. MAPK, AKT, and STAT3 activation was determined by immunoblot and immunoflourescence.
Results: C-Met was constitutively expressed at variable levels in all of nine human GBM neurosphere isolates examined, including established neurosphere cell lines, low passage primary neurospheres, and neurospheres derived from GBM xenograft lines. C-Met was found to support the neurosphere forming capacity of GBM-SCs and neurosphere cell growth as evidenced by their augmentation by exogenous HGF or their inhibition by the c-Met kinase inhibitor SU11274. CD133+ neurosphere cells expressed 10-fold higher levels of c-Met compared to CD133- cells and c-Met inhibition depleted neurospheres of CD133+ cells. High level c-Met expression was also associated with elevated levels of Nestin and SoX2 expression. C-Met activation by HGF induced AKT, MAPK and STAT3 activation and induced the expression SoX2, KLF4, and c-Myc in neurosphere cells.
Conclusions: Together, these findings establish for the first time a link between c-Met expression/function and the neoplastic stem-like molecular fingerprint and phenotype in GBM-derived cells. The findings suggest that c-Met inhibitors may have a role in the development of therapeutic strategies for targeting tumor-initiating stem-like cells in malignant glioma and other solid cancers.

I-7 Critical assessment of cellular heterogeneity in human glioblastoma

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Even though heterogeneity of tumor cells is generally viewed as a quintessential feature of glioblastoma (GBM), very little information is available on the extent, the specifics, and the consequences of this diversity. To elucidate the presence of cellular heterogeneity, we have investigated a series of pediatric and adult GBM tissue specimens applying classic and novel methods for the isolation and observation of adult neural stem cells in vitro and in orthotopic xenograft environments. In a first series of experiments, we clonally expanded a variety of single GBM cells under controlled, adhesive culture conditions for comparative genomic, transcriptomic, and functional analysis. In a second set of experiments, we compared multiple biopsies derived from distinct sites of the tumor center and periphery. Both experimental paradigms revealed unique and co-existing GBM cell phenotypes. Data will be presented with a particular emphasis on current models of tumorigenesis and the need of single cell analysis to optimize diagnosis and treatment of GBM. Supported by the Lichtenberg Program of the VW Foundation.

I-8 Bone morphogenetic protein-7 release from endogenous neural precursor cells suppresses the tumorigenicity of glioma stem cells

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Glioma cells with stem-like properties (GSCs) control tumor growth and recurrence. Here, we show that endogenous neural precursor cells (NPCs) perform an anti-tumor response by specifically targeting GSCs: In vitro, NPCs predominantly express BMP7; BMP7 is constitutively released from neurospheres and induces canonical BMP signaling in GSCs. Exposure of human and murine GSCs to neurosphere-derived BMP7 induces GSC differentiation, attenuates GSC-marker expression, GSC self-renewal and the ability for tumor initiation. NPC-derived BMP or recombinant BMP7 reduces glioma expansion from GSCs by down-regulating the transcription factor Olig2. In vivo, large numbers of BMP7-expressing NPCs encircle glioma in young mice and induce canonical BMP signaling in GSCs. This anti-tumor response is strongly reduced in older mice. Our results indicate that NPCs protect the young brain from glioma by releasing BMP7, which acts as a paracrine tumor suppressor that represses proliferation, self-renewal and tumor-initiation of GSCs.

I-9 Differing onocogenic potentials of notch 1, notch 2 and notch 3 in optic gliomas

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Introduction: While Notch signaling has been widely implicated in tumor growth, direct evidence for in vivo initiation of neoplasia by the pathway is limited.
Materials and Methods: To examine tumorigenic potential of Notch signaling in the brain, we injected retrovirus encoding activated forms of the Notch1, 2 and 3 receptors into the ventricles of embryonic day 9.5 mice under ultrasound guidance.
Results: Proliferative lesions expressing GFAP and nestin were identified in the retina and optic nerve in the majority of animals injected with constitutively active Notch3. The tumor cells appeared bland, with fibrillar cytoplasm, but they contained mitotic figures and invaded extensively into periocular tissues. Activated Notch3 was approximately ten-fold more efficient in inducing glial tumors in the retina and optic nerve than Notch1 or Notch2. Because optic nerve gliomas are predominantly pilocytic, we examined Notch3 levels in a number of Grade I astrocytomas. Overexpression of Notch3 mRNA was identified in 2 of 3 human optic pathway pathway gliomas, and in 7 of 19 pilocytic astrocytoma arising at other sites. While signs of Notch activation have been documented in human malignant gliomas, in our model introduction of active Notch receptors into the brain never induced glial tumors – a distinct contrast with findings in the optic nerve and eye.
Conclusions: Our results demonstrate the ability of Notch3 to efficiently induce gliomas in the optic nerve and eye, highlighting the distinct potentials of Notch receptor paralogs with respect to tumor initiation. They also suggest that glial precursors optic nerve, but not the brain, are susceptible to transformation by Notch3.

I-10 Roles for RAS signaling in neural stem cell homeostasis and malignant glioma progression

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Introduction: Recent evidences have demonstrated that neural stem/progenitor cells (NPCs) are one of the origins of malignant gliomas and activated Ras signaling is involved in the tumorigenesis originated from NPCs. However, molecular pathway regulating the NPC homeostasis as an anti-cancer response to this oncogenic signaling remains to be elucidated. To directly understand the in vivo responses to Ras activation in NPCs, we investigated effects of the conditional expression of an endogenous Kras(G12D) allele in NPCs by a tamoxifen-inducible CreER system.
Material and Methods: We generated Nestin-CreERT2; LSL-KrasG12D (NK) mice, in which the expression of constitutively active Kras is induced by the endogenous Kras promoter, and temporally controlled in NPCs by a tamoxifen-inducible CreER system. We treated NK mice with tamoxifen at several time point of age and analyzed their brain tissue.
Results: Administration of tamoxifen in vivo resulted in suppression of NPC proliferation in subventircular zone (SVZ) and profound defect in brain development, associated with enlarged lateral ventricle. Neither apoptosis nor senescence was observed in the brain of NK mice. The growth suppression was not rescued by loss of p53 or Ink4a/Arf, which are involved in apoptosis and senescence pathway. In contrast to the observation in vivo, constitutive Ras activation induced by tamoxifen treatment stimulated proliferation of NPCs in vitro, consistent with previous reports using MEFs.
Conclusion: The inhibition of NPC proliferation associated with Ras activation is not due to apoptosis or cellular senescence. Constitutive Ras activation in NPCs leads to defective stem cell function associated with growth arrest only in vivo. These data suggest a possibility that environmental factors in combination with oncogenic signals contribute to protect stem cells from tumorigenesis and malignant progression by forced growth arrest in vivo.

I-11 MIF acts as a direct regulator of p53 in glioma cells and is a novel molecular target for brain tumor initiating cells

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Introduction: Recent evidence has suggested that, in several types of human cancer including glioma, only a phenotypic subset of cancer cells called cancer stem cells (CSCs) or tumor initiating cells (TICs) within each tumor is capable of self-renewal and gives rise to tumor. This theory emphasizes the importance of developing effective therapeutics directed towards CSC/TICs because CSC/TICs are origin of the resistance for any therapy including surgical resection, chemotherapy and radiation in cancers.
Macrophage migration inhibitory factor (MIF) is known to be a pleiotropic protein that involves the innate and adaptive immune systems and inflammatory responses, as well as tumorigenesis. Recent studies have suggested a potentially broader role for MIF in growth regulation because of its ability to antagonize p53 functionally. However, the suppression mechanism of p53 by MIF is not well-known.
Material and Methods: We cultured the glioma cells from 24 patients using the sphere formation method and obtained non-brain tumor initiating cells (NBTICs) and brain tumor initiating cells (BTIICs) in mouse xenograft studies. To identify the key molecule in tumorigenesis in glioma, we compare the gene expression of these two types of glioma cells and analyzed the function of the over-expressed gene in BTICs.
Results: MIF was over-expressed in the BTICs compared to NBTICs and neural stem cells in quantitative PCR study and knockdown of MIF by shRNA significantly inhibited cell proliferation inducing severe apoptosis in BTICs. Therefore we analyzed the function of MIF in glioma cells using two glioma cell lines, U87MG (wild type p53) and T98G (mutated p53). MIF knockdown markedly inhibited cell growth of both cells as well as BTICs and we demonstrated that MIF directory bound to p53 and regulated cell growth though p53 dependent pathway in each cells. MIF bound to p53 in the nuclei of U87MG and modulated mainly cell cycle arrest and apoptosis by transcription dependent p53 pathway. Meanwhile, MIF also bound to p53 in cytoplasm in T98G and regulated only apoptosis by transcription independent p53 pathway.
Conclusion: These results illustrate that MIF acts as a direct regulator of p53 in the glioma cells and has critical roles in anti-apoptosis and cell growth. Furthermore, these results strongly suggest that MIF could be a novel therapeutic target for BTICs.

I-12 Modulation of tumour stem cell markers in glioma stem cell-enriched cell cultures by hypoxia

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Introduction: Tumor stem cells are believed to be located in certain niches where they are able to escape conventional treatment such as chemo- or radiotherapy. These niches provide a special environment contributing factors that favor a stem cell phenotype, one of which is hypoxia. Severe hypoxia in distinct regions of the tumour mass is one of the major hallmarks of glioblastomas. We investigated the influence of acute and chronic hpyoxia on the expression of putative glioma stem cell markers, including CD133, SOX2, Oct-4 and CXCR4.
Material and Methods: Glioma stem cell-enriched cell lines (GS lines) were established from 4 different glioblastomas under hypoxic conditions (1% O2, GSH lines) and in parallel under normoxic conditions (21% O2, GSN lines). Cell lines were propagated for at least 10 passages while permanently maintaining these conditions. Furthermore, GSN lines were exposed to short-term hypoxia (48 hrs to 7 days), and conversely GSH lines were exposed to short-term normoxia. Expression of stem cell markers was monitored by quantitative PCR and flow cytometry, and cell motility was studied using modified Boyden chamber migration assays.
Results: Although recent evidence pointed towards a more robust stem-like phenotype of glioma cells under hypoxia as monitored by increased expression of the pluripotency marker Oct-4, only CD133 and CXCR4, both expressed by distinct subpopulations of GS cells, responded to an acute decrease in oxygen concentration. CD133 and CXCR4 were upregulated in a time-dependent fashion at both the mRNA and protein level, with different kinetics and a delayed response for CXCR4. Hypoxia significantly expanded the cell subpopulation immunoreactive for both CD133 and CXCR4. In chronically hypoxic cultures, upregulation of CD133 and CXCR4 was strikingly higher than under acute hypoxic conditions. Furthermore, hypoxia enhanced chemotactic migration of GS cells towards SDF-1, due to increased receptor levels. Reoxygenation of cultures caused an instant decrease of CD133 and CXCR4 protein and mRNA levels within the first few hours, independent of the duration of prior hypoxia. In contrast to recent reports, we detected no influence of hypoxia on Oct-4 or SOX2 expression, neither under acute nor chronic hypoxia. To delineate mechanism of CD133/ CXCR4 induction, we pharmacologically inhibited HIF-1a and HIF2, two major mediators of hypoxia-controlled gene expression, using chaetomin. This led to abrogation of hypoxia-induced expression of both CXCR4 and CD133, while Oct-4 and SOX2 were not affected.
Conclusion: Our findings support the concept that the glioma stem cell-like phenotype, as delineated by the presence of neural stem cell markers, is subject to plasticity which is governed by microenvironmental factors. The adaptive stem-like cells might evade conservative treatment due to their flexibility and call for new strategies for glioma treatment.

I-13 Neural stem cells prevent irradiation brain function losses by replacing damaged endothelial cells

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Introduction: Radiation therapy is an indispensable therapeutic modality for various brain diseases. Though endogenous neural stem cells (NSCs) would provide regenerative potential, many patients nevertheless suffer from radiation induced brain damages.
Methods and Results: Accordingly, we tested beneficial effects of exogenous NSC supplementation. NSCs supplementation indeed inhibited irradiation-induced brain atrophy and thereby preserved brain functions such as short-term memory. Interestingly, NSCs differentiated into endothelial cells as well as neurons in irradiated brains, accompanied by deterioration of the cerebral blood flow by irradiation which was reversed by NSC supplementation. Focused brain irradiation resulted in localized apoptosis of endothelial cells. NSCs implanted into the opposite hemisphere migrated into those areas and selectively repopulated damaged endothelial cells. Furthermore, inhibition of VEGF signaling significantly impaired both the migration and trans-differentiation of NSCs.
Conclusions: Altogether, our data demonstrate that exogenous NSC supplementation could recover irradiation brain function losses by replacing damaged endothelial cells via a VEGF dependent mechanism.

I-14 Regulation of neural stem/progenitor cell maintenance by PI3K and MTOR

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Introduction: The mechanism involved in the control of stem cell state and differentiation of neural stem cells, which is essential for proper development of the nervous system, is presumed to be shared by glioma stem/initiating cells. EGF and FGF2 are known to play important roles in the maintenance of neural as well as glioma stem cells, but the underlying mechanism still remains unclear.
Materials and Methods: We used in vitro primary cultures of mouse neural stem/progenitor cells maintained with EGF/FGF2 and analyzed, in the presence and absence of PI3K and/or mTOR inhibitors, i) the status of the PI3K and mTOR signaling pathways, ii) self-renewal capacity, and iii) expression of stem cell (Sox2) and differentiation (GFAP, betaIII-tubulin) markers, of the neural stem /progenitor cells.
Results: We show that both PI3K and mTOR are activated by EGF/FGF2 but that inhibiting the activation of either PI3K or mTOR alone results in only reduced proliferation of neural stem/progenitor cells without affecting their stem cell state in terms of self-renewal capacity and stem cell/differentiation marker expression. However, significantly, concurrent inhibition of PI3K and mTOR promoted exit from the stem cell state together with astrocytic differentiation of neural stem/progenitor cells.
Conclusions: These findings suggest that PI3K and mTOR are involved in the EGF/FGF2-mediated maintenance of neural stem/progenitor cells and that they may act in parallel and independent pathways, complementing and backing up each other to maintain the stem cell state. Given the similarity between neural stem cells and glioma stem/initiating cells, these findings could also contribute to the development of novel approaches to control glioma stem/initiating cells

Session II
Molecular Classification: Profiling

II-1 Germline polymorphisms in (OR NEAR) CDKN2A/B, RETL1, TERT, and CCDC26 are differentially associated with glioma grade and morphologic subtype

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Introduction: Two recent genome-wide association studies (GWAS), including one from UCSF/Mayo reported that single nucleotide polymorphisms (SNPs) in (or near) CDKN2A/B, RETL1, TERT, or CCDC26 are associated with adult glioma development. These reports did not directly determine if the SNP associations are with all adult gliomas or with specific glioma morphologic subtypes or grades. We report a reanalysis of UCSF/Mayo cases stratified by morphologic grade and subtype of glioma.
Material and Methods: Germline DNA from 681 and 479 patients with gliomas from USCF and Mayo, respectively, were analyzed using the Illumina and Affymetrix SNP platforms. Germline DNA from 602 and 372 controls from USCF and Mayo, respectively, was also analyzed by these platforms.
Results: SNPs in or near CCDC26 (8q24) (e.g. rs 4295627 and rs6985032) are associated with the development of grade 2 astrocytoma (A2)/anaplastic astrocytoma (AA), gliomas with an oligodendroglial component, but not glioblastoma (GBM) (p = 0.0005, 0.01, and 0.26 respectively). SNPs in or near RTEL1 (20q13) (e.g. rs6010620) are very strongly associated with the development of GBM but not A2/AA (p = 2.8 X 10-7 and 0.19, respectively). A SNP within TERT (5p15) (rs2736100) is associated with the development of both GBM and A2/AA (p = 2.1 X 10-5 and 0.007, respectively). SNPs in or near CDKN2A/B (9p13) (e.g. rs497756) are associated with the development of both A2/AA and GBM (p = 0.041 and 0.0009, respectively).
Conclusions: Germline polymorphisms are likely associated with the development of different morphologic subtype and histologic grade of glioma. These observations can be used to generate hypotheses concerning the possible mechanisms by which specific SNPs (or alterations in linkage disequilibrium with such SNPs) are associated with glioma development.

II-2 Identification of a CPG island methylator phenotype that defines a distinct subgroup of glioma

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Introduction: The Cancer Genome Atlas (TCGA) project has profiled glioblastoma (GBM) samples using a variety of platforms. Little is known about global methylation changes in these tumors.
Material and Methods: To date, CpG island methylation data are available for 273 GBM tumors. Clustering methods to identify groups were performed. Correlation of methylation subtypes with expression subtypes was performed. Methylation-specific-PCR was performed to validate individual CpG island methylation sites. IDH1 mutations were detected and correlated with G-CIMP status.
Results: Unsupervised analyses of these data revealed a small subset of GBM samples with highly concordant gene promoter methylation including a large number of hyper-methylated loci, indicating the existence of a glioma-CpG Island Methlylator Phenotype (G-CIMP). These G-CIMP tumors are predominantly of the proneural subtype, and almost completely associated with IDH1 somatic mutations. Patients with G-CIMP-positive tumors were younger at the time of diagnosis and experienced significantly improved outcome. G-CIMP tumors displayed molecular features of low and intermediate grade gliomas. Extension of these data to an independent set of over 300 glioma samples using MSP showed that the proportion of G-CIMP-positive cases was approximately, 80%, 50%, and 10%, for WHO grade II, III, and IV tumors respectively. G-CIMP status was an independent predictor of survival after adjusting for patient age and tumor grade. G-CIMP status appeared very stable and remained unchanged in a sample of 15 matched primary-recurrent pairs. Interestingly, G-CIMP positivity was highly positively associated with a presence of an IDH1 mutation. Gene ontology analysis showed that epigenetically silenced genes in G-CIMP were functionally associated with the mesenchyme gene expression subclass and poor prognosis. Direct correlation of methylation data with expression profiling data indicated that a subset of poor-prognosis genes is in fact epigenetically silenced.
Conclusion: These findings characterize G-CIMP as a distinct subset of human gliomas on molecular and clinical grounds. G-CIMP-positive tumors tend to be lower grade, associated with improved outcome and highly associated with IDH1 mutation status.

II-3 IDH1 and IDH2 mutations in glioma development

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Introduction: Gliomas are the most common type of primary brain tumors. We hypothesized that a comprehensive elucidation of genetic alterations in gliomas would provide novel targets for diagnostic, prognostic, or therapeutic purposes, as well as allow subclassification of patients who may preferentially respond to particular targeted therapies.

Methods and Results: The genome-wide mutational analysis of glioblastomas (WHO Grade IV glioma) revealed somatic mutations of IDH1 in a fraction of such tumors. We then identified mutations that affected the same amino acid of IDH1, R132, in more than 70% of WHO Grades II and III astrocytomas and oligodendrogliomas and in glioblastomas that developed from these lower-grade lesions. Tumors without mutations in IDH1 often had mutations affecting R172, the analogous amino acid of IDH2. Tumors with these mutations had distinctive genetic and clinical characteristics, and patients with such tumors had a better outcome than those with wild-type IDH genes. We have performed a series of biochemical analysis of the mutant IDH1 and IDH2 activities and observed that the IDH mutations changed the enzymatic activity of the encoded protein.
Conclusion: Mutations of NADP+-dependent isocitrate dehydrogenases encoded by IDH1 and IDH2 occur as the earliest genetic event in a majority of malignant gliomas and have important implications for glioma pathogenesis, diagnosis, and therapeutics of these tumors.

II-4 IDH1 mutation defines de novo glioblastomas with a distinct constellation of demographic, genetic, epigenetic, and phenotypic features

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Introduction: Recent molecular profiling studies have identified a set of DNA alterations and expression profiles with prognostic significance in high grade gliomas. Among glioblastoma (GBM) cases, both IDH1R132 mutation and the Proneural gene expression signature have been associated with longer survival, while copy number changes on chromosome 7 and 10 and Proliferative or Mesenchymal gene signatures are associated with poor outcome. Herein, we sought to gain insight into the biology underlying the positive prognostic value of IDH1R132 mutation in GBM by characterization of genetic, epigenetic and phenotypic features of a series of newly-diagnosed de novo GBMs.
Material and Methods: A series of >750 cases of newly-diagnosed untreated brain tumors presenting as de novo lesions with WHO GBM pathology (i.e. clinical presentation as primary GBM) was investigated. Analysis includes data obtained through the Cancer Genome Atlas Project as well as our own molecular profiling and analysis of radiographic images. IDH1R132 mutant (IDH1R132MUT) and wild type (IDH1R132wt) GBMs were compared for features which include patient age, survival time, p53 mutation status, copy number changes on chromosomes 7&10, DNA methylation, gene expression, and appearance of tumors with MR imaging.
Results: As previously reported, IDH1R132 mutation was found to be associated with younger age, better outcome, higher frequency of p53 mutation, and lower incidence of copy number changes on chromosomes 7&10. IDH1R132 mutation was strongly associated with the Proneural expression signature and, in contrast to IDH1R132WT cases, IDH1R132MUT GBMs never presented as or progressed upon relapse to a Mesenchymal signature. DNA methylation patterns showed striking differences between IDH1R132MUT and IDH1R132WT GBMs and significant differences were observed in the radiographic appearance and location of lesions as a function of IDH1R132 mutation.
Conclusion: IDH1 mutation defines a set of primary GBMs with demographic, genetic, epigenetic, and phenotypic features that distinguishes them from other de novo GBMs. Thus, IDH1R132 mutation is a marker that defines two distinct disease entities within lesions that present histologically as WHO GBM. The constellation of correlated features suggests that IDH1-mutant gliomas develop by a mechanism and possibly cell type of origin that is distinct from that of other high grade gliomas and are likely to differ in their response to treatment

II-5 BRAF and IDH1/2 alterations in astrocytomas

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Introduction: Several recent reports have highlighted the contribution of the RAF gene family alterations (KIAA1549-BRAF fusion, BRAF point mutations or SRGAP3-RAF1 fusion) to pilocytic astrocytoma (PA) oncogenesis. Among the diffuse astrocytomas (Astrocytoma WHO grade II [A]; Anaplastic Astrocytoma WHO grade III [AA]; and Glioblastoma WHO grade IV [GB]), mutation of one allele of either the IDH1 or IDH2 genes has also been recently reported. It has been suggested that the identification of these alterations might help differentiate the diffuse astrocytomas from pilocytic astrocytomas.
Materials and Methods: The status of these genes in a total of 43 paediatric tumours (histologically diagnosed as stated below, all <16 yrs) and 268 adult tumours (histologically diagnosed as below; primary GB = pGB and secondary GB = sGB, all >16 years) were correlated with patient survival and other genetic alterations.
Conclusion: There is a notable difference between the total incidence of BRAF/RAF1/NF1 abnormalities in the adult and paediatric PA cases (66% and 85%, respectively). The fusion oncogenes appear highly specific for PAs. A review of the literature indicates focal gain at 7q34 and/or demonstration of the BRAF-fusion gene in 247/369 (67%) PAs but only 15/166 (9%) tumours histologically diagnosed as A. The single paediatric A with the BRAF-fusion recorded above has survived more than 15 years. No IDH1 mutations were noted in any of the PAs or in the small number of paediatric diffuse astrocytomas. This will need confirmation in a larger series, but may limit the potential value of the use of IDH1/2 mutation to support diagnosis in a paediatric population. In the diffuse astrocytomas, TP53 mutation and combined 1p/19q loss were mutually exclusive. The presence of one of these was observed in 93% of IDH1/2 mutated tumours but only 34% of wt IDH1/2 cases ($p < 1 \times 10\text{-}16$).

II-6 Impact of IDH1 analysis on brain tumor classification and grading

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Introduction: Mutations in codon 132 of IDH1 are extraordinary frequent in distinct brain tumor types. Diffuse astrocytomas, oligodendrogliomas and mixed oligoastrocytomas carry these mutations in more than 70% of all cases. Likewise, the vast majority of secondary glioblastomas having developed from lower grade gliomas carry IDH1 mutations. More than 90% of IDH1 mutations lead to replacement of amino acid arginine in position 132 by histidine (R132H). We have developed diagnostic tools allowing efficient detection of IDH1 mutations and applied these methods to human brain tumors
Material and Methods: In order to overcome the need of sequencing, we developed a set of mismatched primers which in combination with restriction endonucleases recognize wild type IDH1 or the R132H, R132S, R132G and R132L mutations. Further we developed a monoclonal mouse antibody capable of detecting mutant IDH1 of the R132H type on paraffin sections. These tools as well as direct sequencing were applied to brain tumor DNA or sections from tumors classified and graded according to the guidelines of WHO.
Results: IDH1 mutations or mutant protein clearly separate tumor entities which frequently pose diagnostic problems. Especially useful is this additional parameter in small tumor biopsies. For example, the differentiation between pilocytic astrocytoma (PAI) and the diffuse astrocytomas is greatly assisted by absence of IDH1 mutations in PAI and presence in the majority of the latter. Similarly, tanicytic ependymoma does not carry IDH1 mutations and therefore is better distinguishable from diffuse astrocytomas. The R132H specific antibody readily detects single tumor cells in otherwise inconspicuously appearing tissue. Further, IDH1 mutations proved to be of major prognostic impact in both, astrocytomas and glioblastomas.
Conclusion: The IDH1 status is a very helpful parameter for classification of brain tumors. IDH1 analysis can be expected to be routinely applied in brain tumor diagnosis and may become an decisive parameter for defining tumor entities in the WHO classification.

< 16yrs									
Dx	n	KIAA1549: BRAF	BRAF mut	SRGAP3: RAF1	Clinical NF1	IDH1/2 mut	1p/19q loss	TP53 mut	10-yr survival
PA	33	23 (70%)	2 (6%)	1 (3%)	2 (6%)	0	0	nd	26/27 (96%)
A	5	1 (20%)	0	0	0	0	0	0	5/5 (100%)
AA	5	0	nd	0	0	0	0	4 (80%)	1/4 (25%)
Total = 43									

16yrs +									
Dx	n	KIAA1549: BRAF	BRAF mut	SRGAP3: RAF1	Clinical NF1	IDH1/2 mut	1p/19q loss	TP53 mut	10-yr survival
PA	12	6 (50%)	1 (8%)	0	1 (8%)	0	0	nd	11/12 (92%)
A	17	0	0	0	0	15 (88%)	3 (18%)	11 (65%)	8/17 (47%)
AA	58	0	nd	0	0	32 (55%)	3 (5%)	38 (66%)	13/58 (22%)
pGB	171	0	nd	0	0	6 (4%)	3 (2%)	59 (35%)	1/171 (0.6%)
sGB	10	0	nd	0	0	5 (50%)	0	6 (60%)	0/10 (0%)
Total = 268									

II-7 Inter-tumoral and intra-tumoral heterogeneities on genomic imbalance in glioblastomas

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Introduction: It is generally accepted that glioblastomas are genetically heterogeneous, but little is known about to what extent intra-tumoral heterogeneity exists at the genomic level. We have recently established a reliable whole genome amplification (WGA) method to randomly amplify small amounts of DNA extracted from paraffin-embedded histological sections with minimum amplification bias (Huang J et al. J Mol Diagn 11:109-116, 2009).
Materials and Methods: We assessed genome-wide chromosomal imbalance by array CGH (Agilant 105K) using WGA-DNA in 2-5 separate tumor areas from 7 glioblastomas.
Results. Chromosomal imbalances detected significantly differed among different glioblastomas; the only alterations that were observed in more than 2 cases included loss of chromosome 10p and 10q (4 cases), amplification of the EGFR locus at 7p (3 cases), and amplification of the 4q12 locus (2 cases). Comparison of different areas of the same tumor revealed that in all cases, there are genetic alterations that are common to all tumor areas analyzed, and those that are area-specific.
Conclusions: Our results reveal a remarkable inter-tumoral and intra-tumoral genomic heterogeneity in glioblastoma. Genetic alterations that are common to all tumor areas are likely to be those that are directly involved in the pathogenesis of glioblastomas, whereas those that are specific to different tumor areas may be a reflection of genomic instability.

II-8 Epigenetic plasticity regulated by polycomb repressive complex as a mediator of heterogeneity in GBM

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Introduction: Functional and morphological heterogeneity characterizes aggressive neoplasms and contributes to invasion and drug resistance. Deciphering the molecular mechanisms underlying this heterogeneity will help invent future therapeutic strategies. Our recent studies uncovered that histone H3 lysine 27 trimethylation (H3K27me3)-mediated gene silencing is mechanistically distinct from DNA methylation-mediated gene silencing, suggesting that these two mechanisms have distinct roles in tumorigenesis (Figure). Given the evidence that Polycomb repressive complex (PRC)-mediated H3K27me3 cellular processes confer stemness and control organism development by regulating the expression of developmental genes, we hypothesized that cancer cells usurp this epigenetic process to mediate adaptation to tumor environments, which results in tumor heterogeneity.
Material and Methods and Results: In human glioblastoma, a highly heterogeneous malignant tumor, we examined epigenetic alterations in glioma-initiating cells (GICs) that have the potential to convert into differentiated cell types. We demonstrated that biological conversion of GICs is reversible, and accompanied by the gain or loss of PRC-mediated H3K27me3 marks. These data suggest that cancer cells usurp this epigenetic mechanism as a mediator of adaptation to their environments, which results in tumor heterogeneity. Consistent with these data, inhibition of EZH2, a PRC component highly expressed in glioblastoma, disrupted the biological conversion and impaired the tumorigenicity of GICs in NOD-SCID mice.
Conclusion: Our global and functional analyses uncover a new layer of cancer epigenetics where labile and flexible PRC-mediated H3K27me3 mechanisms work as a mediator for regulating cancer-initiating cells that may contribute to intratumoral heterogeneity through tumor cell differentiation.

II-9 tMolecular signatures classify astrocytic gliomas by IDH1 mutation status and reveal frequent RPRM silencing in tp53 wild-type tumors

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To identify novel glioma-associated pathomechanisms and molecular markers, we performed an array-based comparative genomic hybridization analysis of 131 diffuse astrocytic gliomas, including 87 primary glioblastomas (pGBIV), 13 secondary glioblastomas (sGBIV), 19 anaplastic astrocytomas (AAIII), and 12 diffuse astrocytomas (AII). All tumors were additionally screened for IDH1 and IDH2 mutations. Expression profiling was performed for 74 tumors (42 pGBIV, 11 sGBIV, 13 AAIII, 8 AII). Unsupervised and supervised bioinformatic analyses revealed distinct genomic and expression profiles separating pGBIV from the other entities. Classifier expression signatures were strongly associated with the IDH1 gene mutation status. Within pGBIV, the rare subtype of IDH1 mutant tumors shared expression profiles with IDH1 mutant sGBIV and was associated with longer overall survival compared with IDH1 wild-type tumors. In patients with IDH1 wild-type pGBIV, PDGFRA gain or amplification as well as 19q gain were associated with patient outcome. Array-CGH analysis additionally revealed homozygous deletions of the FGFR2 gene at 10q26.13 in two pGBIV, with reduced FGFR2 mRNA levels being frequent in pGBIV and linked to poor outcome suggesting FGFR2 as a novel glioma-associated candidate tumor suppressor gene on the long arm of chromosome 10. Furthermore, the RPRM gene, which encodes the p53-induced protein reprimo, was frequently hypermethylated and transcriptionally down-regulated in TP53 wild-type gliomas, including most pGBIV. We suggest that epigenetic silencing of RPRM could constitute a molecular mechanism by which TP53 wild-type gliomas escape from p53 dependent growth control.

II-10 Integrated molecular genetic profiling of paediatric high-grade gliomas reveals key differences with the adult disease

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Introduction: High-grade glioma (HGG) in children is a devastating disease, with a 2-year survival rate of less than 10-30%. Insights into the molecular pathogenesis of pediatric HGG have been limited by the small sample size used for many studies. Our aim was to rigorously define the copy number alterations and gene expression signatures underlying pediatric HGG.

Materials and Methods: We conducted a high-resolution analysis of genomic imbalances in 78 de novo pediatric HGG, including 7 diffuse intrinsic pontine gliomas, and 10 HGG cases arising in children who received cranial irradiation for a previous cancer, using Affymetrix 500K GeneChips. Gene expression signatures for 53 tumors were analyzed with Affymetrix U133v2 arrays. Results were compared with publicly available data from adult tumors.

Results: Pediatric and adult glioblastoma were clearly distinguished by frequent gain of chromosome 1q (30% vs 9%) and lower frequency of chromosome 7 gain (13% vs 74%), respectively. The most common focal amplifications also differed, with PDGFRA and EGFR predominant in childhood and adult populations respectively. These common alterations in pediatric HGG were detected at higher frequency in irradiation-induced tumors, suggesting that these are initiating events in childhood gliomagenesis. CDKN2A was the most common tumor suppressor gene targeted by homozygous deletion in pediatric HGG. No IDH1 hotspot mutations were found in pediatric tumors, highlighting molecular differences in pathogenesis between childhood HGG and adult secondary glioblastoma. Integrated copy number and gene expression data indicated that deregulated PDGFRa signaling plays a major role in pediatric HGG.

Conclusions: Integrated molecular profiling showed substantial differences in the molecular features underlying pediatric and adult HGG, indicating that findings in adult tumors cannot be simply extrapolated to younger patients. PDGFRa may be a useful target for pediatric HGG including diffuse pontine gliomas.

II-11 Medulloblastoma – individualizing therapy based on molecular diagnosis

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Whereas Medulloblastoma (MB) represents one of the most frequent causes of mortality in children, this tumor entity is equally challenging for adult neurooncologists since common treatment strategies are lacking for adult patients and therapy regimens adapted from adult glioblastoma are not successful in MB. Oversimplified stratification schemes only considering age and metastatic stage do not adequately account for the true biological heterogeneity of MB. Thus, markers to identify subgroups of patients with excellent outcome for whom therapy intensity may be reduced and high-risk patients, who cannot be cured by use of current treatment protocols and who require alternative treatment, are urgently warranted.

For childhood MB, we were able to identify and validate five distinct molecular subgroups in a cohort of 340 patient samples, which comprises the largest cohort of MB patients ever investigated for genetic aberrations.

These robust molecular markers have immediate impact on treatment decisions in upcoming European and North American treatment studies: Patients with tumors carrying a monosomy of chromosome 6, who showed a 100% 5-year overall survival rate in our cohort will be treated with reduced total irradiation doses in future trials. Molecular high-risk patients carrying amplifications of certain critical oncogenes in their tumor genomes (even if not considered high risk in terms of clinical variables) will be treated with intensified protocols upfront.

In the successive study in 112 adult MBs, we could clearly show that adult MB is not clinically and genetically distinct from childhood MB. Thus, different molecular staging systems have to be applied including assessment of an oncogene amplification (CDK6) that only occurs in adult MB and defines a subgroup with particularly poor prognosis. Furthermore, we identified a population of adult MB patients (approximately 60% of patients) who had a 5-year overall survival of 96% in our cohort and thus may be cured by use of the pediatric standard-risk protocol.

In conclusion, we have established powerful molecular staging systems for both childhood and adult MB. These molecular staging systems may serve as the basis for tailoring treatment intensity to disease risk in several upcoming multi-center clinical trials in both Europe and North America.

II-12 Classification of medulloblastomas by histopathology and molecular signatures

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The revised WHO classification of brain tumors has been published in 2007. The category "embryonal tumors" includes medulloblastoma and its variants, atypical teratoid/rhabdoid tumors (AT/RT), and rare PNET entities of the CNS. The novel term "CNS-PNET" defines a small group of rare entities including medulloepithelioma, ependymoblastoma, but also rare hemispheric PNETs/neuroblastomas and spinal PNETs. Recent clinical, histological, genetic and biological studies have identified medulloblastoma variants (WHO grade IV) with different cells of origin, genetic alterations and clinicopathological features which are now defined as tumor entities: 1) medulloblastoma (MB; classic), 2) desmoplastic / nodular MB (with typical islands surrounded by reticulin-rich areas at least in parts of the tumor), 3) MBs with extreme nodularity (occuring in young children), 4) large cell MB, 5) anaplastic MB (cases with diffuse and severe cytological anaplasia). Histopathological MB entities indicate different clinical outcome in several studies; histopathology represents an independent prognostic marker in young children with MB. Genetic and cell biological analyses have shown that crucial developmental signalling pathways including Hedgehog, Wnt, Notch and Igf signalling are activated in subgroups of medulloblastomas by genetic, epigenetic or transcriptional alterations. The activity of individual pathways is associated to specific histological variants, and furthermore identifies subgroups with different outcome. Activity can be scored by measurement of pathway related transcriptional targets or accumulation / nuclear translocation of specific transcription factors. In addition to their role as molecular classifiers the pathways and their components may serve as novel therapeutic targets. Interestingly, some of these pathways have been shown to be active in medulloblastoma cells with stem cell characteristics so that pathway inhibition will also target this important subpopulation of tumor cells. Molecular and histological classification may assist ultimately in the stratification in future clinical trials and in the selection of patients for molecular targeted therapies.

III-1 Molecular markers predicting natural course and response to therapy in low-grade glioma: an update from the german glioma network

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Introduction: The German Glioma Network (GGN) was made possible by a 6-year grant from the "Deutsche Krebshilfe" and started its clinical and scientific work on October 1, 2004. The GGN has established a network of interdisciplinary competence centers for the diagnosis, treatment and care of patients with gliomas.
Materials and Methods: The GGN pursues a population-based approach and focuses on scientific projects in the areas of (i) molecular predictors of natural course and response to therapy, (ii) long-term survival in glioblastoma, (III) novel treatment strategies and (IV) the neurotoxicity of multimodality treatment.
Results: The GGN has collected a large population of patients with low-grade gliomas who have not received either radiotherapy or chemotherapy after the initial surgical intervention. An analysis of this patient population has revealed that the 1p/19q codeletion is not prognostic in a population of patients with low-grade gliomas who have not been exposed to genotoxic therapies. A similar dataset is now available to demonstrate the absence of prognostic power of p53 mutations, MGMT promoter methylation and isocitrate dehydrogenase 1/2 mutations.
To identify novel genes of prognostic significance in low-grade gliomas that may help to identify patients with rapid tumor progression who would potentially benefit from more aggressive initial treatment, we have defined two populations of patients with low-grade diffuse astrocytomas treated initially with surgery alone, one consisting of patients with early progression within 12 months after surgery, another consisting of patients without progression by Macdonald criteria within the first five years after surgery. Tumor tissues from these patients as well as from patients with anaplastic astrocytomas were subjected to genome-wide genomic and mRNA expression profiling using array comparative genomic hybridization (aCGH) as well as Affymetrix gene expression chip analyses. Integrated bioinformatic evaluation of the genomic and transcriptomic data was used to unravel candidate gene regions and gene expression signatures distinguishing these patients groups and thereby representing putative molecular biomarkers of prognostic significance.
Conclusions: Non-biased approaches using high-throughput analyses are more likely to disclose molecular prognosticators determining the course of low-grade glioma than the current candidate molecular changes thought to determine course and response to therapy.

III-2 IDH1 mutations are strongly associated with MGMT promoter gene methylation and are prognostic but not predictive for outcome to PCV chemotherapy in oligodendroglial tumors: a report from the randomized eortc study 26951

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Introduction: Anaplastic oligodendroglial tumors (AOD) are chemosensitive tumors. Recent studies have shown the prognostic significance of IDH1 mutations in glioma. Why these tumors have a better outcome is yet unknown, nor is it clear if IDH1 mutations are predictive for outcome to chemotherapy. In the present study we assessed the impact of IDH1 mutations on progression free survival (PFS) and overall survival (OS) in the prospective randomized EORTC study 26951 on adjuvant PCV on 368 patients with AOD.
Material and Methods:
DNA was extracted from formalin-fixed paraffin-embedded tissues from selected areas enriched for a high tumor cell percentage. IDH1 and IDH2 alterations of the mutational hotspot codons R132 and R172, respectively, were assessed by bi-directional cycle sequencing of PCR-amplified fragments. MGMT promoter methylation was assessed using methylation-specific MLPA based on methylation sensitive restriction analysis. Loss of chromosomes 1p, 19q, 10 and 10q and gain of 7 and the EGFR gene were assessed with FISH.
Results: In 160 patients sufficient material was available for IDH1 analysis. In 73 (46%) an IDH1 mutation was found, in one case an IDH2 mutations was identified. The presence of IDH1 mutations was strongly correlated with 1p/19q co-deletion and MGMT promoter methylation (Spearman correlation 0.42 and 0.43) and inversely correlated with loss of chromosome 10, EGFR amplification, polysomy of chromosome 7 and the presence of necrosis (Spearman correlations -0.33, -0.39, -0,30 and -0,33 respectively). IDH1 mutations were found to be equally prognostic in both the radiotherapy (RT) and the RT/PCV treated patients, for both PFS and OS. In univariate analysis for OS, the hazard ratio reduction in the presence of IDH1 mutations was 0.24 [95% confidence interval 0.15-0.38]. With Cox proportional hazard modelling for OS with stepwise selection, IDH1 mutations, the presence of necrosis and 1p/19q co-deletion were found to be independent prognostic factors.
Conclusions: In this homogeneously treated group of AOD patients, the presence of IDH1 mutations was found to carry a very strong prognostic significance for OS but without evidence of a predictive significance for outcome to PCV chemotherapy. IDH1 mutations were strongly associated with 1p/19q co-deletion and MGMT promoter methylation.

III-3 Glioblastoma recurrence is not associated with changes in the MGMT promoter methylation status – a translational study of the german glioma network

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Introduction: Hypermethylation of the MGMT promoter is strongly associated with longer progression-free and overall survival in glioblastoma patients treated with radiotherapy and concomitant and adjuvant temozolomide. We here addressed the question whether glioblastoma relapses are associated with changes in the MGMT promoter methylation status or altered expression of the DNA mismatch repair genes, MLH1, MSH2, MSH6 and PMS2.
Material and Methods: MGMT promoter methylation status was analysed in paired primary and recurrent glioblastoma samples of 80 patients using non-quantitative methylation-specific PCR (MSP). The vital tumor cell content of each primary and recurrent tumor specimen was histologically determined. Quantitative promoter methylation analyses using DNA pyrosequencing were performed, too, for MGMT in 48 patients as well as for the DNA mismatch repair genes MLH1, MSH2, MSH6 and PMS2 in 42 patients. Furthermore, levels of MGMT, MLH1, MSH2, MSH6 and PMS2 proteins were analysed semiquantitatively by immunohistochemistry in 43 patients.
Results: MSP revealed MGMT promoter hypermethylation in 27 patients, borderline methylation in 3 patients and no methylation in 50 patients at diagnosis. In 73 of the 80 patients, the MGMT promoter status of the primary tumor was retained at recurrence. In 6 patients, loss or reduction of MGMT promoter methylation was detected in the recurrent tumor, however, in 3 patients this finding was explained by low tumor cell contents in the recurrent tumor specimen. In one patient, a change from MGMT borderline methylation (8% methylated alleles) to hypermethylation (50% methylated alleles) was detected. None of the investigated primary and recurrent glioblastomas showed MLH1, MSH2, MSH6 or PMS2 promoter hypermethylation. However, immunohistochemical expression scores for MLH1, MSH2, MSH6 and PMS2 proteins were

frequently reduced in the recurrent as compared with the corresponding primary tumor.
Conclusion: The MGMT promoter methylation status does not change from the primary to the recurrent tumor in the vast majority of glioblastoma patients. Our results further suggest that glioblastoma recurrences often demonstrate lower MLH1, MSH2, MSH6 and/or PMS2 immunoreactivity scores. However, MLH1, MSH2, MSH6 and PMS2 promoter hypermethylation does not appear to be involved in glioblastoma recurrence.. Accordingly, acquired changes in MGMT promoter methylation status are unlikely to account for acquired resistance to temozolomide.

III-4 Intrinsic gene expression profiles of gliomas are a better predictor of survival than histology

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Introduction: Gliomas are the most common primary brain tumors with heterogeneous morphology and variable prognosis. Treatment decisions in patients rely mainly on histological classification and clinical parameters. However, differences between histological subclasses and grades are subtle, and classifying gliomas is subject to a large inter-observer variability. To improve current classification standards, we have performed gene expression profiling on a large cohort of glioma samples of all histological subtypes and grades.
Methods: Glioma samples were collected from the Erasmus MC tumor archive (n=276) from patients (1989-2005). Gene expression profiling was performed on Affymetrix HU133 Plus 2.0 microarrays. HOPACH clustering was used to identify intrinsic molecular subgroups. Cluster validation was performed on five external datasets: TCGA, REMBRANDT, GSE12907, GSE4271, and Li et al.)
Results: We identified seven distinct molecular subgroups that correlate with survival. These include two favorable prognostic subgroups (median survival >4.7 years), two with intermediate prognosis (median survival 1-4 years), two with poor prognosis (median survival <1 year), and one control group. The intrinsic molecular subtypes of glioma are different from histological subgroups and correlate better to patient survival. The prognostic value of molecular subgroups was validated on five independent sample cohorts. The power of intrinsic subtyping is demonstrated by its ability to identify a subset of prognostically favorable tumors within an external dataset that contains only histologically confirmed glioblastomas. Specific genetic changes (EGFR amplification, IDH1 mutation, 1p/19q LOH) segregate in distinct molecular subgroups. We identified a subgroup with molecular features associated with secondary glioblastoma, suggesting that different genetic changes drive gene expression profiles. Finally, we assessed response to treatment in molecular subgroups.
Conclusion: Our data provides compelling evidence that expression profiling is a more accurate and objective method to classify gliomas than histological classification. Molecular classification therefore may aid diagnosis and can guide clinical decision making.

III-5 Medulloblastoma comprises four diseases

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Research from Toronto, Ontario, Canada.

Prior attempts to subgroup medulloblastoma using genomics have identified 4-6 distinct subtypes, including distinct groups driven by Wnt and Shh signaling. Both unsupervised hierarchical clustering and principal component analysis of expression data on 103 medulloblastomas reveals very high confidence for existence of four medulloblastoma subgroups: WNT, SHH, Group C, and Group D. Further bioinformatic analyses using Prediction Analysis of Microarrays (PAM), Nonnegative Matrix factorization (NMF), and Subclass Mapping (SubMap) all support the existence of four subgroups. The subgroups have distinct demographics; SHH group tumors occur in infants and adults, Group C tumors occur only in children, and Wnt and Group D tumors are found across all age groups. We identified a number of previously uncharacterized, highly subgroup specific regions of chromosomal abnormality including 9p,3q,20q and 21 q gain in SHH tumors, 1q gain and 5q,16q and 10q loss in Group C tumors, near universal isochromosome 17q and frequent loss of the X chromosome in Group D tumors.
We identified 'signature' genes over-expressed in each subgroup for which there are high quality commercial antibodies. We stained two separate medulloblastoma tissue microarrays containing 294 non-overlapping tumors for DKK1 (WNT), SFRP1 (SHH), NPR3 (Group C), and KCNA1 (Group D) demonstrating that 288/294 (98%) of tumors stained for only a single marker. Analysis of the demographics in these patients validated the data from our discovery set studied at the RNA level. Leptomeningeal dissemination was very common in Group C (47%) followed by Group D (30%) patients. A multivariate analysis of age, extent of resection, histology, M stage and subgroup revealed that only LCA histology and Group C were prognostic. As M0 Group C tumors have a terrible prognosis, we suggest that Group C patients includes many of the children with 'average risk' medulloblastoma who relapse after current therapies.
Our data highly support the existence of four independent types of medulloblastoma that differ in their demographics, rate of metastases, transcription, genetic events, and clinical outcome. Our novel '4 antibody' technique is capable of determining medulloblastoma subgroup through immunohistochemistry on formalin fixed, paraffin embedded material suggesting that it will be broadly generalizable across the globe.

III-6 Improved stratification of children and adolescents with medulloblastoma by histological and biological parameters

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Introduction: Treatment recommendations for medulloblastoma, the most frequent malignant brain tumor of childhood, were traditionally based on the clinical risk factors age, M-stage, and extent of tumor resection.
Material and Methods: The recent prospective brain tumor trials conducted by the GPOH and/or SIOP-Europe have aimed to identify histological and biological prognostic parameters.
Results: In the prospective randomised HIT-SIOP PNET4 trial, MB patients between 3 and 21 years without metastases were treated by conventional or hyperfractionated radiotherapy between 2001 and 2006. Three risk groups were identified by our preliminary analyses in 179 patients with completely analyzed parameters. 1) Favourable risk group: Patients with ß-catenin accumulation (IHC), absence of significant postoperative residual tumor, and without anaplastic-large cell histology, c-MYC or N-MYC DNA amplification (analyzed by FISH and PCR) 2) Intermediate risk group: Patients without ß-catenin accumulation and other criteria as mentioned in (1) 3) High-risk patients with large postoperative residual tumors, large-cell anaplastic histology or myc-amplification. The next European standard risk medulloblastoma trial, using biological and clinical factors for treatment stratification, is under preparation.
In older children with metastatic MB, improved survival rates (4 year-EFS 65%) have been obtained in HIT

2000 by an intensified treatment including systemic and intraventricular chemotherapy, hyperfractionated craniospinal radiotherapy and maintenance chemotherapy. In this cohort, no significant histological or biological risk factors have been identified so far.
In young children with medulloblastoma, the desmoplastic variant and MB with extensive nodularity (MBEN) have been identified as significant independent favourable risk factors: Survival rates higher than 80% (EFS) and 90% (OS) have been achieved by a methothrexate-based systemic and intraventricular chemotherapy without radiotherapy within the trial HIT-SKK'92, and confirmed in the trial HIT 2000. Consequently, treatment is currently stratified according to histology in this age-group. Local conformal radiotherapy has been reintroduced for young children with non-metastatic classic MB. An international trial randomizing chemotherapy with and without intraventricular methotrexate in young children with desmoplastic MB and MBEN is under preparation.
Conclusion: Histological and biological risk factors have been newly identified and will be applied prospectively in upcoming medulloblastoma trials, aiming to refine risk-adapted treatment stratification and improve survival rates.

III-7 Prognostic impact of CD133 mRNA expression in 61 glioblastoma patients treated with concomitant radiochemotherapy: a prospective patient cohort at a single institution

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Introduction: Cancer stem cells are thought to represent the population of tumorigenic cells responsible for tumor development. The CD133 antigen has been described as a putative stem cell marker in malignant brain tumor that could identify such a tumorogenic population in a subset of glioblastoma. To date, the correlation between CD133 expression in primary glioblastoma and patients' prognosis is not clearly established. To address this question we investigated the relationship between CD133 mRNA expression and patient outcome in a glioblastoma patient cohort.
Experimental design: The quantitative expression of CD133 stem cell antigen mRNA using real-time QRT-PCR was assessed in a cohort of 61 consecutive primary glioblastoma patients treated by chemoradiation with temozolomide.
Results: On multivariate survival analysis, high CD133 mRNA expression was a significant ($p=0.007$) prognostic factor for adverse progression-free and overall-survival independent of extent of resection ($p=0.012$), and MGMT methylation status ($p=0.002$). Patient age was also an independent prognosticator of overall survival ($p=0.012$). Furthermore, according to the conjoined expression of CD133 mRNA and MGMT status, the patients were categorized into three groups: patients with methylated tumors and low expression level of CD133 (group I) had the best prognosis, patients with unmethylated tumors and high expression level of CD133 (group III) had the poorest prognosis and others (group II) had an intermediate outcome.
Conclusions: These findings constitute conclusive evidence that the measurement of the mRNA expression of CD133 stem cell antigen actually impact the survival of GBM patients, lending support to the current brain tumor stem cell hypothesis.

III-8 Soluble IL-2 receptor measure in central nervous system lymphoproliferative diseases

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Introduction: Soluble IL-2 receptor (sIL-2R) is used as a tumor marker for systemic lymphoma. However the sIL-2R value in intracranial lymphoproliferative diseases is not well studied. The purpose of this study was to evaluate sIL-2R measurement in central nervous lymphomas.
Materials and Methods: The value of sIL-2R in 103 cases with intracranial diseases, including primary central nervous sytstem lymphoma (PCNSL) 43, CNS invasion of intravascular lymphoma (IVL) 3, other CNS tumors (28) and other CNS non-neoplastic diseases (29) were retrospectively investigated.
Results: In PCNSL 21 cases (49%) showed elevated levels of sIL-2R (182-1650 U/ml), whereas all IVL showed elevated levels (663-1130). The elevated levels were observed in other brain tumors in 8 cases (29%) and 11 cases (55%) in non-neoplastic diseases. The abnormal value was observed when the patients have systemic inflammation or coagulation disorder, which in part might be the reason of elevation in the non-lymphomatous diseases. In PCNSL and IVL patients with elevation, the value correlates with the disease progression in most instances.
Conclusion: The sIL-2R measurement provides useful information in follow up clinical status of PCNSL and IVL in a certain number of patients.

III-9 Fish analysis on oligodendroglial and astrocytic component in oligoastrocytomas indicate 1p loss on top of 19q loss may be a molecular pathway

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Introduction: Definition of oligoastrocytoma remains one of the most arbitrary descriptions even among the current WHO classification, and molecular pathway of this entity has not been described well. Molecular genetic studies to compare the astrocytoma- and oligodendroglioma-like component has not detected significant difference, although some genetic or epigenetic changes should exist.
Materials and Methods: In 160 gliomas treated in our institution since 2004, we encountered 6 gliomas harboring distinctively oligodendroglial islands (O-part) in the astrocytoma-like (A-part) background, and performed microsatellite analysis and FISH analysis. Histological diagnosis was oligoastrocytoma in 2, anaplastic astrocytoma in 1, and glioblastoma with oligodendroglial component in 3.
Results: Microsatellite analysis demonstrated 19qLOH at 19q13 in all 6cases, but 1p LOH was not evident. However, FISH study performed on O-part and A-part separately revealed clear 1p36 loss in the O-part in 4 of 6 cases, while no loss was observed in the A-part in either case.
Conclusion: Therefore, there are a subset of gliomas which carry 19q loss as an earlier event and show morphology compatible with astrocytic tumors, and additional 1p loss in a clone form the portion histology resembling oligodendrogliomas. This pathway of 1p/19p loss is different from the proposed t(1;19)(q10;p10) translocation responsible for most of the oligodendrogliomas, and may explain the histogenesis of oligoastrocytoma at least in a subset.

III-10 Methylation-sensitive high resolution melting analysis: a new quantitative assessment of MGMT promoter methylation in high-grade gliomas.

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Introduction: The methylation status of the MGMT (O6-methylguanine-DNA methyltransferase) gene has been shown to be a predictive marker in malignant gliomas treated with temozolomide. Methylation –specific PCR (MSP) is widely used method for the detection of the MGMT methylation. Despite its widespread use, MSP has several disadvantage. False positives can arise if primers are badly designed or used at too low a temperature. MSP is very sensitive but is not quantitative. Here, we show that high resolution melting analysis (HRM) can detect MGMT methylation with high sensitivity and moreover estimate quantitatively the extent of methylation in tumors.
Materials & Methods: We used genomic DNA derived from high-grade glioma samples and univeral methylated/unmethylated DNA standards. After bisulfite treatment, PCR was carried out in the presence of dye to fluoresce when intercalated with double-stranded DNA. Methylated and unmethylated DNA acquire different sequences resulting in PCR products with markedly different melting profiles. By comparing the melting profiles of unknown samples with the profiles of methylated and unmethylated template ratio, we were able to estimate quantitatively the methylation levels of glioma samples.
Results and Conclusions: It took us only about 90 minutes to get the data from PCR. MGMT methylation could be detected at levels as low as 1 %. Methylation level measured by this assay was inversly correlated to the MGMT mRNA expression level quantified by real-time RT-PCR. High-grade gliomas with MGMT methylation less than 45 % showed significantly short progression-free survival. Methylation-sensitive HRM is the rapid and useful method for predicting the effect of Temozolomide in glioma therapy.

III-11 Clinical application of gene expression-based diagnostic system of gliomas.

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Introduction: Many studies about gene expression profiling of gliomas have been performed so far. Although they aimed molecular classification, no result reached clinical application like MammaPrint. We previously performed gene expression profiling in 110 gliomas using PCR-array and identified prognostically useful 58-gene set whose expression levels closely correlated with progression free survival. Its prognostic predictability was tested in another 42 gliomas. If a diagnostic model is established once, because genes to use for a diagnosis are few, array-platform is not necessary for the diagnosis of the individual case. For a practical diagnostic device, real-time PCR which is superior in robustness and simplicity of the measurement will be the first choice. We show our trial to put a molecular diagnostic system to clinical application by usig simple technique such as real-time PCR.
Material and Methods: As molecular classification by gene expression profiles, linear classifiers are most popular, and used in diagnostic tests such as MammaPrint. In linear classifier, the diagnostic score(DS) is a sum of normalized expression value multiplied by coefficient determined from the learning data set.
We examined correlation between DS calculated using real-time PCR and DS previously determined in PCR array. Then We calculated DS by using real-time PCR for 17 cases of Grade II and 15 cases of Grade III, and analyzed the correlation between DS and survival time of these patients whose median follow-up periods was 55 months(48-116months). We also calculated DS in another set of 33 high grade glioma cases and inspected its utility for a diagnosis of Grade III vs Grade IV.
Results: Coefficient of correlation between DS calculated with real-time PCR and DS calculated in PCR array was 0.90. DS calculated by using real-time PCR could distinguish 3 dead cases of 17 cases with Grade II. DS diagnosed 8 cases as poor prognosis among 9 dead cases with Grade III.. As a result of we used real-time PCR for another set of 33 high grade gliomas, and having calculated DS, it was revealed that diagnosis by DS was better than histological diagnosis in Grade III and Grade IV.
Conclusions: We converted array-based diagnostic system into real-time PCR-based system and confirmed good correlation of both.Gene expression-based diagnostic system showed clinical utility to predict outcome for glioma patients with histologically boader zone.

III-12 Analysis of IDH1 and IDH2 mutations in japanese glioma patients – correlation with other prognostic factors

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A recent study reported on mutations in the active site of the isocitrate dehydrogenase 1 /2 (IDH1/2) genes in several types of gliomas. We analyzed the genomic region spanning wild type R132 of IDH1 and R172 of IDH2 by direct sequencing in 245 glial tumors. A total of 77 IDH1 or IDH2 mutations were observed. IDH1 or IDH2 mutations were frequently in diffuse astrocytomas (70%), oligodendrogliomas (70%), oligoastrocytomas (75%), anaplastic astrocytomas (52%), anaplastic oligoastrocytomas (70%), anaplastic oligodendrogliomas (51%), secondary glioblastomas (75%), gangliogliomas (38%) and anaplastic gangliogliomas (60%). Primary glioblastomas were characterized by a low frequency of mutations (5%) in amino acid position 132 of IDH1. Mutations of IDH1 or IDH2 genes were significantly associated with improved outcome in patients with anaplastic gliomas. In anaplastic gliomas (n=120), IDH1/2 mutation is significantly correlated with 1p/19 co-deletion and MGMT promoter methylation (p=0.001, p=0.0001 respectively). Our data suggest that IDH1 or IDH2 mutations plays a role in early tumor progression of several types of glioma and might arise from a common glial precursor. These subtypes are genetically distinct entities with other favorable prognostic factors from other glial tumors.

III-13 MIR-17-92 expression in differentiated t cells – implications for glioma immunotherapy

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Introduction: Type-1 T cells are critical for effective anti-tumor immune responses. The recently discovered microRNAs (miRs) are a large family of small regulatory RNAs that control diverse aspects of cell function, including immune regulation. We identified miRs differentially regulated between type-1 and type-2 T cells, and determined how the expression of such miRs is regulated.
Material and Methods: We performed miR microarray analyses on in vitro differentiated murine T helper type-1 (Th1) and T helper type-2 (Th2) cells to identify differentially expressed miRs. We used quantitative RT-PCR to confirm the differential expression levels. WST-1, ELISA, and flow cytometry were used to evaluate the survival, function and phenotype of cells, respectively. We employed mice transgenic for the identified miRs to determine the biological impact of miR-17-92 expression in T cells.
Results: Our initial miR microarray analyses revealed that the miR-17-92 cluster is one of the most significantly over-expressed miR in murine Th1 cells when compared with Th2 cells. RT-PCR confirmed that the miR-

17-92 cluster expression was consistently higher in Th1 cells than Th2 cells. Disruption of the IL-4 signaling through either IL-4 neutralizing antibody or knockout of signal transducer and activator of transcription (STAT)6 reversed the miR-17-92 cluster suppression in Th2 cells. Furthermore, T cells from tumor bearing mice and glioma patients had decreased levels of miR-17-92 when compared with cells from non-tumor bearing counterparts. CD4+ T cells derived from miR-17-92 transgenic mice demonstrated superior type-1 phenotype with increased IFN-y production and very late antigen (VLA)-4 expression when compared with counterparts derived from wild type mice. Human Jurkat T cells ectopically expressing increased levels of miR-17-92 cluster members demonstrated increased IL-2 production and resistance to activation-induced cell death (AICD).
Conclusion: The type-2-skewing tumor microenvironment induces the down-regulation of miR-17-92 expression in T cells, thereby diminishing the persistence of tumor-specific T cells and tumor control. Genetic engineering of T cells to express miR-17-92 may represent a promising approach for cancer immunotherapy.
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III-14 Information-guided surgical management of gliomas using low-field-strength intraoperative MRI

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Introduction: Contemporary technological developments revolutionized surgical management of intraaxial brain tumors. The intraoperative MRI (iMRI) and updated neuronavigation permitted for neurosurgeons to perform tumor resection under precise guidance. Neurophysiological monitoring and brain mapping allows precise localization of the cerebral functions and preservation of the important function during removal of the tumor. Intraoperative histopathological diagnosis allows fast direct investigation for identification of the neoplastic cells. Incorporation of these adjuncts provides for the surgeon an opportunity to perform aggressive glioma resection with minimal risk of neurological morbidity. The present report highlights our experience with information-guided surgical management of gliomas using low magnetic field strength iMRI with an emphasis on tumor resection rate and outcome.
Material and Methods:
From 2000 to 2009, 574 surgeries for intracranial gliomas were performed in our clinic with the use of intraoperative MRI (iMRI) with magnetic field strength of 0.3 Tesla, updated neuronavigation, neurochemical navigation with 5-aminolevulinic acid, serial intraoperative histopathological investigations of the resected tissue, and comprehensive neurophysiological monitoring. Nearly half of patients (263 cases; 45.8%) were followed more than 2 years after initial surgery in our institute.
Results: Maximal possible tumor resection, defined as radiologically complete tumor removal or subtotal removal leaving the residual neoplasm within the vital functionally-important brain areas, was attained in 569 cases (99.1%). It included cases of radiologically complete tumor removal as well as subtotal removal leaving the residual neoplasm within the vital functionally-important brain areas detected with neurophysiological monitoring and/or brain mapping. The median resection rate constituted 95%, 95%, and 98%, for WHO grade II, III, and IV gliomas, respectively. Importantly, our low-field-strength iMRI showed high sensitivity for detection of the residual glioma, which was confirmed by postoperative high-field-strength MRI investigations. In no one case of the present series unexpected residual tumor was disclosed. Actuarial five-year survival was significantly worse in WHO grade IV gliomas (19%), but did not differ significantly between WHO grade III and II tumors (69% vs. 87%).
Conclusions: Information-guided management of gliomas using low-field-strength iMRI provides a good opportunity for maximal possible tumor resection, and may result in survival advantage, particularly in patients with WHO grade III neoplasms.

Session IV
Tumor Innitiating Cells

IV-1 Brain tumours can arise from stem/progenitor cells in the adult brain through activation of oncogenic pathways: evidence for pathway-specific tumour phenotype

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Introduction: To understand how brain tumours develop it is essential to know from which cells they arise and to identify events that occur during this transformation. It is possible that they arise by transformation of neural stem cells (NSC) which can self-renew and differentiate into neurons and glia, because they (i) contain multiple cell types, suggestive of an origin from a cell with multilineage potential; (ii) often arise from the ventricular zone; (iii) express certain stem cell markers; (iv) express genes that regulate NSC proliferation and (v) forced expression of oncogenes in neural stem and progenitors cells in mice produces tumours that are similar to primary human tumours.
Material and Methods: We used conditional knock-out mice to target the adult neural stem cell compartment by injecting Adenovirus expressing cre recombinase intraventricularly. Also, we derived either stem cells or astrocytes, expanded them in vitro and finally grafted them into recipient mice to test their growth, differentiation and the capacity to form tumours. We analysed tumours and grafts with gene expression arrays.
Results: Recombination of [Rb/p53] or of [Rb/p53/PTEN] showed features of primitive neuroectodermal tumours (PNETs). In contrast, inactivation of [PTEN/p53] resulted in diffuse gliomas, which arose from the SVZ and extended along the corpus callosum and usually were most prominent in the striatum and surrounding structures. Stem cells derived from brains shortly after intraventricular recombination and after expansion in vitro were grafted into recipient mice formed tumours, providing further evidence that the initial recombination was in the stem cell compartment while in contrast, astrocytes did not give rise to tumours.
Conclusion: We found that there is a strong link between the combination of genetic mutations genotypes in stem cells and the phenotype of the tumour. We also found that only stem cells but not astrocytes gave rise to brain tumours, independent of their location. This suggests a cell autonomous mechanism which enables stem cells to generate brain tumours whilst mature astrocytes do not form brain tumours in adults

IV-2 Marker-independent identification of glioma-initiating cells

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Human gliomas are primary neoplasms of the central nervous system that grow diffusely, show different grades of local aggressiveness and display morphologic and molecular phenotypes of glial lineages but also of less differentiated neural progenitors and stem cells (Wechsler-Reya and Scott 2001; Sanai, Alvarez-Buylla et al. 2005; Read, Hegedus et al. 2006). Although the exact cellular origin of gliomas remains unclear (Sanai, Alvarez-Buylla et al. 2005; Read, Hegedus et al. 2006), it has been proposed that a small fraction of cancer cells constitutes a unique reservoir of glioma initiating cells (GICs) controlling tumor growth (Hemmati, Nakano et al. 2003; Galli, Binda et al. 2004). GICs were prospectively identified as CD133-expressing cells (Singh, Clarke et al. 2003; Singh, Hawkins et al. 2004). However, CD133 might not be sufficient to identify all tumor initiating cells in gliomas as CD133- cells derived from some glioma types have comparable self renewal and tumorigenic properties (Clément et al, 2009; Beier, Hau et al. 2007; Wang, Sakariassen et al. 2007; Zheng, Shen et al. 2007; Ogden, Waziri et al. 2008). For further comprehension of cellular hierarchies in brain tumors it is necessary to develop alternative or complementary selection strategies to identify, isolate and characterize GICs. Here, we show that the combination of a distinctive morphotype and emission of autofluorescence signals upon 488nm laser excitation identifies a subpopulation of cultured and prospectively isolated glioma cells with tumor- initiating and long term self-renewing capacities. Furthermore, glioma cell autofluorescence correlates with enhanced expression of stemness genes, higher metabolic activity and a specific cell cycle profile. The FL1+ phenotype did not correlate with the expression of proposed GIC markers. Our data propose an alternative approach to investigate tumor-initiating potential in gliomas and to advance the development of new therapies and diagnostics.

IV-3 A distinct subset of glioma cell lines with stem cell-like properties reflects the transcriptional phenotype of human glioblastomas and overexpresses CXCR4 as therapeutic target

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Introduction: Glioblastomas contain a subpopulation of cells that display stem-like properties and have tumor-initiating capacity. We investigated whether cell lines which were established under serum-free neural stem cell conditions and maintain stem cell-like properties in vitro (GS cell lines) preserve the expression phenotype of human glioblastomas more adequately than conventional glioma cell lines and express specific genes amenable to therapeutic targeting.
Material and Methods: 75 samples were profiled using Affymetrix HG-U133 Plus 2.0 microarrays, including GS cell lines, original tumor samples, conventional serum-cultured glioma cell lines, and monolayer cultures established from the same tumors as GS-lines using standard serum conditions. In addition, we validated CXCR4 as specific therapeutic target in vitro and in vivo and studied its regulation.
Results: A distinct subset of GS cell lines that displayed a full stem-like phenotype (GSf lines) mirrored the expression signature of glioblastomas most closely. These cell lines are highly tumorigenic and invasive in vivo, express CD133, grow spherically in vitro, are multipotent and display a proneural gene expression signature, thus emerging as the most representative model for human glioblastomas both functionally and transcriptionally. In contrast, GS lines with a restricted stem-like phenotype (GSr lines) exhibited expression signatures resembling conventional glioma cell lines, which are most distant from original tumors by cluster analysis, indicating that the transcriptional resemblance between GS lines and tumors is associated with different degrees of "stemness". We identified CXCR4 as overexpressed in tumors and GSf lines compared with conventional glioma cell lines, and CXCR4 was the only gene that was not expressed in normal brain, thus emerging as a potential therapeutic target. GSf lines contained a minor population of CXCR4hi cells, which partially coexpressed CD133 and was expandable by hypoxia, whereas conventional cell lines contained a major proportion of CXCR4lo cells. Convection-enhanced local treatment with AMD3100, a specific CXCR4 antagonist, inhibited the highly invasive growth of GS xenografts in vivo and cell migration in vitro.
Conclusion: The group of GSf cell lines represents the most appropriate model for human glioblastomas, mirroring the original tumor gene expression signature most closely and maintaining highly invasive growth in vivo as well as stem cell characteristics in vitro. CXCR4 is an attractive specific target to treat the otherweise therapy-resistant, tumor-initiating glioma stem cells within a narrow enough therapeutic window to make this a safe strategy.

IV-4 Knock down of HIF-1a in glioma cells reduces migration in vitro and invasion in vivo and impairs their ability to form tumor spheres

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Introduction: Glioblastoma (GBM) is the most common and the most malignant primary intracranial human neoplasm. GBMs are characterized by the presence of extensive areas of necrosis and hypoxia. Hypoxia and its master regulator, hypoxia inducible factor 1 (HIF-1) play a key role in glioma invasion. We wanted to further elucidate the functional role of HIF-1a in glioma cell migration in vitro and in invasion in vivo.
Material and Methods: we used a shRNA approach to knock down HIF-1a expression complemented with genome-wide expression profiling, performed in both normoxic and hypoxic conditions. Next, we assessed the role that HIF-1a plays in maintaining the characteristics of cancer stem cells (CSCs) using the tumor sphere forming assay.
Results: Our data show that knock down of HIF-1a in human and murine glioma cells significantly impairs their migration in vitro as well as their ability to invade into the brain parenchyma in vivo. We also demonstrate that HIF-1a plays a role in the survival and self-renewal potential of CSCs.
Conclusion: Our expression profiling experiments in glioma cells provide a detailed insight into a broad range of specific biological pathways and processes downstream of HIF-1a and we discuss their role in the migratory and invasive properties as well as the stem cell biology of glioblastomas.

IV-5 Enhancer of ZESTE 2 (EZH2) is up-regulated in malignant gliomas and its targeting decreases cd133 expression

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Introduction: Glioblastoma multiforme (GBM) and other tumors may contain a fraction of cells that express stem cell programs and that in vitro are contained in neurospheres (NS). Genes of the Polycomb Repressive Complex 2 (PRC2), and specifically Enhancer of Zeste 2 (EZH2), may play a key role in stem cell maintenance: here we have investigated the expression of EZH2 in gliomas and the effects of its inhibition.
Material and Method:We used the NS assay and a combination of DNA microarray, real time PCR and immunohistochemistry to study EZH2 expression in gliomas. EZH2 was also targeted by specific siRNA and by the histone deacetylase inhibitor SAHA.
Results: GBM-NS were obtained in 50% of the GBM examined and these GBM were associated to significantly shorter overall survival. As shown by real time PCR and by immunohistochemistry, GBM-NS express high levels of EZH2, similarly to primary GBM but not to low-grade gliomas. Targeting EZH2 by siRNA favoured NS differentiation and decreased expression of the stem cell marker CD133. Treatment of GBM-NS

with histone deacetylase inhibitor SAHA (2 microM) decreased EZH2 and CD133 expression.
Conclusions: These results show that GBM-NS, as a good model of primary GBM biology, are also informative on the prognosis. They show that EZH2 is highly expressed in malignant gliomas and may influence the expression of CD133, encouraging EZH2 targeting by therapeutic molecules including HDAC inhibitors.

IV-6 A neural stem cells marker fatty acid binding protein 7 (FABP7) is involved in proliferation and invasion of glioblastoma cells

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Malignant brain tumours are among the most deadly cancers. Current treatment strategies, such as surgery, chemotherapy and radiotherapy only modestly improve patient survival. The limited success of these strategies is largely due to a high incidence of tumor recurrence after the treatment. Recent evidences suggested that a rare population of stem-like cells (or tumour initiating cells) present in brain tumours might be responsible for the aggressiveness and recurrence of these tumours. These cells, named brain tumor stem-like cells (BTSC), share many properties of normal neural stem cells, including self renewal, extended proliferation, formation of neurospheres (when cultured in vitro in the presence of growth factors) and potential to differentiate into neurons and glial cells. Further investigations have suggested that studying this BTSC population in glioblastoma (GBM) is a more relevant system for exploring glioma biology than the adherently growing glioma cell lines.
In the current study we identified target genes and proteins that are differently expressed between adherent glioma cell lines and sphere growing BTSC, using microarray and differential proteomics, respectively.
Fatty acid-binding protein 7 (FABP7) was identified as overexpressed in BTSC compared to glioma adherent cell lines both at the gene as well as at the protein level. FABP proteins are a family of small, highly conserved, cytoplasmic proteins that bind long-chain fatty acids and other hydrophobic ligands. FABPs are thought to play important roles in fatty acid uptake, transport, and metabolism. Functionally, FABPS were described to play a role in gene regulation, cell signaling, cell growth and differentiation. The functional role of FABP7 in glioblastoma was investigated by assessing the effect of silencing FABP7 on migration and cell proliferation of glioblastoma cells. We observed that down-regulating FABP7 expression with FABP7-specific small interfering RNAs significantly reduced in vitro cell proliferation and even more the migration of BTSC. Since increased tumour infiltration was reported as a consequence of radiotherapy, the potential involvement of FABP7 in this process was evaluated. Irradiated BTSCs displayed significantly higher FABP7 protein levels and therefore we suggest that FABP7 may contribute to the radiotherapy-induced aggressive phenotype of BTSCs. Moreover, further mechanistic investigations indicated that directly or indirectly reducing the expression of FABP7 significantly impacts on the behaviour of glioblastoma derived BTSC.

IV-7 Imaging and role of bone marrow derived progenitor cells in intracranial tumor neovascularization

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Introduction: Glial tumors are the most common and lethal primary adult brain tumor and the histopathological hallmark of their malignant transformation is vascular proliferation. Hence novel strategies to target tumor vascularity are continuously being explored in hopes to improve patient outcomes. More recent interest is focused on the role of bone marrow derived precursors cells (BMDCs) in tumor neovascularization. Whether BMDC differentiate into endothelial cells (EC) or perivascular support cells (PVC) is controversial and whether it is tumor type or tumor stage dependent remains unknown. Finally, impact of ionizing radiation (IR) on recruitment, migration and differentiation of BMDCs in brain tumor vasculature has not been examined to date.
Material and Methods: Animal Models: Chimeric NOD/SCID mice were generated by stably reconstituting the bone marrow (BM) with BM derived from green-fluorescent protein transgenic mice. In these mice brain tumor xenografts in an intracranial window chamber model (ICW) were generated using U87 glioma cells engineered to stably express mCherry fluorochrome. Radiation Therapy (RTx): In order to examine effects of IR U87-mCherry tumors were treated with hemicranial irradiation at two regimens, 3x2Gy or 3x5Gy. The U87-mCherry+RTx arm was compared to RTx alone or U87-mCherry alone (15 mice each). In-vivo imaging: Two-photon laser capture microscopy (2PLM) was used to obtain real-time in-vivo longitudinal images of the tumor cells, tumor vasculature and allow tracing of the circulating GFP+BM cells. Mice were imaged in a longitudinal manner following cell implantation or following completion of RTx in a temporal course, over 1d,2d,3d,7d,10d,14d,21d. Brain Tumor Analysis: Mice were sacrificed in accordance with animal care protocol, using perfusion fixation and brains collected for correlative immunohistochemical (IHC) analysis.
Results: Within 24 hours following tumor cell implantation GFP+ BMDCs can be seen circulating intravascularly and lining the vessel wall. After 7d post implantation number of GFP+BMDCs within vessels decreases whilst cells migrating, differentiating and integrating outside the vessel lumen increases. IHC and 2PLM analysis identified three distinct GFP+ cells: 1) circulating cells in vascular lumen, 2) supporting cells intimately encasing vessel walls that are PVCs and 3) infiltrating cells within tumor microenvironment that are macrophages. Control animals demonstrated a temporary recruitment of GFP+BMDCs to the injection site with no differentiation into cellular processes.
Conclusions: Our results are the first to examine the dynamic evolution of BMDC in glioma vasculature in a real-time and manner. We demonstrate that BMDC differentiate to form macrophages and vascular support structures in the tumor microenvironment and not endothelial cells. Ongoing work focuses on understanding alterations in response to IR and molecular regulators of BMDC.

IV-8 Personalized drug discovery based on cancer stem cell biology

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Introduction: Cancer stem cell (CSC) of glioblastoma (GBM) is an attractive therapeutic target because GBM is maintained and endowed with chemo-radiation resistance by CSC. CD133 was previously identified as a specific marker for GBM CSC. However, whether CD133 can be a reliable therapeutic target has not been elucidated yet.
Results: To address this issue, we tested clinical implications of CD133 using primarily cultured GBM cells and found that CD133 expression could not predict patients' prognosis. In addition, several groups including us reported that both CD133 positive and negative GBM cells harbor in vivo tumorigenic potential. These discrepancies provoked the need for other prognostically significant CSC markers. We postulated that proteins related with chemo-radiation resistance could be more reliable markers. Accordingly, expression of candidate proteins was tested against clinical outcome of GBM patients using tissue microarray technique. It was found that c-Met high GBMs had significantly worse prognosis. Subsequently, we confirmed that c-Met is a reliable CSC marker and that CSCs can be targeted using c-Met in vitro and in vivo. We also established a GBM CSC library that includes tumor tissues, primary dissociated cells and clinical data of patients. Primary CSC culture enables us to exam newly-developed agents in an environment that closely mimics those of GBM patients.
The corresponding clinical data and tumor tissues make it possible to compare pre-clinical results with genetic characteristics, gene expression profiles and prognosis. In addition, we can categorize patients according to responses to therapeutics, genetic characteristics, and gene expression patterns, which would be useful to find

biomarkers that could predict treatment response. Using this library, we found that c-Met could be used to target GBM CSCs in the GBMs that have c-Met amplification and/or secrete hepatocyte growth factor (HGF). This indicates that c-Met amplification/expression and HGF secretion need to be elucidated before the application of c-Met targeting strategies.
Conclusions: Altogether, the application of CSC has great potential to find novel and effective therapeutic targets for GMB, and the drug screening technology using CSC would cast a new light on the personalized diagnosis and targeted therapy.

IV-9 A continuous mesenchymal expression signature is recapitulated in glioma stem cells and correlates with radiation and oncogenic pathway signatures.

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Introduction: To guide new therapies targeting at treatment refractory patients, requires a better understanding of the molecular subtypes of diffuse gliomas. Prior attempts to address these issues from high-throughput array data have been limited to small sample sizes or to a single histologic subtype (e.g. GBM). We proposed to overcome these limitations by combining data from multiple sources into the largest reported unified dataset.
Material and Methods: Affymetrix GeneChip data from published and unpublished sources with raw intensity files and clinical annotation (grade, age, survival time, vital status) were included for analysis after minimizing GeneChip-associated batch effects. Unsupervised clustering was performed to identify major subgroups. Data from 517 tumors were randomly divided into training and validation sets for predictive modeling. Candidate models were evaluated by cross-validation and the molecular-clinical prediction (MCP) model was selected. An independent verification cohort (328 tumors), including data form the Cancer Genome Atlas, was used for further validation. Stem cell data [both normal neural (NSC) and glioma cancer (GSC)] were obtained from published sources. Univariate and multivariate correlations and survival analyses were performed to published radiation-associated and oncogene-pathway gene signatures.
Results: Two molecular subtypes of infiltrating gliomas, each with distinct survival outcomes, were observed by unsupervised analyses. The poorer surviving subtype contained genes associated with extracellular matrix and cell motility, typical of cells of mesenchymal origin (MES). The majority of GBMs belonged to this subtype. The MCP, enriched for MES genes, improved significantly over age and grade alone in predicting survival in all datasets. MES signature scores were highly correlated to radiation-associated as well as Ras- and Src- pathway signatures.
Conclusions: Using the largest reported unified glioma gene expression dataset we identified a mesenchymal gene signature associated with poor survival and which corresponded to genes specifically expressed in GSCs. We used this dataset to develop a robust prognostic marker of patient survival that outperforms existing clinico-pathologic factors, such as age and grade, and validated it on an independent dataset. This signature correlated with radiation-associated genes as well as the Src- and Ras- pathways, suggesting that its survival association may be related to radiation response and oncogene pathway addiction.

IV-10 Remission and relapse in pediatric low grade astrocytoma: an oppositional relationship between stemness and p53?

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Introduction: Central tenets of the cancer stem cell hypothesis remain contentious for adult brain cancers1. However pediatric low-grade astrocytomas (PLGAs) conform very well to one facet of the stem cell model. Specifically, PLGAs frequently recur after an initial response to chemotherapy; however, the recurrent tumors are little changed from the primaries and often respond again to an identical regimen of chemotherapy. Repetitive cycles of chemotherapy-induced remission and relapse are predicted by the cancer stem cell hypothesis but difficult to reconcile with the fact that the majority of PLGAs are p53-positive.
We show here that PLGAs express Olig2 – a bHLH transcription factor that marks neural progenitor cells and regulates self-renewal in the developing CNS. We present evidence that the stereotypical cycles of remission and relapse in PLGA reflects an intrinsic oppositional relationship between p53 and Olig2 in stem-like tumor cells.
Materials and Methods: Archival samples of pilocytic (WHO grade I) and fibrillary (WHO grade II) astrocytomas were obtained from Children's Hospital Boston and Olig2 expression was monitored by immunohistochemistry. Gene targeting methods were used to disrupt Olig2 and/or p53 in murine neural progenitors. Hairpin RNAi was used to knockdown Olig2 expression in p53-positive and p53 mutant neurosphere cultures from adult gliomas.
Results: All low-grade astrocytomas are positive for Olig2 expression. Murine neural progenitors that express both Olig2 and p53 are resistant to ionizing radiation or to genotoxic drug treatment (Temozolomide). Ablation of Olig2 unmasks p53-dependent responses (growth arrest/apoptosis) to radiation or drug treatment. Neurosphere cultures from p53 positive adult gliomas are resistant to radiation unless endogenous Olig2 expression is knocked down by hairpin RNAi. By contrast p53-null human glioma cells are resistant to radiation irrespective of Olig2 expression.
Conclusions:
Pediatric low-grade astrocytomas may be more properly described as tumors comprised of developmentally stalled, Olig2-positive neural progenitors. Recurrence of these p53 positive pediatric tumors following an initial response to chemotherapy may reflect an intrinsic oppositional relationship between p53 and "stemness" which has been noted in multiple other stem cell studies2.

IV-11 Characterization of glioma stem cells and its role in tumor invasion

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Introduction: There is accumulating evidence showing the existence of cancer stem cells (CSCs) in malignant gliomas, and these CSCs are implied to play crucial roles in tumor initiation, resistance to chemo-radiation therapy and tumor recurrence. In the present study, we isolated cancer stem-like cells from U251 human glioma cell line, characterized the biological features and investigated their roles in glioma invasion.
Materials and Methods: Cells forming spheres in the presence of mixture of growth factors were isolated from U251 glioma cells and examined their characterization as CSCs. Proliferation of the sphere-forming cells (SFCs) was examined by Alamar blue assay. Migration and invasion activities of SFCs were analyzed by Boyden chamber assay and a rat brain slice. In addition, gene expression of these cells was investigated.

Results: SFCs had abilities of self-renewal and differentiating into neuron-like cells. SFCs continued to proliferate for months and formed solid tumors when grafted subcutaneously to nude mice even if the number of the grafted cells was two hundred. SFCs possessed much higher migration and invasion activities than the parent glioma cells. Gene expression studies demonstrated that SFCs highly and specifically expressed matrix metalloproteinase-13 (MMP-13). Immunoprecipitation assay showed that the active form of MMP-13 was intensely expressed. Treatment of SFCs with a specific inhibitor of MMP-13 markedly suppressed tumor invasion in the brain slice.
Conclusion: U251 human glioma cells contained a sub-population of cells that are characterized as CSCs. These cancer stem-like glioma cells showed high activities of cell migration and invasion. It was suggested that high activity of MMP-13 expressed in the stem-like glioma cells was responsible for the increased invasion of glioma cells.

Session V
Basic Science: Angiogenesis / Invasion

V-1 Mechanisms of evasive resistance to VEGF inhibition in mouse models of glioblastoma

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Glioblastoma multiforme (GBM) is an aggressive, highly-angiogenic and fast-growing brain tumor for which the current prognosis is dismal. Bevacizumab/Avastin, an antibody against the proangiogenic factor VEGF, has generated much excitement in the brain tumor community as an effective treatment against GBM, and just received approval from the FDA as a single agent for recurrent disease. Despite improving overall survival and quality of life, however, thus far Avastin, either as a single agent or in combination with cytotoxic drugs has shown transitory benefits and is followed by at least two major recurrence patterns in which relapsing GBM tumors develop either a revascularized growth pattern and/or exhibit a more invasive phenotype as inferred by MRI imaging in patients. The currently experimental evidence, which is not yet definitive, suggests that there exist different adaptive mechanisms that manifest the evasive resistance patterns to antiangiogenic therapies. We have revealed that recruitment of specific bone marrow-derived pro-angiogenic cell subpopulations can avert the necessity of VEGF signaling and thereby effect re-initiation and continuance of tumor angiogenesis. Genetic or pharmacological abrogation of VEGF activity in mouse models of GBM and GBM stem cells also provokes a proinvasive phenotype which is qualitatively different from the typical infiltration of tumor cells into the brain parenchyma as tumor cells migrate preferentially along blood vessels deep into the brain parenchyma; this angiotropism is referred to as perivascular tumor invasion. Surprisingly, we found that VEGF itself is a direct and negative regulator of motility and invasion of glioblastoma cells in vitro and in vivo, partly by interfering with the proinvasive cues of the tyrosine kinase receptor c-Met. These results call attention to the need of recognizing evasive patterns in patients before tumor relapse in order to combine anti-VEGF therapies with anti-invasive or additional and alternative anti-angiogenic strategies to sustain response and prolong survival.

V-2 A FURIN/MMP-14 signaling cascade processes bai1 into a novel secreted anti-angiogenic and anti-tumorigenic factor, vasculostatin-40 (VSTAT40)

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Introduction: Mechanisms of vascular development and maintenance in the brain are not well-understood, but vascular integrity is compromised in many neurological diseases, particularly highly aggressive glioblastoma multiforme (GBM), which co-opts the existing vasculature and induces extensive angiogenesis. Brain angiogenesis inhibitor 1 (BAI1), a brain-enriched transmembrane receptor, has been proposed to be an endogenous regulator of brain angiostasis, but its mechanisms of action are largely unknown.
Material and Methods: We investigate the proteolysis of extracellular BAI1 using genetic and molecular biology techniques, and use an inducible orthotopic xenograft model to determine its anti-tumor activity.
Results: We show that the large extracellular domain of BAI1 is proteolytically cleaved to generate an abundant product of 40 kDa we named Vasculostatin-40 (Vstat40). We demonstrate that cleavage of Vstat40 from BAI1 is dependent on a two-step process of proteolytic activation. In the final step of the cascade, Vstat40 is directly processed from extracellular BAI1 by membrane type-matrix metalloproteinase 1 (MMP-14). We show that the upstream activity of proprotein convertases, primarily furin, plays an important role in facilitating this processing event. We confirm the significance of Vstat40 as an important angio-regulatory factor by demonstrating that it inhibits angiogenesis in culture in a CD36-dependent manner. Finally, we present data on the effect of Vstat40 on intracranial glioma growth and tumor-associated angiogenesis.
Conclusions: We characterize a novel BAI1 processing event and demonstrate its significance in order to illustrate its potential as a future brain tumor therapeutic.

V-3 VEGF and angiopoietin signalling pathways regulate angiogenesis and myeloid cell infiltration in gliomas

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Glioblastoma is not only the most common and most malignant but also the most highly vascularized brain tumor. Anti-angiogenesis is considered one of the most promising therapeutic targets for glioblastoma patients. We have previously shown that vascular endothelial growth factor (VEGF) and Tie2/Angiopoietin signalling pathways are crucial for angiogenesis and vascular maturation in glioma models in rodents. Inhibition of VEGF and Tie2 receptor signalling lead to endothelial cell apoptosis, an immature chaotic vasculature, diminished vessel perfusion and inhibition of glioma growth in vivo. However, in clinical trials anti-angiogenic therapy is only partly successfull. In order to investigate potential escape mechanisms we examinded the role of bone marrow derived myeloid cells (BMDC) in glioma angiogenesis. By use of transgenic and chimeric mice we show that I. VEGFR-1 signalling on myeloid cells is crucial for glioma angiogenesis and II. identify Angiopoietin-2 as a novel, endogenous, endothelial derived regulator of BMDC infiltration in gliomas.
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V-4 Bevacizumab changes blood flow and increases cell invasion in glioblastoma xenografts

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Introduction: Glioblastoma (GBM) is a highly vascularised tumour and endothelial cell proliferation is one of the neuropathological hallmarks of the disease. Therefore the concept of interfering with the generation of new blood vessels is an attractive strategy against GBM. Recent clinical trials have shown good response rates with bevacizumab, an antibody against vascular endothelial growth factor (VEGF). Yet, the effect is short lived and the physiological mechanisms of bevacizumab action and the biological consequences are poorly understood.
Material and Methods: Here we assessed the response to bevacizumab of highly angiogenic and invasive GBM xenografts, obtained by serial passaging of human GBM biopsies in nude rats. Animals with orthotopic tumours received weekly i.v. injections of 10 mg/kg bevacizumab for 3 weeks. Before sacrifice, animals were analysed by magnetic resonance imaging (MRI) on a 7T Pharmascan (Bruker). MRI protocols included T1 and T2-weighted sequences to assess tumour morphology and oedema, MR spectroscopy to assess key metabolite concentration, Diffusion Weighted Imaging to assess cellularity, and Dynamic Contrast Enhancement MRI to assess tumour perfusion and vascular permeability. After sacrifice, tumours were harvested for transcriptomic, proteomic and metabolomic studies as well as histology and immunohistochemical analysis.
Results: In agreement with clinical data, we found that Bevacizumab reduces vessel permeability and leakiness of the blood-brain barrier as reflected by the loss of contrast enhancement and reduced Ktrans and Ve parameters. Interestingly, Bevacizumab reduced blood flow and blood volumes, while spectroscopy data point at increased lactate concentration in treated tumours. A reduction in vessel number was observed while the extent of cell infiltration in the brain parenchyme was significantly increased. Key angiogenesis-related genes were upregulated after treatment.
Conclusion: Bevacizumab treatment in GBM leads to a reduction of blood vessels and increased tumor hypoxia which is accompanied by increased cell invasion. A novel model of tumour cell plasticity involving a metabolic switch will be discussed.

V-5 Novel anti-invasive and anti-angiogenic mechanisms of MTOR inhibition in glioblastoma

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Inhibition of mammalian target of rapamycin (mTOR) by temsirolimus (CCI-779) only has modest single compound activity in recurrent glioma. Similar to other targeted therapies a combination with radiotherapy might be interesting. The first part of the presented project aims at analyzing combined mTOR/radiotherapy in the syngeneic, orthotopic VM/Dk/SMA-560 mouse glioma model. The combined treatment of CCI-779, a small-molecule inhibitor of the mTOR kinase complex (mTORC) 1 approved for advanced renal cell carcinoma and mantle cell lymphoma, at 20 mg/kg from day 3 until day 17 and focal irradiation at 6 Gy on day 5 after tumor cell inoculation demonstrated remarkable antiangiogenic and antitumoral activity as well as prolonged survival of tumor bearing animals.
Loss of phosphatase and tensin homologue on chromosome ten (PTEN), which is a common event in glioblastoma, results in activation of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt)/mTOR signaling pathway, leading to neovascularization, cell cycle progression and escape from apoptosis. So far, conflicting data on the sensitivity of PTEN wild-type (wt) versus mutant cells exist. Similarly, the relevance of the feedback activation of Akt by inhibition of mTORC1 is debated. Here, analysis of PTEN on mRNA, promoter methylation as well as protein levels clearly demonstrates for cell lines as well as primary glioma cells that proliferation of PTEN wt cells is also sensitive to mTORC1 inhibition albeight at higher concentrations. Observing differential effects of combined mTORC1 and 2 inactivation in response to shRNA-mediated silencing of mTOR as compared to sole inhibition of mTORC1 by CCI-779, we further demonstrate that feedback activation of Akt, which is more prominent in PTEN mutant than in wild-type cells, may in fact have additional therapeutic antiinvasive and antiangiogenic effects via inhibition of a G-protein-interacting protein and vascular endothelial growth factor receptor (VEGFR)-2, respectively. CCI-779 exerted marked anti-angiogenic effects both by reducing levels of VEGFR and by inhibiting radiation-enhanced proliferation of brain endothelial cells. Moreover, CCI-779 applied after radiosensibilization inhibited glioma invasiveness in a supra-additive way and reverted the proinvasive effect of sublethal irradiation alone. The results support the clinical evaluation of combined targeted mTOR inhibition with CCI-779 and radiotherapy in patients with newly diagnosed glioblastomas that is conducted by the European Organization for Research and Treatment of Cancer.

V-6 NCCTG phase II trial of bevacizumab in combination with sorafenib in patients with recurrent glioblastoma multiforme

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Introduction: Angiogenesis inhibition represents a promising therapeutic strategy in the treatment of GBM. We hypothesized that combination of the anti-VEGF antibody, bevacizumab with sorafenib, a small molecule kinase inhibitor of VEGFR2, PDGFRb and RAF kinases, would result in improved antiangiogenic and antitumor activity in recurrent GBM patients.
Material and Methods: Recurrent GBM patients not on EIACs who have received £ 1 regimens for progressive disease are eligible for the trial. Pertinent exclusion criteria include poorly controlled hypertension, bleeding diathesis, ongoing anticoagulation, and prior antiangiogenic therapy. A one stage, three outcome phase II trial design (Sargent et al, 2001) was applied with six month progression-free survival being the primary endpoint. Patients received bevacizumab 5 mg/kg every two weeks in combination with sorafenib 200 mg bid, weekly, days 1-5.
Results: A total of 35 patients have been enrolled to date. Toxicity analysis after the first 19 patients were accrued, showed a high incidence of grade ³ 3 non-hematologic toxicity: 39.3% in cycles 1 or 2, with most common toxicities being fatigue, thrombosis, hypophosphatemia, and muscle weakness; overall 68.5% of the patients experienced ³ grade 2 fatigue. Despite a high objective response rate of 37% (7/19), toxicity resulted in early treatment discontinuation in 29% of the patients and treatment refusal in 10.5%. An additional 16 patients have been treated following decrease of the starting sorafenib dose to 200 mg qd. This modification has significantly improved treatment tolerance with a 9% incidence of ³ 3 non-hematologic adverse events in the first two treatment cycles. Initial data from the correlative laboratory analysis, including assessment of molecular and cellular markers of angiogenesis, indicated a decrease in circulating endothelial cells starting with the first treatment cycle with subsequent increase at the time of disease progression. Dynamic contrast-enhanced perfusion and permeability MR imaging was performed at baseline, day 2-3, day 30, and every 2 months thereafter. Decreased contrast enhancement was observed as early as 24 hours after treatment initiation.
Conclusions: Combination of bevacizumab at 5 mg/kg every two weeks with sorafenib 400 mg bid days

1-5 has resulted in a high objective response rate, however, with significant toxicity. Sorafenib dose decrease resulted in improved tolerance. Updated toxicity, efficacy, correlative analysis and imaging data will presented at the meeting.

V-7 Vascular targeting strategies using F8-SIP antibody against the ed a domain of fibronectin in glioma angiogenesis

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Introduction: Vascular targeting strategies have become a promising approach for glioma diagnostics and therapy. The alternatively spliced extra domain A (ED-A) of fibronectin represents a promising neoangiogenic marker in this concept. The aim of our study was to characterize biodistribution characteristics of an ED-A small immunoprotein (F8-SIP) during anti-angoiogenesis and to analyze the microcirculatory effects of F8-SIP mediated photodynamic therapy (PDT).
Material and methods: SF126 glioma cells were implanted subcutaneously and intracerebrally into nude mice. Microvascular and interstitial accumulation of F8-SIP, microvascular blood flow rate and preferential binding sites were analyzed after intravenous application of ALEXA555-F8-SIP using intravital microscopy. Treatment with Sunitinib (i.p., 40 mg/kg/day) was initiated on day 6 after tumor cell implantation and was applied daily for 6 days. Intravital microscopic analyses were performed on day 8 (acute phase[AP]) and on day 12 (end phase[EP]) after tumor cell implantation and compared to tumors without therapy (basic group[BG]). In PDT experiments red light irradiation (150j/cm2) was applied on tumors after i.v. administration of photosensitizer-coupled F8-SIP and microcirculatory alterations as well as tumor size were analyzed daily for 4 days.
Results: Antiangiogenic treatment increased microvascular binding (EP, t24: 102±4,9 vs. BG, t24: 85,5±6,8; $p<0,05$). Extravasation of F8-SIP into tumor interstitium was significantly increased in both therapy groups (AP, t24: 76,2±4,9 and EP, t24: 77,5±9,9 vs. BG, t24: 61,4±6,3; $p<0,05$, respectively). PDT resulted in short initial hyperperfusion followed by significantly reduced functional vessel density (Pre-PDT: 200±122 cm/cm2; Post-PDT:80±74cm/cm2), microvascular blood flow rate (Pre-PDT:42±29nl/s; Post-PDT:18±10nl/s) and perfusion index (Pre-PDT: 0,65±0,14; Post-PDT:0,38±0,32). Microcirculatory failure recovered to normal values 24-48h after single PDT. Single PDT led to short-term reduction of tumor growth 48h after treatment (PDT: 115±31mm3; Control: 205±91mm3) followed by recovery of growth rate. Only repetitive PDT induced long term reduction of tumor growth (PDT: 139±44mm3; Control: 322±112mm3)
Conclusion: F8-SIP may represent a useful tool to specifically target glioma microvessels. Our results provide insights into microvascular consequences of F8 vascular targeting strategies useful for future diagnostic and therapeutic interventions.

V-8 Nuclear localization of upa and its interaction with HOXA5 suppresses p53 expression in glioma cells and activates angiogenic response in endothelial cells

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Introduction: Studies have shown that the overexpression of HOXA5 in cancer cells results in cell death through a p53-dependent apoptotic pathway involving caspase 8 expression. Recent data using HOXA5 and p53 null mice have suggested their co-operation as tumor suppressors. Our studies and that of other researchers have also suggested that the expression of uPA by glioma cells influence the surrounding stromal cells to increase expression angiogenesis related molecules, thereby increasing the incidence of angiogenesis. Previously we have demonstrated that downregulation of uPAR and uPA caused the activation of the extrinsic apoptotic pathway. In this study, we have demonstrated that uPA has regulatory function and negatively regulates the expression of p53 in glioma cells and activates angiogenesis in endothelial cells.
Results: Nuclear localization of uPA was determined using standard immuno-localization where we observed that uPA was localized in the nucleus of U87 and SNB19 glioma cells. To further substantiate this finding we performed western blot analysis of nuclear, cytoplasmic and membrane fraction and observed that uPA is localized in the nucleus. To determine the regulatory role of uPA, we performed protein-protein binding assays with known transcription factors and observed that uPA shows strong binding to HOXA5 among other TFs. This was further confirmed by co-immunoprecipitation studies. Binding of uPA to HOXA5 was found to be independent of DNA as determined by EMSA studies using HOXA5 specific oligos. Downregulation of uPA caused increase in expression of p53 accompanied with decreased invasion and migration, whereas the simultaneous downregulation of uPA and HOXA5 did not show increased expression of p53, but a decrease in both invasion and migration was observed in both U87 and SNB19 glioma cells. Supplementing uPA in the growth media of endothelial cells induced expression of Angiogenin, accompanied with the formation of a rudimentary network as observed by standard angiogenic assay and nuclear localization of uPA.
Conclusions: Our results demonstrate for the first time that uPA may have regulatory functions in terms of cell survival and activation of angiogenesis, and that the expression of uPA by tumor cells show a strong pro-angiogenic influence on the surrounding endothelial cells.

V-9 PDGFR stimulates glioma cell invasion through src-dependent tyrosine phosphorylation of a bipartite guanine nucleotide exchange factor dock 180

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Introduction: Platelet-derived growth factor receptor (PDGFR)∂ ranks third among top 11 amplified genes in high-grade gliomas. Dock180, a guanine nucleotide exchange factor (GEF) for Rac1, mediates PDGFR-induced cell migration in Drosophila and is critical in glioma invasion. Here we report that PDGFR∂ promotes glioma invasion through Src-dependent phosphorylation of Dock180, activating the Dock180-CrkII-Rac1 pathway.
Material and Methods: Glioma specimens were analyzed by immuno-histochemical staining. Glioma cell migration and invasion was assessed by in vitro cell migration assays and glioma xenografts in the brain. Tyrosine phosphorylation (p-Y) of Dock180 was determined by Western blot (WB) analysis. Impact of PDGFR∂ Dock180 and p-Y of Dock180 on Rac1 activity, cell motility and interaction of Dock180 to CrkII and p130cas were assessed by inhibitors, RNAi depletion, mutagenesis and immunoprecipitation followed by WB.
Results: PDGFR∂ and Dock180 are co-expressed in invasive areas but not the central regions of clinical glioma specimens. PDGF-A stimulation of glioma cells that express endogenous PDGFR∂ promotes cell migration in vitro and glioma growth and invasion in the brain. Cellular depletion of Dock180 inhibits PDGFR∂-promoted Rac1 activity, cell migration in vitro, and glioma growth and invasion in the brain. PDGFR∂ induces p-Y tyrosine (Y) 1811 of Dock180 that is located in a CrkII-binding domain of Dock180. A Dock180

Y1811F mutant inhibits PDGFRβ-induced p-Y of Dcok180 and Rac1 activity. Co-expression of Dock180, PDGFRβ and CrkII promotes association of Dock180, CrkII, p130Cas and Rac1 activity whereas Y1811F mutant attenuates their association and Rac1 activation. Y1811 is also a predicted Src p-Y site. Inhibition of Src suppresses PDGFRβ-stimulated p-Y of Dock180, Rac1 activity and cell migration while a constitutively active Src induces p-Y of wild-type Dock180 but not Dock180 Y1811F mutant. Finally, re-introduction of wild-type Dock180 into LN444/PDGF-A/shRNA-Dock180 cells rescued PDGFRβ-promoted Rac1 activity and cell motility in vitro, and tumor growth and invasion the brain whereas re-expression of Dock180 Y1811F mutant failed to restore these PDGFRβ-stimulated cellular behaviors.
Conclusion: Our data reveals a molecular mechanism by which PDGFRa stimulates glioma cell invasion through tyrosine phosphorylation of a Rac1 GEF, Dock180, leading to increases in Rac1 activity and glioma cell invasion in the brain.

V-10 Ephrin-a2 modulates glioma cell invasion via EPHA2 signaling and correlates with patients survival in glioblastomas

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Introduction: The Eph receptors and their ligands, ephrins, represent the largest family of tyrosine kinases which are involved in neurodevelopmental processes, such as cell migration. Both Ephs and ephrins are transmembrane proteins and transduce signals in a bidirectional manner resulting in repulsion. Accumulated evidence including our previous reports demonstrated that some of these molecules link to the invading cell phenotype in human malignancy. To reveal the role of Eph/ephrin in glioma biology and patient's survival, clinical relevance of Eph/ephrin genes was determined in a clinically annotated expression data set of glioma surgical specimens. Further the function of Eph/ephrin in glioma invasion was analyzed.
Materials and Methods: The clinical significance of Eph/ephrin expression was investigated by analysis of Eph/ephrin mRNA expression level in 24 nonneoplastic brain and 171 glioma specimens. Candidate gene products were validated in surgical specimens and biological function of target genes was evaluated in migration and invasion assays.
Results: Levels of certain Eph/ephrin family members mRNA were significantly different in glioblastoma (GBM) samples (n=82) compared with normal brain. According to the Kaplan-Meier analysis, only ephrin-A2 level among 13 members of Ephs and 8 members of ephrins emerged to be a powerful predictor of a favorable survival in GBM (n=77, p=0.0069). Depletion of endogenous ephrin-A2 expression by small interfering RNA in high expressor glioma cell lines (SNB19, U251) accelerated migration and invasion, while the migration was inhibited by the addition of ephrin-A2/Fc chimera, which blocks ephrin-A2 and simultaneously stimulates EphA signaling. Forced expression of ephrin-A2 in low expressor cell lines (U87, T98G) slowed migration, treatment with ephrin-A2/Fc further suppressed cell migration concomitant with tyrosine phosphorylation of EphA, especially in T98G cells which express EphA prominently.
Conclusions: This is the first demonstration that ephrin-A2 negatively controls cell invasion through EphA signaling both in vitro and in vivo, identifying ephrin-A2 as a good prognostic factor in GBM.

V-11 Analysis of invasion patterns in a model of malignant brain tumor

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Introduction: The invasive phenotype of malignant brain tumors is a major cause for their recurrence and resistance to therapy. It has been suggested that tumor cells recapitulate the migration patterns of glial progenitors. However, it is still unclear which cells acquire a migratory potential, at which stage they acquire it and whether and how invasion patterns change during tumorigenesis and treatment. The present study aims to define the characteristics of infiltrating cells and the patterns of invasion of genetically-induced brain tumor-initiating cells (BTICs) in the syngeneic mouse adult brain.
Material and Methods: We have established a mouse malignant brain tumor model by overexpressing RASV12 in neural stem cells/multipotent progenitor stem cells derived from the subventricular zone of mice with a homozygous deletion of the Ink4a/ARF locus.
Results: Orthotopic implantation of these BTICs into 6-week-old wild type mice results in formation of highly invasive, hypervascular, serially transplantable glioblastoma-like tumors with a 100% penetrance and a 5-week median survival. Sacrifice of BTIC-injected mice at one week intervals followed by histopathological examination revealed that cellular migration was already detectable as early as seven days post-implantation. Infiltration along fiber tracts and blood vessels occurred long before mass proliferation and necrosis (fourth week), and even before the appearance of cellular atypia (third week) or encasement of blood vessels by tumor cells, macrophages and microglia (second week). Fluorescent microscopy of fixed brain slices revealed three major migration patterns: vascular, intraparenchymal and subpial. Furthermore, timelapse analysis of live cultured brain-slices from BITC-injected mice showed that tumor cells displayed a high motility from the second day after implantation. Movement along blood vessels was quick and directed away from the tumor, while the cells migrating intraparenchymally exhibited a saltatory conduction, with repeated extension and retraction of leading processes. Cells which exited the tumor early usually tested several routes before taking one and then exhibited a to-and-fro movement, creating paths for other cells.
Conclusion: Our results highlight a pronounced intravascular, intraparenchymal and subpial tumor cell migration preceding mass formation in the adult brain, suggesting the need for an early and sustained anti-invasion therapy.

V-12 Glioma associated microglia facilitate tumor invasion due to tumor induced overexpression of MT1-MMP

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Diffuse infiltration of glioma cells into normal brain tissue is considered to be a main reason for the unfavorable outcome of patients suffering from malignant gliomas. Invasion of glioma cells into the brain parenchyma is facilitated by metalloprotease-mediated degradation of the extracellular matrix. Metalloproteases are released as inactive pro-forms and get activated upon cleavage by membrane bound metalloproteases. Here, we show that membrane type 1 metalloprotease (MT1-MMP) is upregulated in glioma associated microglia, but not in the glioma cells. Overexpression of MT1-MMP is even lethal for glioma cells. Glioma-released factors trigger the expression and activity of MT1-MMP via microglial toll-like receptors (TLR) and the p38 MAPK pathway since deletion of the TLR adapter protein MyD88 or p38 inhibition prevented MT1-MMP expression and activity in cultured microglial cells. Microglial MT1-MMP in turn activates glioma derived pro-MMP-2 and promotes glioma expansion, as shown in an ex vivo model using MT1-MMP deficient brain tissue and a microglia depletion paradigm. Finally, both MyD88-deficiency or microglia depletion largely attenuated glioma expansion in two independent in vivo models.

V-13 RTVP-1 regulates glioma cell migration via interaction with N-WASP

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Gliomas, the most frequent primary brain tumor are characterized by increased proliferation and invasion into the surrounding normal brain tissue. In a recent study we reported that RTVP-1 is highly expressed in gliomas and that its expression is correlated with the degree of malignancy of astrocytic tumors. In addition, RTVP-1 is involved in the regulation of the growth, survival, and migration of glioma cells. To further delineate the molecular mechanisms underlying the effect of RTVP-1 in glioma cells we performed a pull down assay using His-tagged RTVP-1 followed by mass spectroscopy, and found that RTVP-1 was associated with the actin polymerization regulator, N-WASP. The association of RTVP-1 and N-WASP was further validated by co-immunoprecipitation and FRET analysis. Since N-WASP regulates cell spreading and migration we examined the role of RTVP-1 in these processes. We found that overexpression of RTVP-1 increased, whereas silencing of this protein decreased cell spreading and migration of glioma cells. The effect of RTVP-1 was mediated by N-WASP since silencing of N-WASP abolished RTVP-1 effects. In addition, RTVP-1 increased podosome formation in glioma cells and this effect was also mediated via its interaction with N-WASP. Moreover, the association of RTVP-1 and N-WASP was increased during cell spreading and appeared to be localized to contact areas of the spreading cells with the extracellular matrix. Another protein that was found to interact with RTVP-1 by the pull down assay is hnRNPK. This protein has been recently reported to associate with and to inhibit the effect of N-WASP on cell spreading. Using co-immunoprecipitation and FRET analysis we validated the interactions of hnRNPK with N-WASP and RTVP-1 in glioma cells. In addition, using co-immunoprecipitation studies, we found that overexpression of RTVP-1 decreased the association of N-WASP and hnRNPK, therefore suggesting that RTVP-1 may activate N-WASP by decreasing its association with hnRNPK. In summary, we report that RTVP-1 regulates glioma cell spreading, migration and invasion and that these effects are mediated via interaction with N-WASP and by interfering with the inhibitory effect of hnRNPK on the function of N-WASP.

V-14 Aberrant expression of microrna-21 triggers diffuse infiltration of human gliomas by disrupting negative feedback regulator, SPRY2

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Introduction: Diffuse infiltration of glioma is the hallmark of its malignancy. Although many comprehensive genomic approaches addressed major molecular mechanisms of tumorigenesis of glioma, it is still unclear how the onset of diffuse infiltration occurs at grade II. Here, we show that the significant loss of Spry2 expression by miR-21 is essential for the onset of this diffuse infiltration.
Material and Methods: miR-21 expression was analyzed in human glioma cell lines, glioma tissues, and normal brain by real-time-PCR and northern blot. Spry2 protein and mRNA was analyzed by immunohistochemistry, western blot, and real-time-PCR human glioma cellines and tissues. The functional significance of miR-21-targeted Spry2 was examined by MAPK activity assay, gelatin zymography assay, and invasion assay in glioma cellines that were transfected anti-miR-21 or miR-21.
Results: The aberrant expression of miR-21, triggered by tumor-environmental factors such as hyaluronan (HA) and growth factors(PDGF, EGF, bFGF), was necessary and sufficient to facilitate glioma invasion by targeting Spry2, a negative feedback regulator of Ras/MAPK signalling pathway. The down-regulation of Spry2 by miR-21 increased duration and amplitude of MAPK singling to induce MMP-9 expression. Consistently with these in vitro results, Spry2 protein levels were significantly decreased in 79.7% (55/69) of invasive WHO grade II-IV human gliom a tissues. The Spry2 protein levels were not correlated with their mRNA levels, although they were inversely correlated with miR-21 (Correlation coefficient of -0.50 with P=0.004) in 28 grade II-IV human glioma tissues.
Conclusions: Post-transcriptional regulation of Spry2 by miR-21 plays an essential role on the onset of diffuse infiltration of glioma and provides novel therapeutic marker for the infiltrative glioma.

V-15 Novel brain metastases models developed in immuno-deficient animals closely mimics the progression of metastatic brain tumors in patients

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Introduction: Brain metastasis is a common cause of mortality in cancer patients, and associated with poor prognosis. There is a need for a more detailed mechanistic understanding of the complex metastatic process, in order to develop better treatment strategies and improve patient outcome. We have developed animal models, where brain metastases from a number of different primary cancers are able to form tumors in the brains of immunodeficient rats. We also show that tumor cell lines obtained from patient brain metastases are able to form new tumors in nod/SCID mouse brains, after being injecting into the arterial circulation.
Materials and Methods: Brain metastases from patients were minced into small fragments, and tumor spheroids where implanted the brains of immunodeficient rats. Tumor growth was monitored regularly after implantation using MRI. We also generated tumor cell lines from a malignant melanoma brain metastases, injected melanoma cells into the left ventricle of nod/SCID mice, and followed tumor development by bioluminescence imaging.
Results: Successful tumor growth was achieved in 7 out of 9 human brain metastases implanted. Tumor pieces from 4 of the 7 metastases developing in the rat brain were serially transplanted into new animals and showed a 100% tumor take. MR imaging showed that the animal brain metastases exhibited similar radiological features as observed clinically. Histological comparisons between the primary tumors from the patients, the patient brain metastases and the xenografted brain metastases exhibited similar histology and growth patterns. Immunohistochemistry displayed similar marker expressions between the patient tumors and the corresponding animal xenografts. DNA copy number analysis showed a complex genomic profile both in the brain metastases from the patient and the corresponding animal brain metastases, and the majority of the aberrations were identical between the two samples. The melanoma cells developed tumors in the mouse brain 5 weeks after the intracardial injections. Tumors were also detected in several other organs of the body.
Conclusions: We have developed representative in vivo models for studying metastatic brain cancer. These models may be used to study in detail the molecular mechanisms underlying metastatic tumor spread, and to assess responses to new treatment strategies.

V-16 Macrophages promote glioma angiogenesis and invasion

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Solid tumors, including glioblastoma, contain large populations of both malignant and non-malignant cells. The tumor microenvironment contains a multitude of different cell types and recent evidence supports the role of CD45+ bone marrow cells such as tumor associated macrophages as vascular modulators. Tumor associated macrophages accumulate in hypoxic or necrotic regions of human tumors where they release VEGF. Recent studies have demonstrated that the number of M2-skewed macrophages (CD163+/CD204+) in human tumors correlates with glioma grade, cell proliferation and microvascular density. Macrophages are thought to promote the malignant phenotype of many solid tumor types but little is known about their influence on glioma biology. To evaluate the impact of macrophages on the malignant phenotype, we used co-culture experiments to determine if these cells enhance glioma cytokine and angiogenesis factor release and glioma cell invasion and proliferation. Mouse macrophages (ATCC) and U87 cells were cultured alone or together using a Boyden chamber to maintain cell separation. Conditioned media was evaluated for expression of both mouse and human chemokine expression using ELISA and quantitative PCR. Co-culture resulted in a significant increase in mouse (but not glioma cell) VEGF expression compared to baseline suggesting that macrophage-glioma crosstalk may promote macrophages to become an important source of VEGF in glioma tumors. However, macrophage and glioma cell co-culture resulted in a significant increase in PDGF expression of both macrophage and glioma cell origin. We next evaluated the impact of macrophages on glioma invasion. At baseline, U87 demonstrates minimal transwell migration in vitro and does not invade normal brain in vivo. When co-cultured, macrophages significantly increased U87 in vitro transwell migration compared to control. Finally, serum free media incubated for 48 hours with mouse macrophages was used as growth medium for U87 cells. Using a SRB cell proliferation assay, U87 cells cultured with macrophage conditioned media did not proliferate significantly faster than controls. These results suggest that macrophages may play an important role in promoting glioma angiogenesis and invasion but appear to be less important in promoting glioma cell proliferation. Targeting macrophages may be an effective approach to inhibiting the glioma malignant phenotype.

V-17 Valproic acid inhibits angiogenesis in vitro and in vivo from lioma cells

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Valproic acid (VPA) is one of the histone deacetylase inhibitors that is undergoing clinical evaluation for anti-cancer therapy. We investigate the effects of VPA in glioma angiogenesis in vitro and in vivo in order to suggest new treatment strategy for gliomas. In vitro, the anti-proliferative effect of VPA on human glioblastoma U87MG cells, rat glioma C6 cells and endothelial cells was assessed by cell proliferation assay. We investigated the effects of VPA on the expression of vascular endothelial growth factor (VEGF) and HIF-1 by glioma cells under normoxic and hypoxic condition. The effect of VPA to inhibit for tube formation in human unbilical vein endothelial cell was assessed by angiogenesis kit. In vivo, a brain tumor model of malignant glioma with Wister rat was used. After administration of VPA and CPT-11 to rat, angiogenic status of tumor tissues were assessed by immunohistochemistry and the expression of VEGF and HIF-1. VPA inhibited human endothelial cell and glioma cell, proliferation in vitro. VPA reduced VEGF secretion in conditioned media and reduced VEGF mRNA expression under normoxic and hypoxic condition. VPA was also found to inhibit tube formation in vivo in the angiogenesis assay. Treatment with either CPT-11 reduced VEGF mRNA expression and CD31 expression of immunohistochemistory of brain tumor in vivo. In conclusion, VPA inhibit angiogenesis by mechanisms involving a decrease in VEGF expression and inhibition of tube formation. VPA would be useful as an adjuvant medicine for malignant gliomas through its anti-angiogenic action.

VI-1 The role of the cofilin pathway in human glioma migration and invasion

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Introduction: The cofilin pathway plays a central role in the regulation of actin polymerization and the formation of protrusions that are essential for cell migration. Phosphorylation of cofilin is a key regulatory mechanism modulating cofilin activity. Cofilin expression is altered in a variety of cancers including ovarian, renal cell, and oral-squamous carcinomas. It is clear that the expression of cofilin and other proteins in the cofilin pathway such as Rac and LIMK are upregulated in invasive tumor cells, and that the activation status of cofilin may be directly linked to tumor invasion. To date, the role of cofilin in human glioma migration and invasion has not been studied or described.
Materials and Methods: We examined the expression of cofilin by immunohistochemistry using a glioma tissue microarray (TMA) containing over 60 specimens. We performed immunocytochemistry for cofilin, phosphorylated cofilin, and LIMK1 in a panel of glioma cell lines. Knockdown of cofilin expression was reliably achieved using siRNAs. The migration and invasiveness of glioma cell lines before and after cofilin manipulation was determined in vitro and in vivo using several model systems.
Results: Cofilin expression was increased on the glioma TMA, and correlated with increasing grade malignant astrocytoma. In addition, both cofilin and LIMK had elevated expression in glioma cell lines. Targetted knockdown of cofilin altered glioma cell morphology and inhibited glioma migration and invasion in vitro. In contrast, overexpression of a cofilin phosphorylation mutant in an in vivo xenograft model of brain tumours, resulted in a marked accentuation of the invasive phenotype in 10/10 (100%) of mice. Invasive features found in vivo included spread to the contralateral cerebral hemisphere across the corpus callosum, penetration along arteriolar spaces, and diffuse leptomeningeal disease.
Conclusion: These data show for the first time the role of cofilin in human glioma invasion. They also indicate that the cofilin pathway, which lies downstream of the small cytoskeletal Rho-GTPases, may represent a novel therapeutic target to ablate invasion in these highly malignant tumours.

VI-2 Pharmacologic inhibition of CDK4/6 suppresses the growth of glioblastoma multiforme in an intracranial xenograft model

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Activation of cdk4/6 occurs in the majority of glioblastoma multiforme, and presents a promising molecular target for the development of small molecule inhibitors. In the current study we investigated the molecular determinants and in vivo response of diverse GBM cell lines and xenografts to PD-0332991, a cdk4/6 specific inhibitor. In vitro testing of PD-0332991 against a panel of GBM cell lines revealed a potent G1 cell cycle arrest and induction of senescence in each of 16 Rb-proficient cell lines regardless of other genetic lesions, whereas each of 5 cell lines with homozygous inactivation of Rb were completely resistant to treatment. shRNA depletion of Rb expression conferred resistance of GBM cells to PD-0332991, further demonstrating a requirement of Rb for sensitivity to cdk4/6 inhibition. PD-0332991 was found to efficiently cross the blood-brain barrier and proved highly effective in suppressing the growth of intracranial GBM xenograft tumors, including those

that had recurred after initial therapy with temozolomide. “No mice, in three separate experiments involving PD-0332991 monotherapy against Rb proficient tumors, either with or without initial treatment with temozolomide, died while being treated with PD-0332991, and survival extension from PD-0332991 treatment was highly significant in each experiment ($P < 0.001$)”. Additionally, the combination of PD-0332991 and radiation therapy resulted in significantly increased survival benefit compared with either therapy alone. In total, our results support clinical trial evaluation of PD-0332991 against newly-diagnosed as well as recurrent GBM, and indicate that RB status is the primary determinant of potential benefit from this therapy.

VI-3 Role of microRNA in glioma pathogenesis

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Introduction: MicroRNAs (miRNAs) are endogenously expressed, non-coding RNAs that are effective post-transcriptional regulators of gene expression by translational inhibition or cleavage of target mRNAs. The fact that miRNAs regulate fundamental cellular mechanisms such as cell proliferation, differentiation, apoptosis and metabolism suggests that aberrations in miRNA activity or biogenesis may contribute to cancer development. We explored the role of miRNA aberrations in human gliomas by miRNA expression profiling and functional analyses of differentially expressed candidate miRNAs.
Material and Methods: The expression of 365 human miRNA was determined in 125 primary adult gliomas and 10 non-neoplastic brain tissue samples by using TaqMan Low Density Arrays. MiRNA expression profiles were correlated with tumor type and grade as well as patient survival. To identify epigenetically regulated miRNAs, we additionally investigated glioma cell lines with and without 5-Aza-2´-deoxycytidine and trichostatin A treatment. Differentially expressed candidate miRNAs were subjected to independent validation, followed by functional in vitro analyses using transient transfection of glioma cell lines with either anti-miRNAs or pre-miRNAs as well as cell biological assays to assess influences on cell proliferation, apoptosis, viability, migration and invasion. Gene expression and proteomic profiling were carried out to identify miRNA-regulated targets, which were then validated by Western blot analysis and 3'-UTR-luciferase assays. Sodium bisulfite sequencing was used to assess epigenetic inactivation of candidate miRNAs by DNA hypermethylation.
Results: Using miRNA expression profiling of primary gliomas and glioma cell lines, we identified a number of interesting candidate miRNAs showing differential expression between different tumor entities and/or associations with glioma progression or survival of primary glioblastoma patients. A novel candidate miRNA located on 19q was identified that was frequenty hypermethylated in 19q-deleted oligodendroglial tumors and diffuse astrocytomas but not primary glioblastomas. Functional in vitro analyses suggested a tumor suppressive role of this miRNA and revealed that it may specifically downregulate oncogenic proteins such as cyclin-dependent kinase 6.
Discussion: Large-scale molecular profiling of miRNA expression in gliomas revealed novel interesting candidates that are aberrantly expressed and likely contribute to glioma pathogenesis. Our results support a major role of miRNA aberrations in gliomas and suggests potential novel diagnostic and therapeutic targets.

VI-4 Identifying novel tumor modifier genes involved in gliomagenesis using retroviral gene-trapping mutagenesis screens

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Introduction: Several gain- and loss-of-function genetic alterations have been implicated in gliomagenesis leading to GBM formation, however, many more genetic alterations exist, as evidenced by recent reports from The Cancer Genome Atlas (TCGA) project on human GBMs. Well-characterized mouse models, especially those that progressively develop gliomas, also, offers an opportunity to discover novel glioma relevant genetic alterations, using viral and non-viral random mutagenesis strategies.
Materials and Methods: Using gene-trap strategies in our spontaneous transgenic mouse RasB8 glioma model, which expresses V12H-Ras under the control of the astrocyte tissue specific human promoter GFAP, we identified GATA6, a member of the GATA family of transcription factors, as a novel tumour suppressor gene (TSG) involved in progression of human GBMs and GATA4, a close family member of GATA6, to also function as a TSG involved in initiation of gliomagenesis.
Results: We now generated novel gain of function and loss of function gene-traps and transduced non-transformed but genetically susceptible primary murine astrocytes harboring either activated V12H-Ras, loss of the tumour suppressor p53, loss of Ink4a/Arf, Pten-/- and over-expression of the human EGFRvIII mutant. Pending verification in our mouse models and human specimens we hypothesized that that trapped clones from V12H-Ras, p53 null and Ink4a/ARF-/- astrocytes would reveal progression factors, as these genetic alterations are associated with human low grade gliomas and occur early on in GBM formation. In contrast, trapped clones from astrocytes with EGFRvIII or Pten-/-, already demonstrated to promote progression, would likely lead to the discovery of initiation factors. Several gene-trapped clones led to transformation as measured by soft agar assays in initial screens using astrocytes with activated V12H-Ras. By means of inverse PCR we identified gene-trap insertion sites in introns of RapGap1, Ikkß, Socs6, and Pink1 leading to loss of function. Of great interest was PTEN induced Kinase 1, PINK1, a mitochondrial Serine/threonine-protein that is frequently mutated in patients in Parkinson disease while its link to cancer and GBM is poorly characterized. Initial screening in GBMs reveals reduced expression of PINK1 protein compared to normal human astrocyte controls. Pink1 gene-trap clones also exhibited increased proliferation and increased transformation compared to empty vector controls providing initial evidence that mouse and human PINK1 may be a tumour supressor gene, with current validation in progress.
Conclusions: Gene-trapping strategies in robust animal models provide an invaluable tool that complement large scale cancer genome sequencing projects in identification of relevant driver GBM modifier genes in random non-biased manner.

VI-5 Hypoxia inducible factor inhibition by nanoparticle delivered siRNA inhibits growth and angiogenesis in an intracranial xenograft mouse model

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Introduction: Hypoxia-inducible factor 1∂ (HIF-1∂) expression is increased in human glioblastoma multiforme (GBM) compared to low-grade tumors. HIF-1∂ induces transcription of glycolytic genes such as carbonic anhydrase IX (CA IX) and glucose transporter-1 (Glut-1) and angiogenic proteins including vascular endothelial growth factor (VEGF). We have previously shown that HIF-1∂ inhibition decreases tumor growth in a flank tumor model. This study examines methods of delivery for similar inhibition in an intracranial model and whether this decreases tumor angiogenesis and growth.
Material and Methods: We use siRNA directed against HIF- 1∂ in an intracranial luciferase reporter tumor model with the ability to measure both HIF-1 activity and tumor growth in vivo. These molecules are delivered with a variety of nanoparticle delivery molecules we call multifunctional carrier (MFC) and chemically modified AccellTM siRNAs. Tumor growth was followed over time and compared between various nanoparticle carrier molecules. Tumors were removed at the end of the study and examined for expression of downstream-regulated molecules well as measures of microvascular density and cellular proliferation MIB-1.

Results: We demonstrated reduced tumor growth and HIF-1 activity in tumors treated with anti-HIF-1∂ siRNA delivered with our MFC nanoparticles compared to other carriers and negative control siRNA injections. We also found reductions of HIF-1∂, VEGF and GLUT-1 expression, as well as decreased MIB-1 index and decreased vascular density.
Conclusions: This preliminary study suggests that intracranially injected siRNA directed against HIF-1∂ complexed to a newly developed carrier with tumor targeting moieties might be used for treatment of malignant brain tumors. We will also discuss future work to develop more specific tumor targeting and potential systemic delivery techniques.

VI-6 Targeting MAPK signaling in malignant glioma

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Signaling via Erk1/2 and p38 mitogen-activated protein kinases (MAPK) is universally active in newly diagnosed glioblastoma multiforme (GBM) patients. Both MAPKs converge on the MAPK integrating kinase (Mnk), which is the sole kinase capable of phosphorylating the cap-binding translation initiation factor, eukaryotic initiation factor (eIF) 4E. eIF4E is the rate limiting factor in ribosome recruitment to mRNAs and, thus, reigns over all protein biosynthesis in the cell. We determined that anti-tumor activity of the prototype oncolytic poliovirus, PVSRIPO, is mediated by abnormal MAPK signaling to eIF4E, favoring cap-independent, viral translation initiation and replication in GBM. Based on our correlative mechanistic investigation of PVSRIPO oncolysis in GBM, we propose a mechanism responsible for the oncogenic effects of eIF4E phosphorylation. PVSRIPO recently passed IND-directed toxicology in non-human primates and is anticipated to enter clinical investigation in patients with recurrent GBM next year. Our recent research revealed that abnormal MAPK signaling to the protein synthesis apparatus is due to ectopic over-expression of the p38-alpha isoform in GBM. This is due to the absence of micro RNA 128
(miR128) in GBM, while in the normal CNS abundant miR128 represses p38-alpha synthesis. Our research has important implications for oncolytic viral therapies based on anomalies of the protein synthesis machinery in GBM and for the use of MAPK inhibitors for targeted therapy of these tumors.

VI-7 MG132 exhibits potent anti-tumor activity in an animal model of malignant glioma when administered via thermoreversible gelation polymers

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Introduction: MG132, a proteasome-inhibitor, has been shown to induce apoptosis of experimental malignant cells in vitro. However, its systemic toxicity prevents further use in clinical settings. To circumvent this toxicity, MG132 can be delivered directly to the tumor. We tested the efficacy of MG132 incorporated into thermoreversible gelation polymer (TGP) for treating experimental gliomas.
Materials and Methods: U87MG and T98G glioma cell lines were treated with MG132 (0.1-10μM) for 24 h in vitro. Cell-viability was measured with MTT-assays. As a caspase activity assay, a total of 40 μg of protein isolated from cells treated with MG132 was incubated in each reaction with 10 μg of corresponding caspase substrates for 1 hour, and the activities for caspases 3, 8 and 9 were measured in a CytoFluor Multiwell Plate Reader. Toxicity of TGP was tested in vivo by implanting MG132/TGP polymers intracranially to C6 rats. The efficacy of 1μM MG132/TGP polymers was determined by treating nude mice subcutaneously 5 days after implantation with U87MG.
Results: MG132 was cytotoxic to U87MG, causing a 16 +/- 8% growth inhibition at 0.5μM, and that increased to 78 +/- 4% at 1μM. Similarly, MG132 inhibited growth of T98G by 18 +/- 8% at 0.5μM and 74 +/- 2% at 1μM in vitro. A significant increase in cleavage activities of caspases 3, 9, and 8 detected in caspase cleavage assay results was observed in both cell lines in response to greater than 0.5μM MG132. Polymers released MG132 for 21 days and intracranial implantation in rats neither generated local nor systemic toxicity. In vivo assay, MG132/TGP polymers with loading concentrations of 1μM inhibited tumor growth for the whole 3 weeks after injection.
Conclusion: MG132 exhibits potent cytotoxic-activity against U87MG and T98G in vitro, it can be efficiently incorporated and delivered using TGP, and at the proposed concentrations MG132/TGP polymers inhibit tumor growth in the subcutaneous nude mice model.

VI-8 A novel NF-KB inhibitor DHMEQ reduces growth of human glioblastoma cells

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Introduction: Glioblastoma is the most frequent malignant brain tumor characterized by rapid growth, extensive invasiveness, and angiogenesis. The prognosis of patients with glioblastoma remains extremely poor in spite of recent innovations of surgical technique, chemotherapy, and radiotherapy. It has been reported that nuclear factor- κB (NF-κB) signaling has been implicated not only in inflammation but also in tumorigenicity or tumor progression. NF-κB signaling is constitutively activated in various cancer cells such as pancreatic cancer, lung cancer, thyroid cancer, malignant lymphoma, and so on. However, the possible involvement of NF-κB signaling in the malignant features of glioblastoma remains to be clarified. In this study, we analyzed the roles of NF-κB signaling in glioblastoma and the effect of a novel RelA inhibitor, dehydroxymethylepoxyquinomicin (DHMEQ).
Maerials and Methods: First, we examined the expression of RelA, one of the important components of NF-κB, in surgically-resected specimens and cell lines of glioblastoma. Next, we attempted to knockdown RelA gene in human glioblastoma cell lines by short hairpin RNA expression retroviral vector. We also tested the effect of DHMEQ on glioblastoma in vitro.
Results: Reverse transcription PCR revealed most glioblastoma specimens and cell lines expressed RelA. The proliferation of glioblastoma cell lines was reduced by RelA inhibition by RNAi, and was also reduced by DHMEQ in a dose dependent manner. The intracranial implantation of glioblastoma cells in nude mice revealed that the tumor formation was impaired by DHMEQ in vivo.
Conclusion: These results show that NF-κB signaling may serve as a potential therapeutic target for glioblastoma, and DHMEQ may be one of the candidate treatment agents.

VI-9 Retrospective immunohistochemical evaluation of high-grade glioma patients treated with bevacizumab and irinotecan, with or without cetuximab

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Introduction: Recent data have shown that bevacizumab (B) and irinotecan (I) as well as cetuximab (C) combined with B and I induce significant responses in recurrent high-grade gliomas (HHG). Amplification and overexpression of the EGFR is observed in 35-45% of primary Glioblastoma Multiforme (GBM). EGFR amplification/overexpression and expression of the constitutively activated EGFRvIII leads to dysregulated downstream signaling through the PI3K/AKT pathway. Furthermore, GBM have inactivating mutations of the tumor suppressor PTEN leading to elevated activity of AKT (pAKT).
GBM tumors are known to be hypoxic and highly vascularized with pronounced tumor vascularity significantly correlated with poor survival. The HIF-1a and HIF-2a subunits initiate transcription of pro-angiogenic factors such as VEGF. In addition, signaling through the PI3K/AKT pathway has been shown to induce the expression of VEGF either by stimulating its transcription directly or by up regulation of HIF-1a.
Materail and Methods: Thirty-seven evaluable patients with primary GBM treated with CBI and twenty-four evaluable patients with HGG treated with BI were investigated by immunohistochemistry using the following antibodies: EGFR, EGFRvIII, pAKT, PTEN, GLUT1, CA9, HIF-1a, HIF-2a, VEGF and CD34. Measures of association between biomarkers were calculated using Spearman's rank correlation and a logistic regression model were used for screening of response. Estimates of survival probabilities and rates were done using the Kaplan-Meier method and logrank statistics respectively. Cox proportional hazards model were used for PFS and OS
Results: GLUT-1 and CA9, GLUT-1 and HIF-1a plus HIF-1a and CA9 showed a tendency of being correlated in both CBI and BI. This could indicate a hypoxic molecular profile of these tumors and suggest that GLUT-1, HIF-1a and CA9 share regulatory mechanisms. The only biomarker showing a tendency toward predicting survival and response was the endothelial marker CD34 (PFS: P = 0.07, HR: 1.04 (95% CI: 0.99-1.08); OS: P = 0.08, HR of 0.99 (95% CI: 0.98 – 1.00), but only in the BI group.
Conclusions: None of the biomarkers tested showed to be significant prognostic markers for response to BI or CBI. Hence, there is still an urgent need for one or more reliable and reproducible biomarkers able to predict the efficacy of anti-angiogenic therapy.

VI-10 Tumour-specific radiosensitization by inhibition of DNA repair pathways

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Introduction: Treatment of glioblastoma multiforme (GBM) is limited by radiation resistance and the risk of neurotoxicity. Since GBM are rapidly proliferating tumours within non-replicating normal brain, and many tumours exhibit DNA repair defects, tumour-specific radiosensitization might be achieved by targeting replication-dependent processes or exploiting DNA repair defects. We previously showed that inhibition of poly(ADP-ribose) polymerase (PARP) increases radiosensitivity of human glioma cells in a replication-dependent manner, creating excess DNA double strand breaks (DSB) that are repaired by homologous recombination (HR). Meanwhile, other groups have shown that cells deficient in non-homologous end-joining (NHEJ) repair utilize an alternative end-joining pathway that is abolished by PARP inhibition. The aims of this project were: (1) Evaluate whether intrinsic defects in DNA repair enhance the radiosensitizing effects of PARP inhibitors. (2) Test whether tumour-specific downregulation of HR using the HSP90 inhibitor 17-AAG would increase the radiosensitizing effects of PARP inhibition.
Materials and Methods: Experiments were conducted in four human glioma cell lines and mouse embryo fibroblasts (MEFs) deficient in Artemis and DNA Ligase IV. Radiation sensitivity was measured by clonogenic survival, DNA DSB repair by immunofluorescence detection of g-H2AX and Rad51 foci, and replication dependence by confluence arrest or aphidicolin treatment. The PARP inhibitor olaparib and HSP-90 inhibitor 17-AAG were used at non-toxic concentrations.
Results: (1) Olaparib increased radiosensitivity only in replicating cells. Radiosensitization was enhanced in Artemis-/- and Ligase IV-/- MEFs. Ligase IV-/- cells exhibited an incomplete DSB repair defect; residual repair was abolished by PARP inhibition. The repair defect of non-replicating Artemis-/- cells was unaffected by PARP inhibition but repair of replication associated damage was significantly impaired.
(2) 17-AAG increased the radiosensitizing effect of olaparib and delayed repair of DSB while maintaining replication dependence.
Conclusions: The radiosensitizing effects of olaparib are observed only in replicating cells, and defects in DNA DSB repair pathways exacerbate radiosensitization. This predicts tumour specificity since tumours exhibit DNA repair defects and normal brain is composed of non-replicating cells. Tumour-specific inhibition of HR can be achieved by 17-AAG, enhancing the radiosensitizing effects of olaparib while maintaining replication dependence. PARP inhibitors have significant potential in the treatment of GBM.

VI-11 Novel role of neurofibromin in transport of RNA granules

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Introduction: Neurofibromatosis 1 (NF1) is the most common tumor predisposing syndrome in humans, with an incidence of 1:3500 live births. Neurofibromin, encoded by Nf1, acts as a p21-Ras-GAP to directly interact with and inactivate p21-Ras, through its' GAP Related Domain (GRD). Evidence suggests that non-Ras-GAP functions mediated through interactions with domains outside of the GRD are of importance. By making a number of NF1-GST domain constructs coupled with differential Mass Spec analysis, we identified LRPPRC and Dynein as previously unreported NF1-TBD interacting proteins. LRPPRC was of interest as it is mutated in the Leigh Syndrome French Canadian (LSFC) variant, a cytochrome-oxidase deficiency syndrome, characterized by neurodegeneration, developmental- mental- and growth-retardation, thereby having some similarities with non-tumor manifestations of NF1.
Materials and Methods and Results: We have established that LRPPRC and neurofibromin interact in vitro by immunoprecipitation and reverse bait immunoprecipitation. Use of a novel Proximity Ligation Assay (PLA) was used to further confirm the interaction in situ in cell lines and clinical tissue cryosections. Towards elucidating the functional relevance of the interaction, we have established that the two proteins complex with microtubules and motor proteins, which indicates a role in microtubule-dependant intracellular transport. Since LRPPRC binds with mRNA and is involved in its nucleo-cytosolic shuttling, we hypothesize that the cargo transported by this complex could be RNA granules. Mass Spec analysis of NF1 immunoprecipitates revealed a number of proteins that are a part of the RNA granule complex which include RNA binding proteins and proteins involved in mRNA translation. By performing RNA Immunoprecipitation (RIP) with NF1 and LRPPRC antibodies, we have demonstrated that the two proteins do complex with 18S and 28S rRNA which is indicative of the presence of intact mRNA. Towards identifying the specific messages transported by the NF1-LRPPRC RNA granule complex we are performing RIP - microarray gene expression profiling (CHIP).
Conclusions: The resultant candidate mRNAs will be further investigated to understand the importance of this novel interaction and novel mechanism towards some of the overlap of neurological and developmental manifestations between LSFC and NF1.

VI-12 Three tesla MRI perfusion image study on differential diagnosis among astrocytic and oligodendroglial tumors

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Introduction: It is often difficult to make different diagnosis between astrocytic tumors and oligodendroglial tumors on the aspect of image diagnosis and histologic findings. Generally, it is well known that if the glioma with oligodendroglioma (ODG) component has good response to the treatment and longer survival time compared to the astrocytic tumors. So it is crucial to make differential diagnosis between the two groups. Histologically we can observe the development of fine micro-capillaries in ODG, so it will be suspected that blood volume in ODG increased. So, we evaluated the intra-tumoral relative blood volume (rBV) using 3 T MRI perfusion images (PIs) and discussed the effectiveness of this procedure to make differential diagnosis between the two groups.
Patients: Total 19 patients including 2 G-II and 3 G-III astrocytoma (A), and 5 G-II and 4 G-III oligo-astrocytomas (OAs), and 2 G-II and 3 G-III ODGs in which 3 T MRI PIs between November 2006 and May 2009. Mean age was 48.4 years old (19 - 81) and 12 males and 7 females.
Methods: Before operation, 3T MRI PI study was done and rBV maps were made. Intra-tumoral maximum rBV / contra-lateral rCBV of the white matter were calculated and discussed.
Results: 1) Significant difference of rBV was found among the 3 groups. 2) It was found that the tendency of the highest rBV in ODG. 3) No significant difference was found among G-II and G-III in all glial tumors.
Discussion: It was indicated that differential diagnosis could be done between A and OA – ODG using this method because glial tumor with the component of ODG has tendency of increased rBV. On the other hand, this method is not useful to evaluate the grades of glial tumors which were as reported useful before. This maybe caused by the difficulty of evaluation of malignancy only by increase of rBV in the tissue of ODG.

VI-13 Cyclin-dependent kinase inhibitor enhances temozolomide-induced cytotoxicity in human glioma cells by suppressing DNA repair associated with g2 checkpoint

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Recent progress in chemotherapy for malignant gliomas was led by a DNA-methylating agent temozolomide (TMZ). Previous studies revealed that TMZ induced prolonged G2/M arrest of human glioma cells, and then non-apoptotic cell death associated with senescence-like phenomenon (in p53-wild type cells) or mitotic catastrophe (in p53-non-functional cells). However, the escape of the tumor cells from the chemotherapeutic agent is still a big problem, and development of safe and effective methods to potentiate anti-tumor activity of the drug is warranted. Previous studies suggested that G2 checkpoint system might regulate the linkage between DNA damage and cell death. We tested the effect of a cdk inhibitor flavopiridol (FP) which has been known to inhibit the action of cdc2 (cdk1), a key protein in G2 checkpoint pathway, on TMZ-treated U87MG human glioma cells. FP (< 10 nM) did not show cytotoxicity or cell cycle arrest as a single compound, but potentiated the cytotoxicity of TMZ. FACS analysis revealed low concentration FP induced accumulation of TMZ-treated cells, but not untreated cells, exclusively at G2 (4N DNA content), which suggested that low concentration FP acted exclusively on cdk1. This FP-induced potentiation of TMZ was clearly associated with suppression of key proteins at G2-M transition including polo-like kinase 1, Aurora and Pin1. Furthermore, this "complete" G2 arrest was associated not only with increased expression of γ-H2AX, a DNA double strand break marker, but also with increased level of phosphorylated ATR, which suggests that FP promoted enhancement of DNA damage and apoptotic cell death signal in TMZ-treated glioma cells. FP also enhanced cytotoxity of TMZ to the cells with over-activated Akt, which has been previously shown to associate with TMZ resistance. In conclusion, our results suggest that TMZ resistance could be promoted by enhanced DNA repair activity in G2-M transition following G2 checkpoint activation, and that cdk inhibitor enhanced TMZ cytotoxicity by suppression of this activity. Further investigation on linkage between cdk1 and DNA repair would provide important information on TMZ resistance, and cdk1-targeted compound might be useful as chemosensitizing agent for TMZ on gliomas.

VI-14 EPH-A signalling in vitro and in vivo

Authors: TBA

Introduction: The Eph receptors and their ligands, ephrins, represent the largest family of tyrosine kinases which are involved in neurodevelopmental processes, such as cell migration. Both Ephs and ephrins are transmembrane proteins and transduce signals in a bidirectional manner resulting in repulsion. Accumulated evidence including our previous reports demonstrated that some of these molecules link to the invading cell phenotype in human malignancy. To reveal the role of Eph/ephrin in glioma biology and patient's survival, clinical relevance of Eph/ephrin genes was determined in a clinically annotated expression data set of glioma surgical specimens. Further the function of Eph/ephrin in glioma invasion was analyzed.
Materials and Methods: The clinical significance of Eph/ephrin expression was investigated by analysis of Eph/ephrin mRNA expression level in 24 nonneoplastic brain and 171 glioma specimens. Candidate gene products were validated in surgical specimens and biological function of target genes was evaluated in migration and invasion assays.
Results: Levels of certain Eph/ephrin family members mRNA were significantly different in glioblastoma (GBM) samples (n=82) compared with normal brain. According to the Kaplan-Meier analysis, only ephrin-A2 level among 13 members of Ephs and 8 members of ephrins emerged to be a powerful predictor of a favorable survival in GBM (n=77, p=0.0069). Depletion of endogenous ephrin-A2 expression by small interfering RNA in high expressor glioma cell lines (SNB19, U251) accelerated migration and invasion, while the migration was inhibited by the addition of ephrin-A2/Fc chimera, which blocks ephrin-A2 and simultaneously stimulates EphA signaling. Forced expression of ephrin-A2 in low expressor cell lines (U87, T98G) slowed migration, treatment with ephrin-A2/Fc further suppressed cell migration concomitant with tyrosine phosphorylation of EphA, especially in T98G cells which express EphA prominently.
Conclusions: This is the first demonstration that ephrin-A2 negatively controls cell invasion through EphA signaling both in vitro and in vivo, identifying ephrin-A2 as a good prognostic factor in GBM.

Session VII
New Therapeutic Approaches

VII-1 Neural stem cell-mediated cancer therapy: towards glioma clinical trials

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Introduction: Despite aggressive multimodal therapy, high-grade gliomas remain incurable and lethal. Neural stem/progenitor cells (NSCs) offer an unprecedented advantage over conventional approaches because of their exceptional ability to cross the BBB, target invasive tumor cells throughout the brain, and provide a platform for localized chemotherapy. Used as a delivery vehicle, NSCs have been engineered to express a variety of anti-cancer agents, demonstrating therapeutic efficacy in pre-clinical models of glioma, medulloblastoma, and melanoma brain metastases. We now propose the clinical use of a well-characterized, human NSC line, HB1.F3, modified to express cytosine deaminase (CD), an enzyme that converts the prodrug 5-Fluorocytosine (5-FC) to the active chemotherapeutic 5-Fluorouracil (5-FU) in patients with recurrent high-grade glioma. We postulate that NSCs will localize to residual and invasive tumor foci following injection into the resection cavity wall, and convert orally administered 5-FC to 5-FU directly at the tumor sites. FDA IND approval for this first-in-human phase I study is pending.
Material and Methods: The HB1.F3.CD clonal NSC line was generated from15 wk fetal telencephalon by retroviral transducion with v-myc. Characterization analysis included LAM-PCR, karyotype, and gene

sequencing. In vivo biodistribution, safety and therapeutic efficacy studies were conducted in normal and glioma-bearing immunocompromised and immunocompetent adult mice.
Results: In vitro cytogenetics, migration and activity assays demonstrate that HB1.F3.CD NSCs are chromosomally and functionally stable. Identification of a single copy and insertion site for both CD and v-myc genes was determined by LAM-PCR. In vivo studies demonstrated retention of NSC tumor-tropism following radiation to the brain or in the presence of dexamethasone. Biodistribution and safety studies indicate this cell line is non-toxic, minimally immunogenic (HLA class II negative), and non-tumorigenic.
Conclusions: Clinical use of this expandable allogeneic NSC line circumvents problems associated with primary stem cell pools. We postulate that our HB1.F3.CD NSCs will localize to invasive glioma foci and convert 5-FC to 5-FU, causing preferential killing of dividing tumor cells and improve clinical outcom in recurrent glioma patients. Demonstration of safety/feasibility in a phase I study will provide the foundation for further therapeutic development and applications to other invasive cancers.

VII-2 Cell-based therapies for malignant gliomas using hematopoietic progenitor cells?

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Introduction: Intracerebral experimental gliomas attract hematopoietic progenitor cells (HPC) in vivo. HPC are therefore promising vehicles for a cell-based delivery of therapeutic molecules to malignant gliomas. Before therapeutic application, however, it is important to investigate (i) potential tools for genetic modification of the cellular vector, (ii) tumorigenicity after genetic modification, (iii) the time-frame of homing and (iv) the spatial distribution of the HPC after intravenous injection.
Materials and Methods: We used lentiviral vectors for genetically modifying HPC. For analyzing the homing characteristics of HPC in glioma-bearing mice after lentiviral transduction (lenti-HPC), we analyzed the glioma-mediated attraction of ex vivo intravenously (i.v.) injected lenti-HPC as well as the tropism of endogenous lenti-HPC after bone-marrow transplantation. Time lapse two photon laser scanning microscopy (2PLSM) and positron emission tomography (PET) are two powerful techniques to visualize cellular migration in living glioma-bearing mice. We prepared a cranial chronic glass window and stereotactically implanted green fluorescent protein-positive glioma cells into the neocortex of mice. Human CD34+ cells isolated from granulocyte-colony stimulating factor-mobilized peripheral blood or murine lineage-depleted Sca1+ Kit+ (LSK) bone marrow cells were stained with PKH26 and injected five days later i.v. to assess the glioma tropism by 2PLSM. Furthermore, we implanted glioma cells into the right striatum. Fourteen days later we injected mutant H. simplex tyrisone-kinase (Hsv-tk)-positive HPC into the tail vein. PET analysis with the tracer [18F] FHBG, a ganciclovir-analogue, was used to determine the biodistribution of injected Hsv-tk-positive HPC by small animal PET imaging.
Results: Lentiviral transduction does not interfere with the glioma tropism of exogenous or endogenous HPC. We did not detect any teratoma formation after injection of lenti-HPC. Further, the combination of 2PLSM and PET enabled us to characterize both, the time-frame of homing and the systemic distribution of HPC after i.v. injection in living glioma-bearing mice. The data processing will be finished until the conference.
Conclusions: Bone-marrow-derived cells are promising vehicles for a cell-based therapy against malignant gliomas.

VII-3 Optimized convection-enhanced delivery platform: experience in canine spontaneous glioma and non-human primates

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The importance of assessing the distribution of agents delivered via convection-enhanced delivery (CED) is critical to the evaluation of efficacy as well as toxicity of intracranial administration of therapeutics. Comprehensive and fully integrated drug delivery system to the brain consisting of magnetic resonance imaging (MRI)-compatible stereotactic system, infusion cannula, navigational software are critical for successful drug and viral vector deliveries into the brain. Critical parameters required for safe, reproducible and robust drug delivery in monkey basal ganglia, thalamus and brain stem have been developed and validated using near real time MRI and are now being implemented in translational studies in neurooncology. Canine spontaneous intracranial tumors bear striking similarities to their human tumor counterparts and have the potential to provide a large animal model system for more realistic validation of novel therapies typically developed in small rodent models. We used canines with spontaneously occurring gliomas to investigate the use of CED of liposomal nanoparticles containing the topoisomerase I inhibitor CPT-11. To facilitate visualization of intratumoral infusions by real-time MRI, we included identically formulated liposomes loaded with Gadoteridol. Real-time MR imaging defined both distribution of infusate within tumor and normal brain tissue. The most important limiting factor for volume of distribution (Vd) within tumor tissue was leakage of infusate into ventricular or subarachnoid spaces, evident on MR imaging. Decreased tumor volume, tumor necrosis and modulation of tumor phenotype correlated with Vd of infusate, infusion location, and leakage as determined by real time MRI and histopathology. Data obtained from infusions monitored in near real time in a large, spontaneous tumor may provide information allowing more accurate prediction and optimization of infusion parameters. Variability in Vd between tumors strongly suggests that near real time imaging along with its ability to predict optimal placement of the CED cannula should be an essential component of CED therapeutic trials to allow minimization of inappropriate infusions and accurate assessment of clinical outcomes.
This study demonstrates the potential for using canine spontaneous glioma as a model system for the validation and development of novel therapeutic strategies for human brain tumors.

VII-4 Convection enhanced delivery of topotecan for gliomas

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Introduction: Chemotherapy drugs for gliomas fail mostly because of inadequate delivery rather than lack of anti-tumor activity. Convection enhanced delivery (CED) is a local-regional strategy for achieving maximal drug concentrations in malignant gliomas. Topoisomerase-inhibitors, such as topotecan, are ideal drugs for CED since they are cytotoxic to glioma cells and nontoxic to normal brain where topoisomerase levels are low. Beginning with small animal models and through to a Phase I clinical trial, we investigated the safety and efficacy of topoisomerase inhibitors by CED for malignant brain tumors.
Material and Methods: Topotecan by CED was investigated in a rat glioma model, a pig model and in a Phase I human clinical trial. Safety and efficacy were measured through radiographic analysis, quality of life analysis, measurement of topoisomerase level response and survival.
Results: In a rat glioma model with invasive features, safe and effective concentrations of topotecan by CED

were established. A pig model was used as a large animal model capable of radiographic monitoring to study the potential for safely maximizing topotecan volume of distribution with chronic delivery. These preclinical findings were used to conduct a Phase I dose escalation study of topotecan by CED in patients with recurrent malignant gliomas. 65% of patients demonstrated favorable treatment response with median survival of 59 weeks. Treatment analysis including neuropsychological testing, quality of life measurements and radiographic imaging confirmed the safety of this therapeutic trial.
Conclusions: Topotecan by convection-enhanced delivery has significant anti-tumor activity at concentrations that are non-toxic to normal brain. Preliminary results indicate survival advantage exceeding historical controls for recurrent malignant gliomas. A maximum tolerated dose has been established to use in a planned multi-center Phase II trial.

VII-5 Evaluation of drug delivery to brain tumors: grind and find is not enough

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Introduction: The ability of a systemically administered drug to reach tumor cells in animal models typically involves the use of non-invasive glioma cell lines and gross assessments of tissue concentration following animal sacrifice. We evaluated the hypotheses that these types of assessments overestimate the real tissue concentrations and that the concentrations that are achievable may not functionally significant.
Material and Methods: Athymic nude rats bearing orthotopic human gliomas (U87MG) with or without transgenic overexpression of wild-type EGFR were treated with erlotinib (20 to 40 mg/kg/day), with or without the Pgp/Bcrp inhibitor GF120918, for 5 days. Multiple tissues including blood, normal brain, and tumor were harvested for analysis of erlotinib concentration by LC/MS/MS, with or without prior saline perfusion to eliminate the intravascular concentration of erlotinib. Evaluation of the activation of the EGFR signaling pathway was performed by Western blot.
Results: Erlotinib failed to produce dephosphorylation of EGFR until animals were exposed to lethal doses. Drug concentration analysis indicated that erlotinib penetrated the tumor core but not the brain immediately surrounding the tumor mass or normal brain (approximately 10% of plasma concentration). Intravascular saline perfusion prior to harvesting significantly influenced the erlotinib tissue distribution values in the brain surrounding tumor and contralateral brain, but not tumor core. Treatment with GF120918 significantly enhanced erlotinib distribution into the tumor core (2.5 fold), brain surrounding tumor (12-fold) and normal brain (10-fold).
Conclusions: Evaluations of the ability of a drug to penetrate the brain which are limited to assessment of drug concentrations only may provide misleading results due to the detection of circulating drug within microvessels. Erlotinib is excluded from the brain by active efflux at the BBB, especially at the tumor edge and normal brain, but this may be overcome by an active efflux inhibitor. Concentrations that reach glioma tissue in this model fail to induce EGFR signaling changes at non-toxic doses. Clinical trials which only evaluate drug concentrations in tumor tissue, and not the biological effects of the drug, cannot be relied upon to determine whether the drug was ever successfully delivered.

VII-6 Continuous development of delta-24-RGD for the treatment of gliomas

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We have previously shown that the replication-competent Delta-24-RGD oncolytic virus is an effective treatment against gliomas. Delta-24-RGD is currently being tested in a Phase I clinical trial in patients with recurrent malignant gliomas. Although intratumoral delivery of Delta-24-RGD may be effective, intravascular delivery would improve successful application in humans. We hypothesized that human mesenchymal stem cells (hMSC) could be harnessed as intravascular delivery vehicles of Delta-24-RGD to gliomas. To determine anti-tumor efficacy, mice were implanted with luciferase-labeled glioma xenografts, treated with hMSC-Delta-24 or controls, and imaged weekly by bioluminescence imaging. Analysis of tumor size by bioluminescence imaging showed inhibition of glioma growth and eradication of tumors in hMSC-Delta-24-treated animals compared with controls ($P < 0.0001$). There was an increase in median survival from 42 days in controls to 75.5 days in hMSC-Delta-24-treated animals ($P < 0.0001$) and an increase in survival beyond 80 days from 0% to 37.5%, respectively. We conclude that intra-arterially delivered hMSC-Delta-24 are capable of delivering Delta-24-RGD into xenografts, resulting in improved survival. Because treatment of gliomas will probably require multimodal therapy we explore the anti-glioma effect of the combination of Delta-24-RGD and temozolomide. Clinical evidence shows that gliomas with silencing of the MGMT promoter are sensitive to temozolomide. Based on the fact that adenoviral proteins inactivate DNA repair genes, we hypothesized that the oncolytic adenovirus Delta-24-RGD could overcome the reported MGMT-mediated resistance. We first observed that Delta-24-RGD treatment overrides the temozolomide-mediated G(2)-M arrest. Furthermore, Delta-24-RGD infection was followed by down-modulation of the RNA levels of MGMT. Chromatin immunoprecipitation assays showed that Delta-24-RGD prevented the recruitment of p300 to the MGMT promoter. Importantly, we showed that Delta-24-RGD interaction with p300 was required to induce silencing of the MGMT gene. Of further clinical relevance, the combination of Delta-24-RGD and temozolomide significantly improved the survival of glioma-bearing mice. Collectively, our data provide a strong mechanistic rationale for the combination of oncolytic adenoviruses and temozolomide, and should propel the clinical testing of this therapy approach in patients with malignant gliomas.

VII-7 Oncolytic HSV-1 (g47) efficiently kills glioblastoma-derived cancer stem-like cells

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Oncolytic virus therapy is an emerging strategy for treating malignant brain tumors. A variety of viruses have been studied in experimental models, and a number of them already used in clinical trials. G47Δ is a third generation oncolytic HSV-1 that has triple modifications in the viral genome. Its efficacy has been shown in experimental brain tumors as well as in other cancer models. In order to further investigate its usefulness for brain tumor therapy, we evaluated the effect of G47Δ on cancer stem-like cells (CSLCs) derived from surgically obtained human glioblastoma specimens. Three CSLC lines (TGS-01, -02 and -04) were studied. In vitro, the cell lines were positive for CD133, nestin and GFAP expressions, but were negative for TujI and O4 expressions by flow cytometry and immunocytochemistry under a serum-free condition. In these CSLC lines, autocrine TGF-Δ signaling was found to play essential roles in retention of stemness. Secondary sphere forming assay revealed that G47Δ killed CSLCs efficiently. In vivo, the CSLC lines were capable of forming tumors when as low as 1x103 cells were transplanted to the brain of athymic mice. Single intratumoral inoculation of G47Δ (1x107 pfu on day 10) significantly prolonged the survival of mice transplanted with CSLCs in the brain. These results indicate that G47Δ may efficiently eradicate glioblastoma including CSLCs.

VII-8 Clinical development of a third-generation recombinant oncolytic HSV-1, g47Δ, for recurrent glioblastoma in japan

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Genetically engineered, conditionally replicating herpes simplex viruses type 1 (HSV-1) are promising therapeutic agents for cancer. We have developed a triple-mutated, third-generation oncolytic HSV-1, G47Δ, by introducing an additional genetic mutation in the viral genome of G207, a second-generation HSV-1 used in multiple clinical trials for glioblastoma in the US. Preclinical studies revealed that G47Δ exhibited increased antitumor efficacy in various tumor models while preserving the safety of G207.
Prior to a clinical trial, we performed G47Δ genome structure analysis, stability tests, and preclinical safety evaluation using HSV-1-susceptible A/J mice. After developing manufacturing processes, we manufactured the clinical-grade G47Δ at the GMP Vector Production Facility at the University of Tokyo. Quality tests under GLP were completed for products at 4 steps of manufacture. The first clinical trial of G47Δ in Japan is planned as an open-label, single-armed, phase I-II study. Patients with a single lesion of recurrent glioblastoma, age 18 or older, and with KPS 70% or higher will be registered. The primary endpoint is to assess the safety of G47Δ, and the secondary endpoint is to assess the efficacy by tumor size and PFS. Three cohorts of 3 subjects each are planed in a dose escalation phase, and 12 additional subjects will be treated at the highest safe dose. G47Δ will be administered stereotactically into the tumor, twice within 14 days. Dealing with regulatory authorities in Japan started in 2006 which included the Ministry of Education for the use of recombinant bioorganisms for non-clinical purposes (manufacturing), the Ministry of Health for evaluation of the gene therapy clinical protocol and for the use of recombinant bioorganisms for clinical purposes, and the Ministry of Economy for clearing the Washington Convention for exporting Vero cell samples for quality tests. The final approval was granted in May 2009, and the first patient was treated with G47Δ 6 months later. We hope overcoming such hurdles of translational research would lead to development of a new drug and eventually to the cure of glioblastoma, and also to an improvement of the system for drug development in Japan.

VII-9 Identification of novel synergistic targets for rational drug combinations with PI3 kinase inhibitors using sirna synthetic lethality screening

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Targeted therapeutics directed towards molecular pathways that underlie the malignant phenotype offer a new attractive cancer therapeutic strategy. Constitutive activation of PI3K/Akt pathway seems to be a prerequisite for a wide spectrum of cancers including glioblastoma. However, targeting aberrant transduction signals with single targeted agent has met with little success. Tumor heterogeneity and complex compensatory or collateral pathways may negate the therapeutic efficacy of suppressing a single target at a time. Therefore systematic platform for identifying targets that have a synergistic effect with PI3K inhibitors (PI3Ki) that silence PI3K activity is very much needed to provide critical information for rational combination targeting of multiple targets simultaneously. We first employed a methodology of combining a lentivirus shRNA library targeting 8500 known human genes with a PI3 kinase inhibitor, and a genome-wide screen was performed using microarrays to identify targets or pathways whose inactivation sensitizes cells to PI3Ki-induced cell death. The GeneNetTM human shRNA library contains 43,800 siRNA sequences targeted 8,500 well-characterized human genes listed in the NCBI with 4-5 shRNAs per gene. In brief, U87 glioma cells were infected with the lentiviral shRNA library for 24 hr; cells were treated with PI3Ki for another 72 hours. Viable cells were harvested for extraction of total RNA. Inserted siRNAs were then PCR amplified for subsequent hybridization to an Affymetric Genome Focus Array. Using a stringent objective statistical algorithm, we screened for genes that showed a > 4-fold increase in untreated cells. We used Ingenuity Pathways Analysis (IPA) software to facilitate identification of relevant targets. So far, we selected five targets as PI3K interacting partners in glioblastoma. We are currently doing secondary validation in 3/5 genes. As a second approach, we performed a high throughput single siRNA synthetic lethal screen using the on-Targetplus SMARTpools from Dharmacon siRNA kinome library targeting 714 genes. We identified 10 genes in the primary screen that's silencing strongly sensitized PI3K inhibition. Secondary screen of positive hits from the original library screen was done using four individual on-TargetplusTM siRNAs that made up the SMARTpool. Hits that show effects with each individual On-TargetplusTM siRNAs were evaluated further. With this strategy, the probability that any siRNAs that show consistent synthetic lethality through all levels of screening are due to off-target effects is significantly reduced. We identified a number of genes involving cell cycle control, apoptosis pathway, and others. The biological function of these genes is of potential interest and further validation is in progress to identify novel target genes. These findings suggest that synthetic lethality screen could be useful in the identification of new targets that would compliment the effects of the drug of interest and could be used as a powerful tool to identify rational combinations for therapeutic intervention.

VII-10 Experimental glioblastoma growth is inhibited by secreted interstitial delivery of ectodomain neuropilin-1 bodies

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Glioblastoma is the most common primary brain tumour in adults, still with a very poor prognosis regardless of significant progress made the last decade. Local relapses are the most common cause of death in spite of aggressive multimodal local treatment. Angiogenesis is one of the hallmarks of glioblastoma biology and vascular endothelial growth factor (VEGF) is the main angiogenic factor identified. Inhibition of VEGF signalling has shown significant effects on glioma progression in experimental as well as clinical settings. The neuropilin (NRP) family of receptors are important co-receptors for VEGF, forming complexes with VEGFR2 and thereby facilitating VEGF signalling. Naturally occuring soluble NRP isoforms have been described , they are able to bind VEGF and act as decoy receptors. Luciferase (luc2) expressing U87 glioma cells (U87-luc2) and baby hamster kidney (BHK) cells were transduced with a fusion protein of the extracellular domain of NRP1 (ecNRP1) and a human Fc fragment (huFc) using a lentiviral vector. U87 tumour cells were intracerebrally implanted in NOD/SCID mice and tumour growth was assessed either using ex vivo MRI or quantification of the bioluminescence signal from U87-luc2 cells. To mimic a therapeutic situation, ecNRP1 producing bioreactors were generated by encapsulating BHK-ecNRP1 cells in alginate beads and co-implanted with U87 tumour cells. Gene transfer of the soluble ecNRP1 construct completely inhibited growth of U87 glioblastoma in vivo (92% growth inhibition). No effect on U87luc2 cell proliferation was observed in vitro. Endocthelial cell (HUVEC) tube formation was inhibited in vitro by conditioned media from ecNRP1 producing cells. Alginate bioreactors producing ecNRP1 reduced U87luc2 glioblastoma growth with 97% at 21 days.
In conclusion, we provide evidence that ecNRP1 inhibits experimental glioblastoma growth at least partly by inhibiting tumour angiogenesis. Alginate bioreactors delivering ecNRP1 inhibits tumour growth in vivo and may represent a promising strategy for postoperative adjuvant anti-angiogenic treatment of glioblastoma.

VII-11 GSTP1 tyrosine-phosphorylation by egfr mediates a novel mechanism of drug resistance in glioblastoma

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The EGFR gene is frequently amplified, mutated, and/or overexpressed in malignant gliomas. The resulting aberrant EGFR signaling cascade plays a key role in the development of an aggressive malignant phenotype with a dismal prognosis and high resistance to therapy. To date, however, the essential cellular and molecular mechanisms underlying the association between activated EGFR signaling and drug resistance remain not fully understood. Similar to EGFR, the GSTP1 gene, which encodes a major drug metabolizing and cell signaling regulatory protein, is aberrantly overexpressed in higher grade gliomas and other human tumors and the overexpression is associated with a more malignant histology and poor therapeutic outcome. We recently made the important finding that GSTP1 is a, heretofore, unrecognized downstream target of EGFR and that, in vitro and in vivo, GSTP1 undergoes EGFR-dependent phosphorylation on tyrosines 3, 7 and 198, resulting in an enhancement of its phase II metabolic function. In the present study, we sought to better understand the impact of the EGFR-GSTP1 crosstalk on the drug resistance phenotype in brain tumors, by examining the effect of EGF ligand-dependent activation of EGFR on the drug sensitivity of human glioma cells. Using the GBM cell line, U87MG, and its isogenic counterpart, U87MG-EGFRwt, engineered to express high levels of EGFR, we showed that a 100 ng/ml EGF-pretreatment increased both the level of tyrosine-phosphorylation and the catalytic activity of GSTP1 by approx. 3-fold and was associated with a significant increase in cellular resistance to cisplatin and temozolomide The resistance. A similar EGF induced increase in drug resistance was observed in cells of four other GBM cell lines and was reversible by treatment with the clinically active EGFR inhibitor, gefitinib. Differential results with four anticancer agents (cisplatin, mitomycin C, doxorubicin and BCNU) indicated that the mediation of drug resistance by EGFR-GSTP1 interaction differs with the type of drug. These findings provide strong evidence that the GSTP1-EGFR crosstalk is a critical mediator of resistance to chemotherapy in human glioblastoma and may be targeted for improved GBM therapy. Supported by grants RO1 CA127872, RO1 CA112519, P50CA108786 and P30 CA014236 from the National Institutes of Health.

VII-12 sdf-1 and cxcr7 are key molecules for glioma angiogenesis and invasiveness

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Anti-angiogenic treatment is a promising therapy for malignant gliomas. Principal target should be endothelial cells composed of tumors. In this study, the genetical and functional characteristics of glioblastoma derived endothelial cells (GBMECs) are investigated especially focusing on chemokine SDF-1/ CXCR7 system. Immunohistochemical detection of SDF-1, CXCR4, and CXCR7 were obtained in 54 astrocytic tumors. SDF-1 and CXCR7 were strongly positive both in glomeruloid and small angiogenic vessels in high grade gliomas. Three ECs from 13 glioblastoma patients were isolated and cultured with endothelial growth medium (GBMEC-1, 7, 8). GBMEC showed strong expression of VEGF, SDF-1, and CXCR7 and no expression of CXCR4. GBMECs that secreted a large amount of SDF-1 into the conditioned medium migrated and constructed tube formation very well. Their SDF-1 induced migration was inhibited by CXCR7 antibody. Their tube formation was inhibited by CXCR7 antibody and dalteparin and stimulated by AMD3100 that is CXCR4 antagonist as well as CXCR7 stimulator. Also co-culture system with glioma cells / endothelial progenitor cell (EPC)s and GBMECs demonstrated that glioma cells/ EPCs attracted to GBMEC containing chamber through CXCR4. Finally, dalteparin associated with VEGF antibody inhibited glioma growth in vivo in subcutaneous as well as brain tumor model
In conclusion, SDF-1∂/CXCR4/CXCR7 were key target molecules in glioma angiogenesis and invasiveness. The characteristics of glioma derived ECs were completely different from those of normal ECs. GBMECs secrete SDF-1, resulting enhancement of autocrine angiogenesis through CXCR7 as well as paracrine glioma invasiveness. In order to inhibit glioma angiogenesis and invasiveness, GBMECs should be targeted, for example by antagonizing SDF-1 or CXCR7 expression.

VII-13 The influence of temozolomide dose-intensity on treatment efficacy in a panel of GBM xenografts

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Introduction: Dose-intense temozolomide (TMZ) schedules may enhance the efficacy of therapy in GBM by increasing DNA damage or suppressing MGMT activity.
Material and Methods: The efficacy of different TMZ dosing regimens were compared in 5 primary xenograft lines and 3 secondary TMZ-resistant lines derived from the Mayo GBM xenograft panel. All survival studies were performed with oral dosing of TMZ or placebo in mice with orthotopic xenografts.
Results: The efficacy of cyclical TMZ dosing was evaluated using dosing regimens similar to those tested on RTOG 0525: 'standard dose' (50-66 mg/kg/day, days 1-5 every 28 days) and 'dose-dense' (25-33 mg/kg/day, days 1-5, 8-12, and 15-19 every 28 days). The results of these studies are summarized in Table 1. Of the 5 primary xenograft lines tested, dose-dense therapy appears more effective in only one tumor line GBM12 (follow-up is ongoing). Of the secondary models of TMZ resistance, dose-dense TMZ therapy provided a clinically marginal, but statistically significant survival benefit in at least 2 of the 3 xenograft lines tested (GBM12TMZ and GBM39TMZ). There was no correlation between MGMT protein expression and the efficacy of dose-dense or standard TMZ treatment regimen. Interestingly, TMZ resistance in GBM12TMZ was associated with a marked upregulation of MGMT protein level, in comparison to the parental GBM12, and a corresponding increase in histone H3K9 acetylation within the MGMT promoter without a change in the DNA methylation status. Additional studies evaluating the influence of dosing intensity on the induction of TMZ resistance will be presented.
Conclusions: These data suggest that dose-dense TMZ therapy may provide a clinically significant additional survival benefit in only a limited sub-set of GBM tumors.

Median survival, days								
GBM line	GBM6	GBM8	GBM12	GBM39	GBM59	GBM12T-MZ	GBM39T-MZ	GBM59T-MZ
placebo	47	36	15	34	42	25	30	62
standard	57	156	79	219	79	31	36	77
dose-dense	52	176	103	189	74	37	41	NA
p-value	0.05	0.09	NA	0.77	0.98	0.01	0.003	NA
MGMT level	++	-	-	-	-	+++	-	-

VII-14 Chemo-immunotherapy with osmotic blood-brain barrier opening for recurrent or refractory primary CNS lymphoma

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Introduction: High-dose methotrexate (HD-MTX)-based chemotherapy with whole brain irradiation improves the prognosis of PCNSL, however up to 30% of patients are refractory to primary therapy and 60% relapse. Fewer than 50% of patients enter a second remission. Thus, novel treatment regimens for relapsed PCNSL are needed. As the majority of PCNSL are B-cell type expressing the CD20 antigen, treatment with the monoclonal antibody (mAb) rituximab might be reasonable. However, delivery of mAb to the brain would be limited by the blood-brain barrier (BBB). Osmotic BBB disruption (BBBD) is currently being used to increase the delivery of chemotherapeutic agents for the treatment of brain tumors. We hypothesize that BBBD would enhance delivery of mAb to the CNS. Here we report our experience with i.v. rituximab with BBBD followed by ICE (ifosfamide, cisplatin, and etopside) or temozolomide (TMZ) chemotherapy in patients with relapsed or refractory PCNSL.
Materials and Methods: Five patients who failed therapy with HD-MTX based chemotherapy were treated with a R-ICE regimen in a 28-day cycle. Two patents with relapse after R-ICE regimen and two elderly patient relapse after HD-MTX and WBRT were treated with a R-TMZ regimen in a 28-day cycle. BBBD was performed with 20% mannitol infused through catheter placed into the artery of the tumor site selectively at a rate of 3ml per second for 30-60 seconds.
Results: Three complete remissions (CR), one partial remission (PR) and one stable disease were achieved by R-ICE. Two CR and two PR were achieved by R-TMZ. Median progression-free survival (PFS) and median overall survival (OS) from first R-ICE were 4 months and 7.7 months, respectively. Median PFS and median OS from first R-TMZ were 4.5 months and 6 months, respectively. Adverse events possible related to osmotic BBB opening procedure were observed in two cases, I.e. one CNS hemorrhage and one ischemic stroke, both of which were grade 2 minor events. Hematotoxicity was prolonged in an elderly patient by R-ICE.
Conclusion: Chemo-immunotherapy regimens with osmotic BBB opening, R-ICE and R-TMZ, are tolerable with limited non-hematological toxicity and active in recurrent or refractory PCNSL.

Session VIII
Basic Science: Pathways / Metabolism

VIII-1 Stem-like cells and the perivascular niche

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eNOS expression is elevated in human glioblastomas and is correlated with increased tumor growth and aggressive character. We investigated a potential role for nitric oxide (NO) in the perivascular niche (PVN) using a genetic mouse model of PDGF-induced gliomas. We show that eNOS expression is highly elevated in the tumor vascular endothelium and closely aligned with perivascular glioma cells that express nestin, notch, and the receptor for NO. We also show that the NO/cGMP pathway drives notch signaling in vitro, and induces the side population phenotype in PDGF-induced glioma primary cultures. NO enhances the neurosphere forming capacity of these PDGF-driven glioma primary cultures, and enhances their tumorigenic capacity in vivo. We show that this mechainsm is conserved in a subset of human PDGFR gene amplified gliomas. This novel role of NO signaling in the glioma PVN presents attractive novel therapeutic targets for this devastating disease.

VIII-2 Tumor heterogeneity in glioblastoma is an active process driven by a mutant EGFR-induced paracrine circuit

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Introduction: A powerful oncogenic event in GBM is amplification and rearrangement of EGFR (EGFRvIII, de2-7EGFR, hereafter DEGFR), yet notably, only a minority of primary tumor cells possess this lesion while the remainder maintain expression of wildtype EGFR (wtEGFR). This disconnect between tumorigenic potential and the frequencies and proportions of these receptors might arise simply from a stochastic process wherein independent genetic events arise in these rapidly fatal tumors which never have the time to become homogeneous. Another, possibility would be that DEGFR occurs later in tumor progression where the minority of cells that express it not only enhance their own intrinsic tumorigenic abilities, but also potentiate the proliferation of neighboring majority cells expressing amplified wtEGFR. If this proved to be the case, then elucidation of such a potentiation loop would provide a potential novel therapeutic point of attack for high-grade disease. Here, we hypothesized that the ΔEGFR-expressing subpopulation provides enhanced tumorigenicity to the entire tumor cell population through a paracrine mechanism.
Material and Methods: We engrafted wtEGFR-expressing glioma cells into mice and examined the ability of small numbers of co-injected DEGFR-expressing cells to influence tumorigenic growth. We also examined conditioned media produced from DEGFR-expressing cells (DEGFR-CM) for its ability to activate signaling pathways in wtEGFR-expressing cells and for the presence of potential paracrine factors.
Results: We determined that human glioma tissues, glioma cell lines, glioma stem cells and primary mouse astrocytes, that express DEGFR each secrete IL-6 and/or LIF cytokines. This then prompts a novel interaction between the receptor that is common to these cytokines, gp130, and wtEGFR in neighboring cells that express amplified levels of EGFR, resulting in co-receptor activation and tumor growth enhancement mediated through activation of gliomagenic signature molecules, Akt, MAPK and STAT3. siRNA knockdown of IL-6, LIF or gp130 uncouples this cellular cross-talk and potently attenuates tumor growth enhancement.
Conclusions: These findings demonstrate that the heterogeneity which characterizes GBM, and perhaps other tumors with this feature, does not occur stochastically, but instead can be an actively maintained feature and illuminates a heterotypic cancer cell interaction of potential therapeutic significance.

VIII-3 Pathway analysis of glioblastoma tissue after preoperative treatment with the EGFR tyrosine kinase inhibitor gefitinib

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Introduction: Amplification of the EGFR is one of the most common oncogenic genetic alterations in primary glioblastoma making it a prime target for therapy. However, clinical trials in glioblastoma testing small molecule inhibitors of the EGFR tyrosine kinase failed, although some responses were observed. Here we aimed at investigating the molecular effects of preoperative treatment with the tyrosine kinase inhibitor Gefitinib on the EGFR signalling pathway in glioblastoma of patients enrolled in a clinical trial (phase 0).
Material and Methods: Patients selected for re-operation of a glioblastoma were treated at least 5 days with 500 mg Gefitinib prior to surgery, followed by post-operative Gefitinib until recurrence. Resected glioblastoma tissues were evaluated for drug concentrations and EGFR-pathway activity using a panel of phosphorylation specific assays (Bioplex, Biorad).
Results: Glioblastoma tissues exhibited high concentrations of Gefitinib (median, 4100 ng/g) that were more than 20 times higher than in the plasma (median, 181 ng/ml). The frequency of EGFR amplification in the patient cohort was 7/22. The EGFR was efficiently dephosphorylated in the treated patients as compared to a control cohort of 12 patients (7/12 with EGFR amplification), while downstream pathway constituents did not seem to be affected. In contrast, in vitro treatment of a glioblastoma cell line, BS153, with endogenous EGFRwt amplification and EGFRvIII expression resulted not only in dephosphorylation of the EGFR, but also of key regulators in the pathway like AKT. Intriguingly, a respective in vivo model, treating nude mice with established subcutaneous BS153 xenografts (1cm diameter) using the human dosing scheme over 5 days, showed dephosphorylation of the EGFR, however, similar to the human glioblastoma, downstream constituents of the pathway were not affected.
Conclusions: Gefitinib reaches high concentrations in the tumor tissue and efficiently dephosphorylates its target. However, regulation of downstream constituents in the EGFR pathway seems to be dominated by EGFR phosphorylation independent regulatory circuits. Beside negative feed-back loops of the pathway other pathophysiologic signals such as hypoxia may contribute to this effect under steady state conditions in vivo. Thus, additional rate limiting constituents of the pathways influencing EGFR downstream signalling need to be targeted for effective glioblastoma treatment.

VIII-4 Molecular therapy targeting sonic hedgehog and hepatocyte growth factor signaling in a mouse model of medulloblastoma

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Introduction: Medulloblastomas are malignant brain tumors that arise by transformation of neural stem cells in the cerebellum in children. Treatment-related neurotoxicity has created a critical need to identify signaling molecules that can be targeted therapeutically to suppress tumor growth and minimize collateral neurologic injury. In genetically engineered mice, activation of Sonic Hedgehog (Shh) signaling in neural stem cells in the developing cerebellum induces medulloblastomas. Hepatocyte growth factor (HGF) and its cell surface receptor c-Met are highly expressed in human medulloblastomas, and elevated levels of c-Met and HGF mRNA predict an unfavorable prognosis for patients. Previously, we showed that systemic administration of an HGF-neutralizing monoclonal antibody (mAb L2G7) prolonged survival of mice bearing Shh+HGF–induced medulloblastomas. We asked whether Shh+HGF–driven medulloblastomas were also responsive to Shh signaling blockade and whether treatment response could be enhanced by combination therapy, targeting both HGF and Shh signaling pathways.
Materials and Methods: we carried out a survival study in mice, in which we induced medulloblastomas by retrovirus-mediated expression of Shh+HGF, after which we treated the mice systemically with the following agents: (1) mAb L2G7, (2) cyclopamine, a plant alkaloid that inhibits Shh signaling by binding Smoothened, (3) Shh-neutralizing mAb 5E1, (4) L2G7+cyclopamine, (5) L2G7+5E1. Kaplan-Meier analysis showed that monotherapy, targeting either HGF signaling or Shh signaling prolonged survival.
Results: Anti-HGF therapy had a more durable treatment response than Shh-targeted therapy. Surprisingly, combination therapy was less effective than monotherapy. Histochemical analysis of the tumors showed that the principle mechanism by with Shh– and HGF–targeted therapies inhibited tumor growth was a potent apoptotic death response in tumor cells, supplemented by a weaker suppressive effect on proliferation. We also observed that L2G7 treatment shifted the tumor cytoarchitecture from an undifferentiated, classic medulloblastoma pattern to a more differentiated, extensive nodularity pattern.
Conclusions: These findings demonstrate efficacy of Shh- and HGF-targeted therapy in a mouse model of endogenously arising medulloblastomas. These results underscore the need for preclinical testing in animal models of tumors, in which the targeted pathway is activated.

VIII-5 EGFRVIII translocates to the nucleus and regulates gene transcription with STAT5

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Dysregulation of receptor tyrosine kinases is a major contributor to cancer, including glioblastoma, where amplification and mutation of epidermal growth factor receptor (EGFR) is common. The most common mutation is in-frame deletion of exons 2-7, known as EGFRvIII, EGFR* or ΔEGFR. EGFRvIII confers enhanced tumorigenicity on glioma cells in vivo, reducing apoptosis and increasing proliferation. EGFRvIII signals constitutively and at approximately 5-to-10-fold lower intensity than wild-type. We are investigating the pathways that are preferentially activated by EGFRvIII, using a phosphotyrosine-directed, mass spectrometry-based shotgun phosphoproteomics approach in two different cell lines, LNZ308 (PTEN null) and LN428 (PTEN wt). We identified 433 proteins with 772 peptides out of which 249 proteins corresponding to 354 peptides showed tyrosine phosphorylation. Statistical analysis revealed several signals that were prevalent in EGFRvIII expressing glioma cells, including phosphorylation of STAT5, Gab1 and MIG6. Identification of STAT5 as a target of EGFRvIII prompted an investigation of whether EGFRvIII is active in the nucleus. We have identified EGFRvIII associated with phosphorylated STAT5 on chromatin, and capable of activating the expression of STAT5 target genes, including Aurora A kinase, which positively regulate glioblastoma malignancy. STAT5 promotes expression of the EGFRvIII target gene Bcl-XL, and knockdown of STAT5 reduces the transformation induced by EGFRvIII. Furthermore, we have mutated signals in EGFRvIII that regulate nuclear localization, and are examining the impact of these on its ability to promote tumor growth. In a related study we have used artificial receptor dimerization, mediated by technology from Ariad Pharmaceuticals, to enhance the EGFRvIII signal. A chimeric version of EGFRvIII was created, in which the receptor was fused N-terminally with two FKBP-F36V domains that could be activated by the chemical inducer of dimerization, AP20187. This has resulted in a more oncogenic form of EGFRvIII that emits a stronger signal, and we are using this to deepen our phosphoproteomics analysis to seek additional preferential targets of EGFRvIII action, which will be reported.

VIII-6 Reveral of temozolomide resistance by modualtion of a ubiquitin-controlled apoptotic pathway

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Introduction: DNA repair in response to methylating agents such as temozolomide (TMZ) requires monoubiquitination of proliferating cell nuclear antigen (PCNA) and the Fanconi anemia D2 protein (FANCD2), and

loss of either protein, or inhibition of their monoubiquitination enhances methylating agent-induced toxicity. USP1 is a ubiquitin hydrolase that removes monoubiquitin from PCNA and FANCD2 and as such may be a key regulator of TMZ response. Material and Methods: To address the possible role of USP1 in TMZ action, U251 (TMZ-sensitive, low levels of MGMT) and GBM8 (TMZ-resistant, high levels of MGMT) GBM cells were transfected with control or USP1-targeted siRNA, exposed to TMZ (100mM, 3 hours), washed, and placed in TMZ-free medium. The effect of USP1 downregulation on TMZ-induced G2 arrest, cell death, and clonagenicity were then monitored.
Results: siRNA-mediated suppression of USP1 alone (>80% relative to controls) had minimal effects on cell cycle progression and cell death in either cell line. Suppression of USP1 levels similarly did not alter the extent of TMZ-induced G2 arrest, which remained significant in the TMZ-sensitive U251 cells, but minimal in the TMZ-resistant GBM8 cells. Surprisingly, however, USP1-knockdown increased TMZ-induced loss of clonagenicity in both TMZ-sensitive and TMZ-resistant cells. Further examination of the mechanism of cell death showed that while USP1-suppressed control cells, or TMZ-treated cells with normal levels of USP1, rarely underwent apoptotic cell death (9% and 4% average, respectively) TMZ-treated cells in which USP1 levels were suppressed underwent high rates of apoptotic cell death (34% average).
Conclusion: The deubiquitinase USP1 has been indirectly suggested to inhibit the function of PCNA and FANCD2, and in so doing to inhibit the repair of methylating agent-induced DNA damage and to sensitize cells to DNA damage. The present studies, however, show that USP1, rather than sensitizing cells to DNA damaging agents, appears to suppress latent apoptotic pathways and to protect cells from TMZ-induced apoptosis. These results suggest novel functions for USP1 beyond the control of PCNA/FANCD2 ubiquitination and DNA repair, and suggest that suppression of USP1 and/or USP1 controlled pathways may be a means to enhance the cytotoxic potential of TMZ and to sensitize previously resistant GBM.

VIII-7 Synthesis of cytochrome c oxidase 2 (SCO2): a p53-dependent metabolic regulator that promotes respiratory chain function and protects glioma cells from hypoxia-induced cell death

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p53 has an important role in the processing of starvation signals. p53-dependent molecular mediators of the Warburg effect regulate glucose consumption and mitochondrial function. We therefore hypothesized that the presence of wild-type p53 in glioma cells serves to limit metabolic demands induced by deregulated signal transduction processes in the presence of hypoxia and nutrient depletion. We here report that shRNA-mediated gene suppression of wild-type p53, or introduction of the dominant-negative p53V135A mutant, increase glucose consumption and lactate production, decrease oxygen consumption and enhance hypoxia-induced cell death in p53 wild-type human malignant glioma cells. Further, antagonism of p53 represses synthesis of cytochrome c oxidase 2 (SCO2), an effector molecule for respiratory chain function. A SCO2 transgene reconstitutes glucose consumption, lactate production and oxygen consumption and resistance towards hypoxia in a rotenone-sensitive fashion, demonstrating that this effect depends on intact oxidative phosphorylation. Finally, gene suppression of SCO2 in p53 wild-type glioma cells sensitizes these cells towards hypoxia. These findings suggest that glioma cells may benefit from retaining p53 wild-type status by reducing their vulnerability towards tumor hypoxia and that SCO2 is the major mediator of this effect. Targeting metabolic regulators may be a valuable strategy to enhance glioma cell sensitivity towards spontaneously occurring or therapy-induced hypoxia.

VIII-8 Developmental profile and regulation of the glycolytic enzyme hexokinase 2 and its association with aerobic glycolysis

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Introduction: Proliferating embryonic and tumor tissues rely on aerobic glycolysis, or the metabolism of glucose to lactate under oxygenated conditions, to assist in the synthesis of biosynthetic precursors necessary for proliferation. The reliance on aerobic glycolysis may be mediated by the expression of specific metabolic enzymes. Mammalian Hexokinases HK1 and HK2 are 100kDa proteins that phosphorylate glucose to glucose-6-phosphate as the first step of the glycolytic pathway. In normal adult tissues, HK1 is ubiquitously expressed but is particularly prominent in the brain and kidney. HK2 is generally expressed at low levels within adipose tissue and skeletal tissue and negligently in normal brain. In this study, we wished to determine the ontogeny of these isoforms within the developing brain and determine how their expression relates to the extent of aerobic glycolysis in GBM. Mining of existing published microarray data found strong expression of HK1 in 1, 2, 4 cell stage but a switch to stronger expression of HK2 in the blastocyst stage, previously reported to rely heavily on aerobic glycolysis.
Materials and Methods and Results: We performed HK2 immunohistochemistry in mice from gestational age E12, E16, post natal 1 month and 2 months. Our results demonstrated an age and cell specific HK1 and HK2 immunoreactivity in embryonic brain tissue with decreased expression of HK2 post-natally. Subsequently, we investigated the expression of HK1 and HK2 isoforms in a panel of GBM cell lines with varying levels of dependence on aerobic glycolysis, as measured by lactate levels and O2 consumption. HK2 but not HK1 expression was higher in GBM cells that had low O2 consumption and high extracellular lactate levels, supporting an association of HK2 with aerobic glycolysis. As HK2 expression is nearly silent in adult brain but expressed in fetal tissue and GBM cells, we hypothesized that DNA methylation/demethylation events may be playing important in its regulation. Adult normal human brain and GBM cell lines that had no HK2 expression (U343, A172) were found to be methylated at CpG islands within intron 1 by bisulfite sequencing in contrast to fetal tissue and HK2-expressing GBM cells. The degree of methylation correlated with transcript expression in GBM cell lines. Treatment of U343 and A172 cell lines with 5-aza cytidine and Trichostatin A restored HK2 transcript expression, supporting that HK2 maybe epigenetically regulated.
Conclusions:
Overall, our results demonstrate that the expression of the HK2 isoform, in contrast to HK1, may be particularly important in tissues relying on aerobic glycolysis for proliferation including embryonic tissue and GBMs.

VIII-9 Microrna-451 regulates LKB1/AMPK signaling and allows adaptation to metabolic stress in glioblastoma

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Introduction: To sustain tumor growth cancer cells must adapt to a dynamic and challenging microenvironment. We hypothesized that gliomas microRNAs may control this process.
Materials and Methods: Using glioma spheroids, we sought out microRNAs that were up-regulated or down-regulated during microenvironmental changes, such as glucose levels or migration.
Results: Amongst several microRNA changes, we identified one microRNA that controls glioma cell proliferation, migration and responsiveness to glucose deprivation. Abundant glucose promoted high miR-451 expression and stimulated glioma spheroid proliferation. However, in low glucose, miR-451 levels decreased, slowing proliferation but enhancing migration and survival of cells from the glioma spheroid. Mechanistically,

the effects of miR-451 were mediated by LKB1. LKB1 is an activator of AMPK, a highly conserved sensor of cellular energy availability, that also regulates the mTOR pathway. Mir451 targeted LKB1's binding partner, CAB39 (MO25a), destabilizing and inactivating the LKB1 complex. Over-expression of miR-451 sensitized cells to glucose deprivation suggesting that its down-regulation is necessary for robust activation of LKB1 in response to metabolic stress. Downstream effects of mir451 de-regulation affected AMPK activation and the mTOR pathway. In human glioblastoma patients elevated miR-451 was associated with significantly shorter survival

Conclusions: miR-451 is a novel regulator of the LKB1/AMPK pathway, allowing glioma cell adaptation to micro-environmental fluctuations. mir451 represents a microRNA-mediated sensor mechanism of glucose levels that allows glioma cells to survive metabolic stress and seek out favorable growth conditions. This may represent a fundamental mechanism with broad implications, in normal and cancerous cells, that couples glucose availability with glioma proliferation vs. glioma migration (the " go" or "grow" effect).

VIII-10 Impact of differentiation resistance in gliomas

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Introduction: The so-called brain tumor stem cell concept postulates the occurrence of immature cell populations that are irresponsive to physiological differentiation stimuli. Such a loss of differentiation capacity could occur at multiple levels involving e.g. loss of specific receptors for endogenous differentiation inducers like retinoic acid (RA) or up-regulation of degrading enzymes. Additionally, it has been proposed that RA can be diverted into an alternative pro-survival pathway by fatty acid binding protein 5 (FABP5). Here we investigated key molecules of the RA pathway involved in a loss of differentiation and analyzed their influence on patient outcome.

Material and Methods: We made use of a tissue microarray (TMA) comprising tumor samples from 288 patients suffering from astrocytic gliomas WHOII-IV. Protein expression was correlated with tumor grades, differentiation and survival data. Finally, expression and function of RA signaling molecules was analyzed in vitro in a set of primary glioma cells lines.

Results: Contrary to previous findings on other tumor entities, analysis of RA pathway revealed a severe up-regulation of RA synthesis and metabolism in glioma, which increased with tumor malignancy and was associated with poor patient survival. This included up-regulation of 1) the intracellular RA-binding protein CRBP1 catalyzing the up-take of RA-precursor molecules, 2) Aldehyde Dehydrogenase 1 family turning RA-precursors into the active RA metabolite, 3) the intracellular RA-binding binding protein CRABP1 and the metabolizing enzyme CYP26B1, which are involved in RA inactivation and 4) the intracellular RA-binding protein FABP5 diverting RA into a survival-promoting pathway. On the other hand we found a loss of CRABP2, a RA-binding protein shuttling RA into the nucleus, where RA can bind to differentiation receptors. The expression of CRABP2 decreased with malignancy and both down-regulation of CRABP2 as well as loss of differentiation receptors RARalpha and RXRalpha was significantly associated with poor patient outcome.

Conclusions: Our data support the notion that different mechanisms are involved in the loss of differentiation capacity and in the maintenance of an undifferentiated phenotype in glioma and that such a differentiation resistance influence the clinical prognosis of glioma patients.

VIII-11 NG2/MPG promotes resistance to ionising radiation by elevated peroxiredoxin-1 and dna damage response in glioblastoma multiforme

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Glioblastoma Multiforme (GBMs) are lethal cancers that respond poorly to radiotherapy and the mechanisms may involve stem/progenitor cells. Several studies proclaimed that brain tumours enriched in CSCs were preferentially resistant to ionising radiation and chemotherapy due to altered checkpoint and DNA repair pathways compared to conventional tumour cells. Others have claimed that these cells are associated with increased reactive oxygen species and that this is an additional mechanism for radiation resistance. Since the progenitor marker NG2, or melanoma proteoglycan (MPG) has been shown to regulate tumour response to chemotherapy, we examined whether it also affected response to radiotherapy. Quantification of NG2/MPG expression in 96 patient GBM biopsies revealed that high NG2/MPG expressers had shorter survival outcomes than low expressers. Two-dimensional (2D) proteomics of 11 of these biopsies showed that peroxiredoxin-1 (PRDX-1) was upregulated in the shortest surviving patients, and was associated with reduced oxidative damage. Furthermore, NG2/MPG expressing GBMs were highly resistant to ionising radiation (IR) in vitro and in vivo and increased PRDX-1 levels in a dose dependent manner. shRNA mediated NG2/MPG knockdown sensitised the tumour cells to IR and attenuated dose dependent induction of PRDX-1. Moreover, NG2/MPG expressing cells rapidly induced DNA damage response signalling as indicated by phosphorylation of H2AX, ATM, and Chk2 proteins compared to NG2/MPG negative cells. PRDX-1 knockdown transiently slowed tumour growth rates in vivo and partially sensitised the tumours to ionising radiation in vitro. These data demonstrate a novel role for NG2/MPG in mediating radioresistance in human GBMs by interaction with PRDX-1 and DNA damage response machinery.

Session IX
Immunology / Immunotherapy

IX-1 Dual-specific immunotoxin D2C7-(SCDSFV)-PE38KDEL for brain tumor therapy

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Introduction: Glioblastoma multiforme (GBM) is the most malignant and most frequently occurring brain tumor. The epidermal growth factor receptor (EGFR) is expressed by normal epithelial cells in most tissues but is not expressed in normal brain; it is overexpressed in 60%-90% of all GBMs. In addition, 58%-61% of all GBMs also express the EGFR variant III mutant (EGFRvIII), which is not found in normal tissues. Monoclo-

nal antibodies targeting either the wild-type EGFR (EGFRwt) or EGFRvIII have been developed. D2C7, a murine IgG1κ, recognizes both the EGFRwt and tumor-specific EGFRvIII receptors. We tested the in vitro and in vivo efficacy of D2C7-(scdsFv)-PE38KDEL, a recombinant immunotoxin recognizing both EGFRwt and tumor-specific-EGFRvIII receptors.
Material and Methods: The reactivity of D2C7 antibody was tested in GBM patient samples by immunohistochemistry. D2C7-(scdsFv), cloned from the D2C7 hybridoma, was fused to -epidermoid-carcinoma, and glioblastoma cells. In vivo activity was evaluated with A431, NR6M Pseudomonas exotoxin A, carrying a C-terminal KDEL peptide (PE38KDEL). The D2C7-(scdsFv)-PE38KDEL binding affinity and specificity for EGFRwt and EGFRvIII were measured by surface plasmon resonance and flow cytometry. In vitro cytotoxicity was measured in EGFRwt-transfected (NR6W), EGFRvIII-transfected (NR6M), A431, 43, and D270MG tumor models.
Results: D2C7 antibody positively stained virtually all cells in the GBM samples: 100% (50/50) of those with and 76% (39/51) of those without EGFRwt amplification. The affinity of D2C7-(scdsFv)-PE38KDEL for EGFRwt and EGFRvIII extracellular domain was 6.3×108(mol/L)–1 and 7.8×108(mol/L)–1, respectively. Flow cytometry with NR6W and NR6M cells confirmed the dual-specificity of D2C7-(scdsFv)-PE38KDEL. The D2C7-(scdsFv)-PE38KDEL IC50 on NR6W and A431 cells was 0.7 and 0.17 ng/mL, respectively. The IC50 of D2C7-(scdsFv)-PE38KDEL on NR6M, D2159MG, D270MG, and D256MG cells was also determined (0.36-0.52 ng/mL). A431–tumor-bearing, D2C7-(scdsFv)-PE38KDEL-treated animals demonstrated a significant tumor–growth delay, averaging 112 days. In a tumor model with NR6M tumors, D2C7-(scdsFv)-PE38KDEL exhibited growth delays of ≈10 days (P<0.001). Significantly, D2C7-(scdsFv)-PE38KDEL delayed growth for 75 and 15 days in glioma models, 43, expressing EGFRwt, and D270MG, expressing both EGFRwt and EGFRvIII, respectively.
Conclusion:
In preclinical studies, D2C7-(scdsFv)-PE38KDEL, an EGFRwt/EGFRvIII-targeting immunotoxin, exhibited significant potential for treating astrocytic tumors.

IX-2 Autologous heat shock protein vaccine for patients with newly diagnosed and recurrent glioblastoma

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Introduction: Autologous heat shock protein vaccine HSPPC-96 (Oncophage®) is derived from a patient's individual tumor and contains glycoprotein-96 (gp96) polypeptide associated with cancer-specific antigenic peptides. HSPPC-96 has been shown to elicit innate and adaptive immune response in patients with recurrent glioma. In ongoing investigations, we evaluated the use of HSPPC-96 for treatment of Recurrent Glioblastoma Multiforme (GBM) and in combination with chemotherapy in patients with Newly Diagnosed GBM.
Materials and Methods: Two ongoing, phase 2, open-labeled investigations designed to evaluate clinical efficacy and immunologic response of HSPPC-96 treatment in patients with recurrent GBM (multi-center investigation) and newly diagnosed GBM (single-center investigation). Patients with recurrent GBM receive HSPPC-96 treatment within 5 weeks post-surgery and patients with newly diagnosed GBM are treated with concurrent HSPPC-96 and temozolomide following surgery and standard of care radiation and temozolomide therapy.
Results: HSP vaccine was well tolerated with no serious adverse events attributable to vaccine and no related grade 3 or 4 toxicities. In patients with recurrent GBM (n=32) the overall median survival approximated 44 weeks post-resection and the majority (94%) of patients survived beyond the historical median of 26-weeks. Approximately 70% of patients survived beyond 36 weeks and 41% survived up to or longer than 1-year. All patients with immunological endpoints tested to date exhibited a significant innate immune response following vaccine administration and 92% of patients demonstrated CD8 T cell IFN gamma production upon restimulation with recombinant gp-96 (p<0.01). The majority of patients (92%) exhibited significant Th1 type responses as measured by multi-cytokine qPCR. The presence of an adaptive immune response and minimal residual disease was associated with survival beyond 36 weeks. In patients with newly diagnosed GBM (n=8; trial initiated 2009), there have been no significant toxicities associated with concurrent treatment of HSPPC-96 and temozolomide. Clinical and immunologic evaluation is ongoing.
Conclusions: HSPPC-96 is safe for patients with recurrent glioma and appear to be safe with concurrent treatment with chemotherapy. HSPPC-96 evokes a specific and innate immune response in patients with recurrent glioma and survival data available to date indicates HSPPC-96 vaccine provides a possible clinical benefit with a favorable safety profile.

IX-3 Targeting glioma tumorigenesis and immune suppression with p-STAT-3 blockade

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Introduction: The phosphorylated signal transducer and activator of transcription (p-STAT)-3 pathway is frequently expressed in malignant gliomas, especially within cancer stem cells (CSCs) that are mediators of chemo- and radiation resistance. This pathway drives the fundamental components of tumorigenesis such as proliferation, angiogenesis and invasion and becomes induced in the various immune populations upon encountering the glioma microenvironment resulting in global, profound immune suppression.
Materials and Methods: CSCs, macrophage/microglia and T cells were isolated from human malignant gliomas and were phenotypically and functionally characterized. A small molecular inhibitor of the p-STAT-3 pathway (WP1066) and siRNA were used to reverse the CSC-mediated immune suppression and in murine models of established intracerebral tumors.
Results: The CSCs by both a variety of secreted products (TGF-ß, Galectin-3, macrophage inhibitory cytokine) and by cell-to-cell contact via expression of B7-H1 trigger T cell apoptosis, inhibit T cell proliferation and effector function, induce Tregs and induce immune suppressive macrophage/microglia that then further perpetuate immune suppression via IL-23 – an inducer of Tregs. Treatment of the CSCs with physiological doses of WP1066, STAT-3 siRNA or differentiation reversed the CSC-mediated immune suppression and restored immunological responses. Oral administration of WP1066 resulted in long-term survival of 80% of mice with established intracerebral tumors (p<0.05) by inhibiting Tregs and enhancing CD8 anti-tumor cytotoxic responses.
Conclusions: Inhibition of p-STAT-3 pathway is a promising immune therapeutic approach that exerts marked in vivo activity, including against CSCs, and could also be used in the context of restoring chemo- and radiation sensitivity. Our intention is to bring the "first-in-class" p-STAT-3 inhibitor, WP1066, into phase I/II clinical trials within the next 18 months for patients with CNS malignancies.

IX-4 Interleukin-2 receptor ∂ (il-2r∂ / cd25)-specific antibodies eliminate regulatory t-cells (t_{regs}) and enhances tumor-specific immune responses against cytomegalovirus (CMV) in the context of therapeutic temozolomide (TMZ)-induced lymphopenia in patients with glioblastoma (GBM)

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Introduction: Immunotherapy is an attractive therapeutic alternative for glioblastoma (GBM), but is limited by the lack of frequent and homogeneously-expressed tumor-specific antigens. The nearly universal presence and homogeneous expression of antigens derived from Cytomegalovirus (CMV) in GBM, but not normal brain, has now been well-established and may provide ideal antigens for tumor-specific immunotherapy. Despite the potential immunogenicity of CMV antigens, CMV immune responses in patients with GBM are blunted which may be a result of immunosuppressive regulatory T cells (TRegs) which are present at high levels in patients with GBM. Monoclonal antibodies (MAbs) that block IL-2Ra have been shown to abrogate TReg function in animal models, but also inhibit effective antitumor immune responses in the context of established tumors and attenuate immune responses in humans with autoimmune disorders or allogeneic organ transplants.
Material and Methods: The interleukin-2 receptor a (IL-2R a)-specific MAbs, PC61 (250 µg/mouse) and daclizumab (1 mg/kg), were administered during the recovery from TMZ-induced lymphopenia in mice and humans with GBM, respectively, concurrent with tumor-specific vaccination targeting the model antigen OVA or CMV pp65.
Results: As expected, in normal mice, anti-IL2Ra MAb treatment depletes and functionally impairs TRegs, but it also abrogates vaccine-induced immune responses. However, in direct contrast, when IL-2Ra-specific MAbs are administered during recovery from temozolomide (TMZ)-induced lymphopenia, anti-IL2Ra MAb administration depletes TRegs while simultaneously augmenting vaccine-induced effector T-cell responses and antitumor efficacy. Similarly, in a randomized Phase II trial, administration of the humanized IL-2Ra-specific MAb daclizumab after therapeutic TMZ dramatically reduces TReg levels (73.9% + 8.2 S.D.) and increases pp65-specific CD8+ T-cell responses (4.3 fold) in response to vaccination with CMV pp65 mRNA transfected DCs. Time-to-progression in the cohort of patients treated with daclizumab exceeds 24 months with a likelihood of 2 year progression-free survival of 66.7% (CI95 19.5%, 90.4%).
Conclusions: Therapeutic TMZ-induced lymphopenia provides a unique window of opportunity in which the presence of anti-IL-2Ra antibodies selectively inhibits TRegs, but potentiates ongoing anti-tumor effector T cell responses.

IX-5 A bispecific t cell-engaging antibody effectively eradicates EGFRVIII expressing glioblastoma multiforme

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Introduction: Glioblastoma multiforme (GBM) is the most common primary brain tumor and remains uniformly lethal despite the progress of conventional therapies, which lack specificity and thus result in high toxicity to normal cells. Immunotherapy promises to induce robust tumor-specific immune responses that eliminate neoplastic cells with unparalleled specificity and with no additional toxicity to multimodality therapy. Recently, an approach using recombinant bispecific T-cell engagers (BiTEs), which consist of a tumor-targeting, single-chain antibody conjugated to a single-chain antibody directed against a T-cell activation ligand such as CD3, has arisen as a promising means for treating tumors. Human trials using a CD19XaCD3 BiTE confirmed the potency of these constructs by producing tumor regression in 7/7 patients with non-Hodgkin's lymphoma. The most significant limitation of these promising constructs, however, is the lack of tumor-specific targets that are frequently and homogeneously expressed. The epidermal growth factor receptor mutation EGFRvIII is a consistent 801-base-pair in-frame deletion in the extracellular domain of EGFR, not expressed in normal tissues but widely expressed in GBM and other common neoplasms. EGFRvIII also produces a constitutively active tyrosine kinase that enhances neoplastic cell growth and migration and confers radiation and chemotherapeutic resistance. Thus EGFRvIII appears to be a perfect anti-tumor target for these novel constructs.
Materials and Methods: Here, we hypothesize that by targeting EGFRvIII through the T-cell engager MR1-1XaCD3, GBM will be efficiently eradicated. To test our hypothesis, we constructed the molecule MR1-1XaCD3, which consists of MR1-1, the murine anti-human EGFRvIII single-chain Fv, and aCD3, the murine anti-human CD3 single-chain Fv. MR1-1XaCD3 was expressed in and purified from bacteria BL21 (DE3), and the activity of this double- function molecule was confirmed by flow cytometry, showing its specific binding to EGFRvIII expressing cell lines as well as to human T cells. The cytotoxicity of MR1-1XaCD3 on EGFRvIII-expressing GBM D54MG.EGFRvIII cell lines was measured in vitro by standard chromium release assay.
Results: Preliminary results show that the MR1-1 construct is highly cytotoxic and antigen-specific, with an 8-fold increase in specific lysis for D54MG.EGFRvIII over the wild-type control. Currently, the efficacy of MR1-1XaCD3 is being evaluated in NOD/SCID gamma mice where human EGFRvIII-expressing xenografts have been implanted.
Conclusion: In summary, our experiments showed that a human EGFRvIII-specific T-cell engager, MR1-1XaCD3, is effective on EGFRvIII-expressing tumor cells and provide new insights into GBM treatments.

IX-6 Clinical and immunological evaluation of wt1 (wilms' tumor gene 1) peptide vaccination for patients with recurrent or refractory malignant brain tumors

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Introduction: Wilms' tumor gene (WT1) possesses oncogenic functions and is expressed in various kinds of malignancies, which suggests that the gene's product, the WT1 protein, should be one of the most promising cancer antigens. Clinical trials of the WT1 peptide vaccination were initiated against malignant brain tumors, and immunological and clinical responses were observed.
Material and Methods: WT1 peptides that could induce WT1-specific CTLs were identified. Patients with WT1/HLA-A*2402 positive recurrent glioblastoma and recurrent adult PNET were evaluated in a study of WT1 vaccine therapy. Patients were intraderrmally injected with an HLA-A*2402-restricted, modified 9-mer WT1 peptide emulsified with Montanide ISA51 adjuvant at 3.0 mg per body every week for 12 weeks. The responses were analyzed by RECIST criteria. Patients who achieved an effective response continued to be vaccine until tumor progression occurred. Immunohistochemical study of the tumor specimen was analyzed. Immunological responses were assessed by ex vivo immuno-monitoring, such as the tetramer assay.
Results: The protocol was well tolerated; only local erythema occurred at the WT1 vaccine injection site. The disease control (PR and SD) rate of 57.1% was obtained especially in 21 cases of recurrent glioblastomas, with a median progression-free survival period of 20.0 weeks and progression-free survival rate at 6 months of 33.3%. The disease control rate of 60 % was obtained in 5 cases of recurrent adult PNET. Immunohistochemical study showed that malignant gliomas and PNET highly expressed WT1 protein. The frequencies of WT1-specific cytotoxic T lymphocytes (CTLs) were higher in patients with glioblastoma than in healthy donors, already before the vaccination. Changes of phenotype in WT1-specific CTLs during the WT1 vaccination were observed. Conclusions: Although small uncontrolled nonrandomized clinical trial, it showed that WT1 vaccination for malignant brain tumors was safe and elicited a favorable clinical and immunological response. Further clinical studies of WT1 vaccination in patients with malignant brain tumors as well as other cancers are warranted.

IX-7 GD3/GD3A as putative therapeutic targets for glioma

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Introduction: The ganglioside GD3 is upregulated in neoplastic cells and plays a pivotal role in the regulation of tumour growth and invasion. Whilst in non-neoplastic cells, the build up of GD3 induces mitochondrially-mediated apoptosis, this does not occur in tumour cells due to the acetylation of the terminal sialic acid to form GD3A. Haemaglutinin esterase (HE) from Influenza C virus has been shown to deacetylate GD3A and restore pro-apoptotic GD3. We are investigating the efficacy of exogenous addition of recombinant HE and transfection and transduction with the HE at deacetylating GD3A in gliomas and inducing mitochondrially-mediated apoptosis.
Methods: Recombinant HE and HE were supplied by R. Vlasak, cells used in all assays are glioblastoma multiforme biopsy-derived early passage cells and normal human astrocytes are used as a control. GD3/GD3A expression was determined by flow cytometry and immunocytochemistry, Cell viability was determined using the MTS and Annexin V assays western blotting for cytochrome c. The effect on invasion was assessed using the modified Transwell Boyden Chamber™ assay.
Results:
Exogenous addition of recombinant HE causes a decrease in expression of GD3A and an increase in GD3 expression, this occurs with a simultaneous increase in cytotoxicity and apoptosis and a decrease in invasion. We have generated pcDNA3.1-HE and pBacPAK-CMV-HE for transfection/transduction studies. Our previous studies have shown the baculovirus to be effective at transducing brain tumour cells.
Conclusions:
HE deacetylates GD3A in glioma to a critical threshold and restores apoptotic ability. GD3A and GD3 are onco-fetal antigens and are not expressed in astrocytes thus HE has no effect on these cells. Deacetylation of GD3A may be a potential therapeutic strategy for glioma.

IX-8 Elimination of regulatory t-cells alters the isotype and affinity index of the antigen specific humoral immune response following vaccination with CDX-110 in patients with EGFRVIII expressing gliomas

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Introduction: Regulatory T-cells (Tregs) are increased in patients with GBM. These Tregs are uniquely dependent on the high affinity interleukin (IL)-2 receptor (IL-2R∂) for their function and survival. Monoclonal antibodies that block IL-2R∂ have also been shown to abrogate Treg function in animal models but can also inhibit effector antitumor immune responses in mice and attenuate immune responses in humans. This inhibition of effector T-cells is likely due to a requirement for IL-2R∂ signaling by effector T-cells in these contexts as well. TMZ-induced lymphopenia, however, may provide an environment where surges in cytokines that share receptors with IL-2 such as IL-7 and IL-15 can bypass the need for IL-2 signaling in effector T-cells. If so, lymphodepletion may provide a unique context wherein effector T-cells may not be susceptible to inhibition by IL-2R∂ blocking antibodies while Tregs may remain susceptible because of their dependence on IL-2.
Materials and Methods: Patients with an EGFRvIII expressing glioblastoma underwent resection followed by external beam radiation with concurrent TMZ. Patients were randomized to receive a single dose of daclizumab (1 mg/kg) or saline. All patients received vaccinations with an EGFRvIII-specific peptide (PEPvIII) conjugated to keyhole limpet hemocyanin (CDX-110) along with granulocyte-macrophage colony-stimulating factor and monthly cycles of TMZ. Subjects received vaccinations until tumor progression or death. Blood was drawn at each vaccination for determination of Treg levels and anti-PEPvIII titers.
Results: EGFRvIII-specific immune responses were generated in all patients despite treatment with TMZ and daclizumab, and all immune responses were sustained or enhanced during subsequent TMZ cycles. There were no drug-related adverse events. Furthermore, preliminary analysis suggests that daclizumab reduced Treg numbers (change 82.4 ± 7.1% from baseline (P=0.011; t-test)). There was an increase in the titer and affinity index of EGFRvIII-specific IgG1 isotype antibodies compared to the saline treated group (P=0.003; t-test). Clinical endpoints of time-to-progression and overall survival in both arms have not been reached.
Conclusion: In the context of TMZ-induced lymphopenia, the blockade of the high affinity IL-2∂ receptor reduces Tregs, and increases the IgG1 isotype of PEPvIII specific antibody along with the affinity index of these antigen specific antibodies.

IX-9 Therapeutic targeting of the NG2 proteoglycan with mab 9.2.27 and adoptively transferred NK cells lyses human glioblastoma multiforme in vivo

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Glioblastoma Multiforme (GBM) is a lethal subgroup of intracranial tumours with a median survival of less than 15 months, despite multimodal treatment involving surgery, concurrent radio- and chemotherapy. We have previously shown that several treatment resistant tumour types aberrantly express the neural progenitor marker NG2. We aimed to therapeutically target NG2 in GBM tumours using the 9.2.27 mAb and adoptively transferred autologous NK cells and to determine the mechanisms of anti-tumour effect at the immunological synapse. We designed a novel cocktail of rat antibodies to obtain NK cells of high purity and yield from acutely dissociated splenocytes. The NK cells and mAb were infused intratumourally by convection-enhanced delivery (CED) in rat brains transplanted with human GBM xenografts, as well as syngeneic rat gliosarcoma. Magnetic resonance imaging was used to longitudinally monitor the tumour growth characteristics. The mAb bio-distribution to the tumour bed in vivo was visualised by optical imaging. Flow cytometry and immunohistochemistry were used to evaluate the tumour cytotoxicity in vitro and the cell types at the immunological synapse in vivo. Combined targeting of NG2 with NK cells and mAb 9.2.27 resulted in significantly longer survival times compared to the vehicle, NK and mAb 9.2.27 only control groups (U251-NG2: logrank test, p=0.0081); (U87: Logrank test, p= 0.0003). Histological analyses revealed strong presence of cell types expressing myeloperoxidase, granzyme B cytotoxic granules, and IFNy, in focal areas of strong necrosis/ apoptosis in the NK+mAb and mAb treated groups. While CD3 positive cells were markedly reduced in the NK+mAb treated animals p<0.01, CD8+ cells were abundantly recruited, p<0.001. ED1 positive cells were equally present in the tumours from all groups. However, double labelling revealed the greatest numbers of ED1/CD8 positive cells that penetrated deep into the tumour of the NK+mAb treated animals. Animals treated with NK cells only recruited uniformly double ED1/CD8 positive cells that were less abundant and remained at the tumour brain boundary. More abundant single positive ED1 cells were present in the mAb treated tumours, while single CD8 positive cells were more abundant in the vehicle control tumours. . MRI revealed tumour growth arrest in some of the animals receiving mAb and NK cell therapy, whereas others displayed tumour regions with reduced contrast enhancement centrally, suggesting necrosis. Necrotic regions

or growth arrest were not observed in any of the control animals. An intermediate therapeutic effect was observed with the mAb alone.
Conclusion: Combined 9.2.27 mAb and NK cell therapy strongly affected tumour growth and vascular parameters. Thus, adoptive cell therapy with NK cells may be an amenable therapy for treatment resistant GBMs. However, a deeper investigation into the mechanisms of combination therapy in immune competent animals is required to confirm the therapeutic effects and safety observed in nude rats.

IX-10 A high-affinity anti-3'-isoLM1/3',6'-isoLD1 igg monoclonal antibody, gmab-1, raised in lacto-series ganglioside-defective knockout mice

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Introduction: The lacto-series gangliosides 3'-isoLM1 and 3',6'-isoLD1 have been identified as tumor-associated antigens whose formation is initiated by the Lc3 synthase. Until now, high-affinity IgG monoclonal antibodies (mAbs) against 3'-isoLM1 and 3',6'-isoLD1, which are highly expressed in gliomas, have not been developed, although mAbs against lacto-series gangliosides are powerful tools for functional studies or antibody-based therapy of glioblastomas.
Material and Methods: We previously produced the Lc3-synthase gene n3Gn-T5 knockout mice. In this study, we immunized n3Gn-T5 knockout mice with purified 3'-isoLM1/3',6'-isoLD1 coupled to Salmonella minnesota, and screened hybridomas by ELISA, flow cytometry, and immunohistochemistry. We determined the specificity of the mAb by ELISA, and the binding affinity by surface plasmon resonance (BIAcore).
Results: We produced an anti-3'-isoLM1/3',6'-isoLD1 mAb, GMab-1, of the IgG3 subclass. GMab-1 specifically recognized 3'-isoLM1 and 3',6'-isoLD1 among 15 gangliosides in ELISA. GMab-1 also reacted with D54MG glioblastoma cell line in flow cytometry. Furthermore, GMab-1 immunoreactivity was detected in 26 of 33 (78.8%) glioblastomas in immunohistochemistry. BIAcore analysis revealed that affinity constants (KA) against 3'-isoLM1 and 3',6'-isoLD1 are 1.85 s 108 (mol/L)-1 and 6.58 s 107 (mol/L)-1, respectively, indicating that GMab-1 has high affinity against 3'-isoLM1 and 3',6'-isoLD1.
Conclusions: We immunized n3Gn-T5 knockout mice with 3'-isoLM1 and 3',6'-isoLD1. GMab-1, of the IgG3 subclass, specifically recognized both 3'-isoLM1 and 3',6'-isoLD1 and showed high binding activity. GMab-1 was also reactive against glioblastomas in immunohistochemical analyses, which indicates that GMab-1 should be useful in antibody-based therapy of glioblastomas.

IX-11 Adoptive cellular therapy targeting CMV antigens in patients with glioblastoma

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Introduction: Conventional therapies for GBM fail to target tumor cells exclusively. Adoptive cellular therapy targeting tumor-specific antigens holds the promise of eliminating invasive tumor cells with exquisite precision and minimal toxicity. The discovery (Cobbs. et al. 2002) and recent confirmation (Mitchell et al. 2008) that GBM, but not surrounding normal brain tissue, serves as a refuge for Cytomegalovirus (CMV) reactivation provides an unparalleled opportunity to subvert, as tumor-specific antigens, the highly-immunogenic viral proteins expressed by CMV. In this study, we explored the capacity expand CMV-specific T cells from patients with GBM to clinical scale using pp65 RNA pulsed autologous dendritic cells (DCs) in vitro. A phase I dose-escalation trial of adoptive cellular therapy targeting CMV antigens has been initiated in patients with newly-diagnosed GBM (ERADICATe Trial- FDA IND 13240, Duke IRB Protocol 0580).
Materials and Methods: Peripheral blood leukapheresis samples from patients with GBM were used for in vitro generation of pp65 RNA-pulsed DCs and expansion of CMV-specific T cells. A phase I/II randomized clinical trial was initiated to assess the immunogenicity and safety of targeting the immunodominant CMV integument protein, pp65, in patients with newly-diagnosed GBM using adoptive T cell transfer with or without concurrent DC vaccination.
Results: While patients with GBM exhibited deficits in CMV-specific polyfunctional immune responses in vivo, highly polyfunctional immune responses were restored by ex vivo stimulation and expansion with pp65 RNA-pulsed autologous DCs. Feasibility of clinical-scale expansion (>2 x 10e9 cells) has been demonstrated in 4 out of 5 patients enrolled on the ERADICATe trial. Adoptive lymphocyte transfer and DC vaccinations have been well tolerated in all patients.
Conclusions: Patients with GBM exhibit deficits in CMV-specific polyfunctional T cell responses in vivo that are restored with ex vivo activation and expansion using autologous RNA-pulsed DCs. The adoptive transfer of expanded CMV-specific T cells is feasible, safe, and may be an effective means to restore polyfunctional T cell responses in patients with GBM.

IX-12 Stem cells, tumor stem cells, TGF-ß and treatment concepts

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Transforming Growth Factor beta (TGF-ß) has a strong regulatory effect upon normal neuronal stem cell migration and differentiation: as shown in neurodegenerative model systems (e.g. Huntington`s disease), TGF-ß may arrest precursor cells in the subventricular zone therebye preventing repair processes. In addition, TGF-ß is a major player in the crosstalk between inflammatory cells, like myeloid cells, microglial cells, T- and B-lymphocytes and even granulocytes within solid tumors. Therebye, it seems to interfere with normal immmune function and more importantly, immunosurveillance of solid tumors. Effects on the composition and funtion of the extracellular matrix (ECM) are being further elucidated, however, seem to interfere with migratory potential of tumos stem cells. Tumor metabolism is critical in regulating the expression of TGF-ß – which is essential, as the TGF-ß system is largely defined by dose. Finally, TGF-ß has numerous functions upon the generation of tumor vessels, potentially enhancing tumor recurrence and progression.
Over several years our group has tried to target TGF-ß within malignant gliomas by convection enhanced delivery of antisense oligonucleotides against TGF-ß2 into tumor tissue over a maximum of 6 months. Very promising data of Phase 1 and randomized Phase 2 trials will be discussed, also in the context of our increasing understanding of the mechanisms of tumor progression. Further analysis of the neuroradiological outcome will be given, indicating that modes of tumor response may be indicative for prolonged immune response, possibly aiming at tumor stem cells. The current randomized international Phase 3 trial of TGF-ß2 antisense in recurrent malignant glioma will also be discussed, as well as futher treatment strategies.

X-1 Correlation of physiologic and tissue characteristics for newly diagnosed and recurrent glioma using image guided biopsies

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Introduction: Gliomas are heterogenous on standard magnetic resonance imaging (MRI) and with respect to biological behavior. One of the major research efforts at UCSF is to integrate physiologic imaging with morphologic features of glioma both at the time of initial presentation and at recurrence using an image guided surgical approach.
Material and Methods: Patients undergoing a resection of a glioma at initial presentation or at "recurrence" were consented to participate. Uniformly acquired preoperative physiologic imaging with diffusion, perfusion and spectroscopy parameters with surgical navigation imaging were performed. Based on pre-specified metabolic parameters, targets were identified preoperatively and image guided biopsies acquired at the time of planned surgical resection. Histopathological characteristics of the biopsies are then analyzed for correlative biological properties e.g Factor VIII and microvessel density and perfusion parameters. Ex vivo spectroscopic examination was also performed.
Results: 272 patients have been accrued (average of 2.3 image guided biopsies per patient acquired). Patients include newly diagnosed non-enhancing glioma (n=61, 67% had grade II histology), newly diagnosed high-grade glioma (n=82), recurrent low-grade glioma (n=56) and recurrent high-grade glioma (n=73). Diffusion-weighted imaging was useful for distinguishing non-enhancing astrocytoma from oligodendroglioma and for predicting the 1p/19q status of mixed oligoastrocytoma. There was also a significant difference between the ADC values for upgraded versus non-upgraded recurrent low-grade gliomas. The ex vivo spectroscopic patterns of non-enhancing astrocytoma were associated with histologic grade, Ki-67 proliferation index, and p53 status. There were significant differences in ex vivo levels of Myo-Inositol/Choline ratios for GBM versus low grade tumors. Patient accrual and correlative studies including ex-vivo spectroscopic analyses are ongoing.
Conclusions: Determining the extent of infiltrative tumor, the accurate evaluation of response to therapy and the ability to predict clinical outcome using imaging parameters are important challenges in Neuro-Oncology. The imaging and tissue correlative studies described here will be critical to the assessment of the utility of novel physiologic imaging as noninvasive, easily acquired biomarkers of the biological behavior of gliomas- both low and high grade, at the time of initial presentation and at the time of recurrence.
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X-2 Motor mapping findings and correlation with dti data during surgical removal of lesions involving motor areas or pathways

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Introduction: Surgery of lesions involving motor areas or pathways requires their intraoperative identification to guide resection and preserve functional integrity. The brain mapping technique allows performing such a identification. DTI-FT reconstructs tracts, including CST, and SMA. This work reports the correlation of neurophysiological findings obtained during removal of lesions located in or within M1, SMA or CST, and DTI data.
Material and Methods: The correlation was performed on 280 patients with gliomas. The neurophysiology includes mapping, with Direct Electrical Stimulation (DES, bipolar and monopolar), and monitoring (EEG, ECoG, MEP) procedures. Motor responses were evaluated by multichannel-EMG. DTI-CST (3T DTI maps) was avalaible in all patients, and in 40 also with different FA thresholds. DTI-CST and SMA was loaded onto the neuronavigation system and available intraoperatively for correlation with DES data.
Results: Motor responses were registered in all patients. They appeared as focal when CST was stimulated close to the surface, or affecting multiple muscles with deep stimulation. The near-cortical stimulation induced overt movements, deep subcortical stimulation induced muscle activations detected by EMG. In more than 95% of patients a high level of correlation was observed between DTI FT data and DES findings. When CST was highly infiltrated, DTI FT failed to show fibers in the upper part of CST, where DES induced responses. In such cases, bipolar stimulation didn't evoke responses, which were induced by monopolar stimulation. Monopolar stimulation evoked responses in a large cortical and subcortical area, even faraway from CST: indeed, bipolar stimulation allowed a more precise localization of CST, well correlated with DTI-CST. Monopolar stimulation was useful in patients with a long seizure history to successfully localize the CST and decrease the occurrence of intraoperative seizures. FA varied among the same area of the tumor and in its deep portion, accordingly with the degree of infiltration of tract, and results varied according to the stimulation modality.
Conclusions: DTI-CST data showed a good correlation with DES findings. DES techniques, bipolar and monopolar, evoked motor responses in all patients. The combined used of DES and DTI-FT allowed to effectively and safely trace the tract.

X-3 Intraoperative evaluation of the optic pathways using subcortical recordings, DTI tractography, and real-time 3-D ultrasound based navigation- a work in progress

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Introduction: Surgery for brain tumors in regions adjacent to the optic pathways harbors the risk of post-operative visual fields deficits. While dominant hemianopsia is a debilitating condition, it is often accepted as a "reasonable risk" in the attempt to achieve maximal tumor resection. To date there is no described intraoperative method for identifying the subcortical visual pathways.
Methods and Materials: 12 patients with tumors adjacent to the optic radiation (6 HGG, 2 LGG, 2 metastases, 1 cavernous malformation, 1 infiltrating meningioma) underwent detailed pre-operative neuro-ophthalmological evaluation, fMRI for detection of the primary and associated visual areas, and DTI tractography of the optic radiation (OR). Anatomical and functional images were used for navigation using the SonoWand 3D ultra-sound (US) system. Awake craniotomy was performed in 7 patients and general anesthesia was used in the remaining patients. Throughout surgery, 3D ultrasound scans were performed to update the navigation system and compensate for brain shift allowing precise assessment of distance to the OR. Flash stimulation was delivered to both eyes using a standard LED embedded goggle system. Evoked potentials were collected at preoperative stages using scalp electrodes for preoperative baseline. Following durotomy, an 8-contact silastic embedded electrode (Ad-Tech) was slid subdurally in direction of the calcarine sulcus and associated visual cortex for recording of potentials emanating in the visual cortex. During later stages of tumor excision, a monopolar probe was used as a recording device to record VEP activity from the lining of the tumor cavity. Concurrent cortical responses were used as controls. Waveforms were analyzed for appearance of VEP waveforms, as well as recognition for early-latency, fast frequency activity thought to represent white matter VEP conduction through the OR. Stimulation of the white matter visual tracts was done in awake patients at the same locations used for recordings. Stimulation was applied through the electrode in an increasing, stepwise manner, from 0 to 25 mA max., cathodal stimulation. Visual threshold was determined by subjective

response to a visual event, usually the appearance of unformed visual hallucinations in a corresponding visual field. The distance of the OR from the recording and stimulation points was done using the pre-operative DTI on the 3D US-adjusted navigation images.
Results: VEP data were successfully recorded in all patients. Subcortical mapping revealed fingerprint waveforms with correlation to close proximity to the OR. Subcortical stimulation produced visual phenomena with low threshold stimulation intensity corresponding with subcortical OR recordings. Stimulations at distances > 25 mm from the OR did not produce any visual responses.
Conclusions: This describes a new electrophysiologic technique for detecting the OR and assessing the proximity to these tracts during surgery. Intraoperative verification of OR using electrophysiologic technique appears to be feasible and may help the surgeon identify and preserve the OR and avoid post-operative visual deficits.

X-4 Development of the habib hexablate 10 radiofrquency ablation device for resection of brain tumours

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Introduction: The Habib Hexablate is a radiofrequency ablation device used routinely in resection of inoperable vascular tumours. The aim of this pre-clinical study was to test a miniaturised version of the device Habib Hexablate 10, designed for use in the brain. The device was bench tested in in vitro experiments using jelly and egg white and subsequently developed in a porcine model prior to a planned clinical study.
Materials and Methods: The Habib Hexablate 10 surgical device consists of 6 concentrically placed electrodes which is temporarily inserted into tissue and high frequency current from a radiofrequency generator is passed between the electrodes to create heating. There is an integral central suction system for aspiration of liquefied tissue debris.
Experiments were performed at the Foundation for Biomedical Research in Athens, Greece in a porcine model in which the Habib Hexablate 10 was used to create lesions in the adult brain. The pigs were fully anaesthetised and burr holes created in a standard way and the dura was opened to expose the brain. The welfare of the animals was paramount and all the procedures were carried out in conjuction with the local veterinary surgeon and anaesthetist. After the pig was euthanized, the brain was exposed and the ablated areas were excised en bloc with a margin of surrounding brain and then fixed in formalin.
Results: In the initial acute studies ablations were done at varying power settings and for various times and then the pig was euthanized and the ablated brain with surrounding cerebral tissue was removed.
Using this method a suitable power output and time for producing acute tissue coagulation was identified.
In further experiments a single ablation was done unilaterally in 2 pigs, who were then allowed to recover from anaesthesia. Both pigs behave normally with no distress or complications observed. Twenty-four hours after the surgery they euthanized and the brain including the ablation site was removed for neuropathological examination. There were necrotic and ischaemic change within the ablated areas with a penumbra limited to 0.5 to 1 mm only.
Conclusions: The Research Ethics Committee has recently given approval for a Phase I safety trial of the use of the Habib Hexablate 10 radiofrequency device in patients undergoing resection of superficial vascular brain tumours in a series of 5 cases.

X-5 Imaging of hypoxic lesion in glioma patients by PET with [18f]frp-170, a new 18f-labeled 2-nitroimidazole analog

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Introduction: A new hypoxia imaging agent, 1-(2-[18F]fluoro-1-[hydroxymethyl]ethoxy)methyl-2-nitroimidazole ([18F]FRP-170), with higher image contrast and faster clearance compared to preexisting hypoxia tracers for positron emission tomography (PET), was used to visualize hypoxic tissues in eight patients with glioma.
Material and Methods: [18F]FRP-170 was injected and PET imaging was performed 2 hours later in 8 patients, including three with glioblastoma, two with oligodendroglioma, and one with each of diffuse astrocytoma, anaplastic ganglioglioma, and recurrent anaplastic astrocytoma. All eight patients also underwent magnetic resonance (MR) imaging, and some patients underwent [11C]methionine ([11C]MET) or [18F]fluorodeoxyglucose ([18F]FDG) PET, and proton MR spectroscopy for comparison. Tissues obtained at biopsy or radical surgical resection were immunostained with hypoxia-inducible factor 1 (HIF1)-alpha antibody for the confirmation of hypoxia, except for the patient with recurrent anaplastic astrocytoma treated by gamma knife surgery.
Results: [18F]FRP-170 PET imaging showed marked uptake with upregulation of HIF1-alpha in the three glioblastomas, and moderate uptake in the recurrent anaplastic astrocytoma and one oligodendroglioma, but no uptake in the other tumors. [18F]FRP-170 PET imaging showed positive correlation with HIF1-alpha immunoreactivity, and some correlation with [18F]FDG PET and MR imaging enhancement, but no correlation with [11C]MET PET. [18F]FRP-170 PET imaging seemed to be more sensitive for detecting hypoxia than identifying the lactate peak on proton MR spectroscopy.
Conclusions: [18F]FRP-170 PET imaging can visualize hypoxic lesions in glioma patients, as confirmed by histological examination. This new method can assess tumor hypoxia preoperatively and noninvasively.

X-6 Possible role of vascular endothelial growth factor in radiation necrosis in the brain -treatment strategy of radiation necrosis -

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Introduction: With the advancement of high-dose radiation technologies for brain tumors, radiation necrosis (RN) has become a great problem. Here we describe the strategy to treat RN with special reference to possible role of vascular endothelial growth factor (VEGF).
Material and Methods: Twenty seven cases of symptomatic RN were reviewed, who were treated from June 2004 to July 2009 in Osaka Medical College. They were followed up with medical treatments, mainly by steroids, anticoagulants, vitamin E, and so on for at least a month. For 18 patients who were refractory to medical treatments, we applied surgical excision of the enhanced area on MRI. Six cases were treated with bevacizumab. Intracerebral hemorrhage was observed in 3 cases during the observation of RN. We histopathologically examined these surgical specimens with hematoxylin and eosin (H&E) staining and anti-VEGF immunohistochemistry.
Results: In all surgically treated cases, H&E staining showed marked angiogenesis at the peri-ncerotic area. The vasculatures of this area consisted of a thin endothelium, mimicking venules, which is called telangiec-

tasis. They seemed as a cause of edema and hemorrhage. Immunohistochemistry showed that VEGF was produced mainly in the reactive astrocytes in this peri-necrotic area in all specimen. 5-ALA guided surgery was effective to identify this area as faint fluorescence. Surgical excision of the necrotic mass including the peri-necrotic area or medical treatments with bevacizumab decreased peri-lesional edema remarkably just after the treatment. Taken together, the findings suggest that VEGF at the peri-necrotic area might be the cause of edema in radiation necrosis in the brain, and that medical or surgical treatment to diminish VEGF at this area can immediately decrease this edema with symptomatic improvements.
Conclusions: In our hypothesis, radiation causes hypoxia in initial stage of radiation injury in the brain. This hypoxia induces VEGF production in reactive astrocytes, which cause fragile and leaky telangiectasis. In the early stage of RN or for the prevention of RN, anticoagulants or vitamin E might be effective. While, in severe RN cases, decrease of VEGF by surgery or bevacizumab should be necessary and results in rapid shrinkage of the peri-focal edema with improvements of the symptoms.

X-7 MR physiological and metabolic imaging for assessing response to therapy

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Introduction: There is a critical need for advanced imaging methods to characterize changes in the biological properties of brain tumors as a result of intervening therapy and to assess these parameters as noninvasive predictive or prognostic biomarkers. We investigated serial changes in such data and their relationship to progression free survival (PFS) and overall survival (OS) for patients with newly diagnosed glioblastoma (GBM).
Material and Methods: Ninety-nine patients with newly diagnosed GBM were studied with MRI, perfusion weighted (PWI), diffusion weighted (DWI) and spectroscopic imaging (MRSI) prior to treatment, at one month and two month follow-ups. All patients received treatment with radiation and adjuvant chemotherapy with temozolomide; 64 received no anti-angiogenic agents (Cohort 1); 35 received a PKC inhibitor (Cohort 2). MR parameters included changes in the volume of contrast enhancement and T2 abnormality of the lesion, ADC and FA, rCBV and peak height (PH), and the choline to NAA index.
Results: Median PFS for cohort 1 was 27 weeks and median OS was 84 weeks. Baseline and post-RT values of rCBV and PH were associated with worse PFS. Baseline T2 lesion volume, T2 and contrast-enhancing lesion volumes post-RT and time dependent changes in ADC values were associated with worse OS. The median PFS for cohort 2 was 31 weeks, data to evaluate OS are still being collected. Fourteen patients from cohort 2 were assessed as responders based upon a reduction in the volume of the contrast-enhancing lesion and the other 21 as non-responders. There were differences in the pattern of changes in ADC and rCBV between baseline, one month and two month scans for responders versus non-responders. Higher baseline values of the choline to NAA index were associated with worse OS for cohort 1 and with shorter PFS for patients in cohort 2.
Conclusions: The physiological and metabolic imaging parameters evaluated in this study provided new information for predicting clinical outcome and assessing changes in the magnitude and temporal response to different combination therapies for patients with GBM.
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X-8 Variants in the CDKN2B and RTEL1 regions are associated with high-grade glioma susceptibility

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The causes of glioblastoma and other gliomas remain obscure. To discover new candidate genes influencing glioma susceptibility, we conducted a principal component–adjusted genome-wide association study (GWAS) of 275,895 autosomal variants among 692 adult high-grade glioma cases (622 from the San Francisco Adult Glioma Study (AGS) and 70 from the Cancer Genome Atlas (TCGA)) and 3,992 controls (602 from AGS and 3,390 from Illumina iControlDB (iControls)). For replication, we analyzed the 13 SNPs with P<10-6 using independent data from 176 high-grade Glioma cases and 174 controls from the Mayo Clinic. On 9p21, rs1412829 near CDKN2B had discovery P=3.4 X 10-8, replication P=0.0038 and combined P=1.85X10-10. On 20q13.3, rs6010620 intronic to RTEL1 had discovery P=1.5 X 10-7, replication P=0.00035 and combined P=3.40X10-9. For both SNPs, the direction of association was the same in discovery and replication phases. (We will summarize this work which we published in Nature Genetics, 2009, along with pertinent updates.)

X-9 Treatment of central nervous system lymphomas with temozolomide – preliminary results with MGMT promotor methylation status

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Introduction: The prognosis of primary central nervous system lymphoma (PCNSL) has improved with the use of pre-irradiation methotrexate (MTX)-based chemotherapy. However, benefit from the addition of other chemotherapeutic agents to MTX has not been proven. The addition of agents utilized in the treatment of systemic lymphoma does not appear to improve response rates or survival, while increasing toxicity. The lack of penetration across the blood-brain barrier is the likely explanation. Temozolomide is an imidazotetrazine derivative that readily crosses the blood brain barrier. While approved for the treatment of glioblastoma, reports of efficacy in PCNSL have been documented. The purpose of this study is to evaluate the effects of TMZ treatment for PCNSL.
Material and Method: 1. Firstly, three patients with PCNSL were treated with TMZ. All the tumors were recurrence after standard MTX-irradiation therapy. 2. Secondly, nine patients with PCNSL were treated with TMZ in an adjuvant setting after standard MTX-irradiation therapy. 3. MGMT promoter methylation status was evaluated in 30 PCNSL tumors surgically resected.
Results: 1. All the three recurrent PCNSLs responded to TMZ and two achieved CR after 2 and 3 cycles of TMZ. 2. Of the nine cases treated with adjuvant TMZ after MTX-irradiation therapies, one recurred after 6 months, two were lost for follow-up, and the remaining 6 cases are keeping CR after 7 to 13 months with TMZ. 3. MGMT promoter methylation was not detected in 15/30 tumors analyzed. Promoter methylation was detected in the remaining 15 cases; methylation ratio was from 5 to 100%. One of the responders who achieved CR showed 100% methylation. The relationship between survival time and methylation status in the patients with adjuvant TMZ cannot be analyzed because most of the patients are alive without recurrence.
Conclusions: TMZ treatment including adjuvant TMZ was effective for PCNSL. A phase III study of high-dose methotrexate and whole brain radiotherapy with or without concomitant and adjuvant temozolomide in patients with primary CNS lymphoma, METTLE study, is now at the protocol development stage by the Japan Clinical Oncology Group (JCOG) Brain Tumor Study Group.

X-10 Development of a pediatric perceived cognitive function (pedsPCF) instrument for children with CNS tumors

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Introduction : Decrements in neurocognitive function are commonly experienced by children with brain tumors Standardized neuropsychological testing batteries are used to determine the presence of neurocognitive deficits. However, such batteries are expensive, time-consuming and labor intensive. When administered repeatedly to monitor change, the reliability and validity of neuropsychological tests can be compromised by practice effects . Another approach to sampling neurocognitive functioning is simply to ask the patient, or a family member, about the patient's mental abilities and relevant behaviors. Because responses to such questions do not comprise direct measurements of cognitive skills, it is most accurate to consider them indicators of a patient's Perceived Cognitive Functioning (PCF). By providing information about PCF, a patient and their family members can take environmental characteristics and demands into account when describing the cognitive difficulties or changes they have encountered. Several recent studies have supported the validity of PCF ratings For example, a population-based studying Alzheimer's , found that PCF changes preceded measured cognitive dysfunction and even dementia. The extent to which these findings apply to children is unknown it is worthwhile to consider the assessment of PCF in children, particularly for those whose cognition are at risk of developing atypically due to brain tumors.
Objective: To develop and evaluate a pediatric perceived cognitive function (pedsPCF) instrument.
Material and Methods: Based on feedback from clinicians, parents, and children we developed a scale that assesses children's cognitive concerns. We then administered the scale to 1,409 parents of children aged 7-17. Dimensionality of the scale was evaluated by using factor analyses and its clinical utility was evaluated by determining how well scores discriminated between relevant patient groups.
Results: Forty of 45 item met the criteria of unidimensionality. Results showed that the pedsPCF instrument significantly differentiated samples defined by medication use, repeated grades, special education status, neurological diagnosis, and relevant symptom clusters.
Conclusions: Results showed the pedsPCF is a potentially promising clinical tool to monitor cognitive difficulties in children with Brain Tumors. Initial psychometric analyses suggest that further development using modern measurement theory/computerized adaptive tests may be possible.

X-11 Family history and meningioma risk

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Introduction: Few investigators have examined the relationship between meningioma risk and a family history of meningioma or breast cancer.
Material and Method: Data are the first 600 cases and 500 controls drawn from the Meningioma Consortium Study, a large population-based case/control study of meningioma within the United States. Cases are diagnosed among residents of the states of Connecticut, Massachusetts, and North Carolina as well as the Alameda, San Francisco, Contra Costa, Marin, San Mateo, and Santa Clara counties of California and Harris County, Texas from May 1, 2005 to April 30, 2011 and are 20 and 79 years at time of diagnosis. Controls are selected by random-digit-dialing methods and matched to cases by five-year age interval, race, sex, and state of residence. Telephone interviews are used to collect information on risk factors. Logistic regression is used to provide maximum likelihood estimates of the odds ratios (OR) with 95% confidence intervals (95CI) adjusted for age.
Results: Among female subjects, 2.5% of cases and 0.6% of controls reported a family member affected with meningioma. Among male subjects, 3.6% of cases and no controls reported a family history of meningioma. Overall, cases were significantly more likely to report a first degree family history of meningioma (OR: 5.9, 95CI: 1.3, 26.2) than were controls. This association did not appear to vary by age at onset of the study subject. An elevated risk of meningioma was associated with both a personal history of breast cancer (for females) (OR: 2.0, 95CI: 0.9, 4.6) and a first degree family history of breast cancer (OR: 1.2, 95CI: 0.8, 1.6) but did not reach statistical significance in either instance.
Conclusions: A family history of meningioma is associated with an increased risk of meningioma. An association between breast cancer and meningioma is suggested but warrants confirmation in the final, larger data set.

X-12 Clinical manifestation of dissemination in glioblastomas

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Introduction: Dissemination is one of the most difficult problems in the treatment of Glioblastoma (GBM) patients. The objective in this study is to elucidate the clinical manifestation of disseminated GBMs and to discuss the treatment for these patients.
Material and Methods: One hundred twenty-three newly diagnosed GBM patients were treated in National Cancer Center Hospital in Japan from 1999 to 2008. The patterns of dissemination of GBMs are classified on MRI as follows; (1) multiple lesions including multi-focal GBMs, (2) subarachnoid spread (SAS) including intracranial leptomeningeal metastases, (3) subependymal spread (SES) around ventricles, (4) intraventricular spread (IVS) and spinal spread (SS).
Results: Twenty patients (16%) among 123 GBM patients have multiple lesions at initial diagnosis. Median survival time (MST) of these multiple GBM patients with dissemination was 12.3 months and it was shorter than 15.5 months of GBM patients with a single lesion. Twenty-seven patients have dissemination at the first progression and 11 patients at the second progression among the patients with a single lesion. Totally 60 patients (51%) among 123 GBM patients have dissemination in the clinical courses. SES was often seen in 103 GBM patients with a single lesion and was associated with 30% of those patients. Time to progression (TTP) of the first local recurrence (n=44) was 5.2 months, but TTP of the first SES (n=18) was 3.7 months. MST of GBM patients with SES was 7.5 months and it is much shorter than 17.9 months of GBM patients without SES at the first progression. Dissemination was seen 61% among 76 patients with a lesion adjacent to a ventricle and 36 % among 47 patients without a para-ventricle lesion. MST of these patients with a para-ventricle lesion was 11.5 months and it is much shorter than 20.1 months in the patients without a lesion adjacent to a ventricle.
Conclusions: SES is a common feature of GBMs and a cause of worse prognosis of GBM patients. It is possible to occur at the early stage in GBMs with a para-ventricle lesion. Whole ventricle irradiation might be performed to improve the prognoses of these patients.

X-13 Safety of radiotherapy plus concomitant and prolonged-term adjuvant temozolomide for glioblastoma in Japan – a japanese multicenter phase II clinical study

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Introduction: An open-labeled phase II study was conducted to evaluate the safety of temozolomide (TMZ) in Japanese adult patients with newly-diagnosed glioblastoma (GBM).
Material and Methods: Adult patients with newly diagnosed, histologically verified, supratentorial GBM were assigned to receive radiotherapy (fractionated focal irradiation in daily fractions of 2 Gy for a total of 60 Gy) concomitantly with continuous daily TNZ (75mg per square meter of body-surface per day, 7 days per week from the first to the last day of radiotherapy), followed by up-to 11 cycles of adjuvant TMZ (150 to 200 mg per square meter for 5 days during 28-day cycle). The primary end point was safety. Results:
Total of 30 patients (median age of 55.5 years) were enrolled from Oct. '05 to Nov '06. Twenty-eight patients underwent debulking surgery. Twenty-three received both radiotherapy and TMZ as planned, and 7 prematurely discontinued these therapy, because of disease progression (in 4 patients), and toxic effects (in 3 patients). After radiotherapy, 17 completed more than 6 cycles of adjuvant TMZ therapy (Median cycle number of eleven). Main cause of incompletion of adjuvant therapy was disease progression. The adverse events with Grade 3/4 included neutropenia (17%), lymphocytepenia (53%), and constipation (7%) with no pneumocystis pneumonia. Complete/partial response was observed in 9 of 19 patients with measurable lesion. Median PFS was 8.3 months, and median OS was 18.1 months with median follow-up period of 20.6 months.
Conclusions: The addition of TMZ to radiotherapy followed by prolonged-term adjuvant TMZ therapy was safe for Japanese adult patients with newly-diagnosed GBM.

X-14 Usefulness of methyl-[11c]-l-methionine PET in the extensive tumor resection in glioma

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Introduction: Positron emission tomography (PET) using Methyl-[11C]-L-methionine (MET) has been reported to be an useful tool in the diagnosis of glioma. In the present study, we examined relationship between MET-PET uptake area and MR imaging, and investigated histologic features of MET-PET uptake area .
Material and Methods: 37 patients with newly diagnosed and histlogically confirmed glioma (Grade 2: 2, Grade 3: 16, Grade 4: 19) were investigated with MET-PET before surgery. MET-PET uptake value was normalized to the contralateral white matter. Differences between the MET-PET uptake area (MET area) and abnormal area on MR image were assessed. Tumor area (T area) was defined as a Gd-enhancing area in glioblastoma and T1 low intensity area in grade 3, 2 glioma and peritumoral edema area (PE area) was defined as a T2 high intensity area. To assess the biological feature, we examined MIB-1 LI, microvessel density (MVD) and cell density (CD) in the histological sections.
Results: There was a discrepancy in volume between MET area and T area and between MET area and PE area. MET area was greater than T area in all cases and lesser than PE area in 35cases. In two glioblastoma cases, MET area was greater than PE area. MET uptake area was heterogeneous in uptake values in all cases. MET-PET uptake ratio was significantly correlated with MIB-1 LI (R2=0.62), MVD (R2=0.39), and CD (R2=0.30).
Conclusion: These results suggest that MET-PET uptake area shows potentially high proliferative and angiogenesis activity area in all grade glioma and should be an actual target area for tumor resection in gliomas.

X-15 Neoadjuvant approach for gliomas

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Introduction: Neoadjuvant chemotherapy (NAC), in comparison to adjuvant chemotherapy, could confer better survival and/or functional preservation, and is the standard therapy in several cancers such as advanced esophageal and breast cancers. The disadvantage of NAC includes the possibility of progressive disease and unexpected toxicity during chemotherapy, however, recent findings of markers for better response and emergence of temozolomide might enable us to consider application of NAC in neuro-oncology.
Methods: According to Keio protocol Feb/08, gliomas (grade 2-3) with 1p/19q codeletion were treated by upfront chemotherapy regardless of histology. At maximum response, second-look removal (SLR) was considered if subtotal removal deemed possible. Grade 3 tumors were irradiated after maximum response or after second-look removal if performed.
Results: Twenty gliomas with the codeletion have been treated by upfront chemotherapy (9 PR/MR, 8SD, 3NE). There was no PD within 6 months after initiation of chemotherapy, and three tumors showed local recurrence: 2 after PR (AO at 12 months; OD at 17 months), and 1 after SD (OA at 23 months). Second-look removal was performed in four tumors at maximum response, and radiotherapy was given in an AO at maximum response. There has been no recurrence noted in the tumors that have undergone treatment intervention (SLR or RT) at maximum response.
Conclusion: NAC might be beneficial to some portion of glioma patients in improving survival times and/or functional sequela. Drawing out cases that benefit from NAC would be of great importance.

Session XI
Clinical Trials

XI-1 An early phase clinical trials consortium

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Hypothesis: Treatment of specific patient subgroups, in a surgically based approach, enriched for targetable pathways will improve trial efficiency and increase the rate of positive trials. The approach allows early rejection of agents with no chance of success, ensure rapid selection of agents of true potential benefit for larger trials, and will be achieved in small sample size protocols.
Background: Despite years of clinical trials research involving thousands of patients and hundreds of trials, agents tested rarely produce clinically meaningful benefit to patients or reach the threshold for FDA approval. Large clinical trials are typically used to reduce false-positive or false-negative results, which possibly (although rarely) may reach statistical success but still not be clinically meaningful in terms of actual duration of progression-free or overall survival and may "miss" important subsets of patients because of patient heterogeneity. As a result, trials are expensive and inefficient, typically based upon preclinical models that have not been predictive of success in humans, involve irrelevant patient groups, and include drugs that may not even enter into brain tumor targets.
Goals: To significantly increase the efficiency of therapeutic trials, increase the likelihood of success, and minimize exposure of drugs to patients with little chance of success. We will achieve this goal by using small sample size (10-15 patients) phase-2 trials giving therapeutic agents to patients prior to and following surgery. Patient selection will require prior knowledge of specific molecular features relevant to the agent(s) being tested. Surgically based studies will allow rapid assessment of drug delivery into tumor tissue and modulation of pathway being targeted. Drugs that do not enter tumor tissue or modulate proposed pathway targets will be rejected for further testing. Initial studies will use an IHC-based approach for molecular profiling, derived from paraffin tissue sections obtained from patients at the time of initial diagnosis. Expanded phase-2 trials will also use more extensive genomic profiling data. All clinical studies will be done in the relapsed setting. The hope would be that homogeneous patient selection of targetable molecular pathways in surgically-based trials will increase the likelihood of success, increase efficiency, and minimize exposure of potentially toxic agents to patients unlikely to benefit.

XI-2 RTOG 0625: a randomized phase II trial of bevacizumab with either irinotecan (CPT) or dose-dense temozolomide (TMZ) in recurrent glioblastoma (GBM)

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Introduction: Angiogenesis, a hallmark of glioblastoma, can potentially be targeted by inhibiting the Vascular Endothelial Growth Factor (VEGF) pathway. Bevacizumab is a humanized monoclonal antibody against VEGF-A; it rapidly reduces the concentration of circulating VEGF. The addition of chemotherapy may enhance efficacy by synergistic tumor endothelial cell death or enhanced chemotherapy efficacy related to improved tumor delivery via "normalized" tumor vasculature. This study was designed to determine the efficacy and safety of these regimens in the cooperative group setting.
Material and Methods: Eligibility included age ≥ 18, centrally confirmed recurrent or progressive GBM after treatment with radiation and TMZ. Enzyme-inducing anticonvulsants were not allowed. Treatment was intravenous bevacizumab 10 mg/kg and either CPT 200 mg/m2 every 2 weeks or TMZ 75-100 mg/m2 d 1-21 of 28 d cycle. Accrual goal was 57 eligible patients per arm. Primary endpoint was 6-mPFS rate where an estimate of ≥ 35% would be the definition of efficacy (an improvement of 15% over historical data).
Results: 60 eligible pts were enrolled on TMZ arm and 57 pts on CPT arm. Median age was 57, median KPS was 80, and all had prior radiation and temozolomide. For TMZ arm, 6m-PFS rate was 40% (95% CI: 27-53%); for the CPT arm, the 6m-PFS rate was 39% (95% CI: 26-52%). Objective responses: TMZ arm with 1 (2%) CR, 11 (19%) PR; CPT arm with 2 (4%) CR, 13(24%) PR. Moderate toxicity was noted: TMZ arm with 30 (50%) grade 3, 10 (17%) grade 4, and 3 (5%) grade 5 (fatal) toxicities; CPT arm had 22(39%) grade 3, 7 (12%) grade 4, and 3 (5%) grade 5 (fatal) toxicities.
Conclusions: These results corroborate the efficacy of the bevacizumab and CPT combination and confirm activity for bevacizumab and protracted TMZ for recurrent/progressive GBM, even after prior temozolomide exposure, as the 6m-PFS surpassed the predetermined efficacy threshold for both arms. Toxicities were within anticipated frequencies and were as previously described with a moderately high rate of venous thrombosis, moderate hypertension and one intracranial hemorrhage. Studies to determine the efficacy of bevacizumab in newly diagnosed GBM are underway. Supported by NCI Grants U10 CA21661 and U10 CA37422.

XI-3 Phase II trial of bevacizumab and erlotinib in patients with recurrent malignant gliomas

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Introduction: Combination of bevacizumab and erlotinib has demonstrated safety and efficacy in several cancers. In this phase 2 study, we evaluated the efficacy of bevacizumab and erlotinib in patients with recurrent malignant gliomas (MG).

Patients and Methods: Fifty-seven patients with recurrent malignant gliomas (n=25 for glioblastoma multiforme [GBM] and n=32 for anaplastic gliomas [AG]) were enrolled. The primary outcome measure was 6-month progression-free survival (PFS-6). Overall survival, radiographic response, pharmacokinetics and correlative biomarkers were secondary outcome measures. Patients were stratified based on concurrent use of enzyme-inducing antiepileptic drugs (EIAEDs). Bevacizumab was dosed at 10 mg/kg intravenously every two weeks. Erlotinib was orally administered daily with 150 mg/day for patients not on EIAEDs and 450 mg/day for patients on EIAEDs.
Results: Fifty-six (98%) patients were assessable for outcome. The PFS-6 rates were 29% for GBM and 44% for AGs. The median overall survival rates were 45 weeks and 76 weeks for GBM and AG patients, respectively. Twelve (50%) GBM patients and ten (31%) AG patients experienced at least partial radiographic responses. Pharmacokinetic analyses showed no difference of erlotinib exposure between EIAED and non-EIAED groups. Rash, mucositis, diarrhea and fatigue were common but mostly grade 1-2. Serious side effects were rare and included single patients who developed either intestinal perforation, arterial thrombosis, pulmonary embolism or nasal septal perforation. Grade 3 rash was found in 39% of patients and was associated with survival benefit in GBM patients. High levels of HIF-2 alpha and VEGFR-2 in archival tumors were associated with poor survival outcome in GBM patients.
Conclusion: Bevacizumab plus erlotinib was tolerated in recurrent MG patients. However, the addition of erlotinib to bevacizumab did not improve progression-free survival, when historically compared with other bevacizumab-containing regimens. (ClinicalTrials.gov: NCT00671970)

XI-4 Safety and efficacy of bevacizumab with hypo-fractionated radiotherapy in malignant gliomas- a sensitizer and a protector?

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Introduction: Data from glioma models suggests that bevacizumab sensitizes tumor endothelium to RT and disrupts vascular niches that harbor tumor stem cells. We completed a trial of concurrent bevacizumab and hypofractionated stereotactic RT (HFSRT) for recurrent malignant gliomas and initiated a trial in newly-diagnosed glioblastoma. To investigate radio-protective and/or synergistic effects of bevacizumab in combination with HFSRT, we pooled available clinical data from both trials.
Materials and Methods: Data from two prospective MSKCC clinical trials were reviewed. The trial for recurrent malignant gliomas has been completed and consisted of HFSRT (5X 6 Gy), in combination with single agent bevacizumab (10mg/kg every 2 weeks). The trial for newly diagnosed GBM is ongoing, consisting of HFSRT (6x6 Gy to contrast-enhancing tumor and 6x4 Gy to FLAIR over 2 weeks) with concomitant bevacizumab (10 mg/kg every 2 weeks) and temozolomide (75mg/m2), followed by adjuvant bevacizumab and temozolomide (150-200 mg/kg for 5/28 days).
Results: The recurrent GBM cohort (N=20) achieved a 6m-PFS of 65% and 1y-OS of 54% with no radionecrosis and and one grade 3 CNS hemorrhage. In the newly diagnosed GBM trial, 21 patients have been enrolled with a median followup of 6 months, too early for efficacy evaluation. However, no pseudo-progression has been observed and no hemorrhages reported. Other expected bevacizumab toxicities have been seen. MGMT promoter was unmethylated in 11/15 patients tested.
Conclusions: In the recurrent disease setting, concurrent HFSRT and bevacizumab was effective and safe. No radiation necrosis was observed despite high cumulative doses of radiation. Efficacy results in newly-diagnosed patients are pending, but interim analysis demonstrates reasonable safety. An absence of pseudo-progression was observed, including in tumors with MGMT methylation. Moreover, the two-week total RT schedule is more convenient to patients, and the necessity of corticosteroids was significantly decreased as a result of decreased VEGF-mediated vascular permeability. These results suggest that bevacizumab may be suppressing radiation injury, providing the rationale for a new protocol testing bevacizumab with escalating doses of HFSRT.

XI-5 Phase III anti-EGF-receptor antibody (OSAG-101) for newly diagnosed glioblastoma: safety and current status

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The epidermal growth factor receptor, EGF-R, is considered a highly relevant therapeutic target for glioblastoma resulting in a wide spectrum of approaches directed against the intercellular signalling pathway, the ligand binding capacity of the receptor or the specific immunogenicity of the vIII splice variant. Because of promising preclinical and early clinical findings, the evaluation of the therapeutic effect of a monoclonal antibody against the EGF-R (nimotuzumab) which has a lower affinity than cetuximab, thus binding more specifically to highly overexpressing cells was undertaken in a phase III design. Nimotuzumab (OSAG-101) was tested in an open label, randomized, multicenter phase III trial in patients with newly diagnosed glioblastoma. OSAG-101 is administered by i.v. infusion (weekly infusion of 400 mg) in addition to the current standard therapy with concomitant radiochemotherapy using temozolamide followed by biweekly infusions of 400 mg temozolamide thereafter. Nimotuzumab administration in this trial was to continue until progression. Patients with histologically confirmed glioblastoma were included without specification of resection status. Patients under the age of 18 and over 70 years were excluded. Primary endpoint was time to progression as determined by centralized review of standardized MRI and a prespecified evaluation protocol. Overall survival was chosen as a secondary endpoint with quality of life and safety as additional parameters. Between August 2008 and March 2010, 145 patients were enrolled in 10 sites with 165 subjects. All except one patient were GBM on central histopathological review. Just less than 50% of the patients had a gross total resection with no residual contrast enhancement whereas the larger group had partial resections with residual contrast enhancement, including patients with biopsy only. The observed adverse reaction pattern was the same in both study arms and both strata and reflecting the event pattern of the disease and its standard treatment. Specifically, no rash, conjunctivitis or mucositis as known for anti EGF-R reagents were reported. We conclude from the trial so far that the intravenous administration of OSAG-101 for newly diagnosed glioblastoma is safe and free of additional toxicity to the standard radiochemotherapy regimen. 75 patients have reached their primary endpoint at this point and an interim analysis is currently conducted to provide first indications for efficacy.

XI-6 Efficacy and safety results from the aspect study: gene therapy for operable high-grade glioma with herpes simplex virus-thymidine kinase (Adv.HSV-tk) gene therapy and ganciclovir; a phase III, randomized trial of 250 patients

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Having strong evidence that complete resection provides for superior outcome in the treatment of high-grade glioma, enhancement of local control is attempted by the delivery of the Adv.HSV-tk gene into the healthy tumor bed of resected HGG using a serotype 5 adenoviral vector (Cerepro®, sitimagene ceradenovec) followed by intravenous administration of gancyclovir. Between November 2005 and April 2007 250 patients with high-grade glioma thought to be suitable for gross total resection were recruited from 38 centres in Europe and Israel. The randomization was between standard therapy, with or without Cerepro® injections.

On days 5-19 post operatively, the active group received ganciclovir (5mg/kg i.v. twice daily). At that time treatment with temozolomide as adjuvant was permitted but not standard yet, depending on local treatment practice resulting in heterogeneous use. Time from surgery until re-intervention or death was used as the primary endpoint. Overall survival (OS), as well as safety were secondary endpoints. A statistically significant improvement in the primary endpoint between the active (Cerepro/ganciclovir) versus control arms HR=1.53 (1.13-2.07) p=0.0057 was seen when accounting for baseline covariates like KPS, Age, MGMT status, Extent of Resection, Intended use of temozolomide as well as actual temozolomide use as time dependant covariate. The primary outcome measure represents the time to treatment failure. The principal secondary endpoint of OS outcome showed a favourable hazard ratio but as the study was not powered for this endpoint did not reach significance. The treatment effect was most obvious in the group of patients with non-methylated MGMT gene. The safety assessment showed that the treatment is safe with a low risk of transient neurological deficits as to be expected from a local treatment so that it is concluded that there is a favourable risk/benefit ratio.

XI-7 Phase I trial of arsenic trioxide chemoradiotherapy in the treatment of infiltrating astrocytomas of childhood

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Introduction: The prognosis for children with infiltrating astrocytomas including high-grade gliomas (HGG) and diffuse intrinsic pontine gliomas (DIPG) remains poor. Overall survival for these patients is unacceptable despite the use of chemoradiotherapy strategies such as irradiation (XRT) in combination with temozolomide followed by adjuvant therapy and/or salvage regimens. Novel approaches are needed. A multi-institutional Phase I trial utilizing arsenic trioxide (ATO) in combination with XRT was undertaken stemming from a variety of preclinical studies demonstrating synergy between ATO and XRT in xenograft models.
Material and Methods: Newly diagnosed patients with a diagnosis of HGG or DIPG were eligible. ATO was given at a fixed dose of 0.15 mg/kg/day IV over one hour followed by XRT. The frequency of doses per week was escalated after 3 patients were enrolled at a given Dose level with a plan to escalate ATO to daily dosing (5x/wk) during XRT as tolerated.
Results: Twenty-one children ≥ 3 years of age were enrolled on the study. 12 subjects had DIPG and 9 subjects had HGG. There was one dose-limiting toxicity (DLT) at Dose Level 4 of Grade 4 neutropenia which resolved spontaneously. This cohort accrued an additional 3 subjects without a subsequent DLT. Pending the completion of treatment for the final two subjects on Dose Level 5, it appears that the expanded Cohort of 6 subjects at this Dose Level will be without a DLT. Response to the use of ATO/XRT was difficult to determine because of the concomitant use of XRT during arsenic administration and varying adjuvant regimens.
Conclusions: ATO is well tolerated given at a dose of 0.15 mg/kg/day given daily during focal XRT in children diagnosed with infiltrating astrocytomas. No DLT was identified, but the dose was not escalated above 0.15mg/kg/day because of the known toxicity of higher doses in children receiving ATO for the treatment of leukemia in the absence of XRT. ATO/XRT will be one of the arms of the upcoming Children's Oncology Group (COG) trial for children with HGG.

XI-8 Phase II study of ifosfamide, carboplatin and etoposide for patients with glioblastoma at first relapse

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Introduction: The prognosis of patients with recurrent glioblastoma is remains unsatisfactory. We conducted a phase II study of ifosfamide, carboplatin and etoposide (ICE) for patients with glioblastoma at first relapse to prolong the useful life.
Material and Methods: This was an open-label, single-center phase II study. Forty-two patients with first glioblastoma at first relapse after surgery followed by standard radiotherapy (60 Gy) with a first-line temozolomide-based or ACNU-based chemotherapy, were enrolled. The primary endpoint was progression-free survival at 6 months (PFS-6), and secondary endpoints were response rate, toxicity, and overall survival. Chemotherapy consisted of ifosfamide (1000 mg / m2 on day 1, 2 and 3), carboplatin (110 mg / m2 on day 1), etoposide (100 mg / m2 on day 1, 2, and 3), every 6 weeks.
Results: PFS-6 was 35 % (95%CI, 22 % to 50 %). The median PFS was 17 weeks (95% CI; 10 to 24 weeks). Response rate was 25 % (95% CI, 9 % to 34%). Adverse events were generally mild and consisted mainly of alopecia. Conclusions:This regimen is well tolerated and has some activity and could be one of the options for patients with recurrent glioblastoma. This study was supported in part by The Tazuke Kofukai medical research foundation.

XI-9 Clinical trial with temozolomide and interferon-beta in an alternating weekly regimen against recurrent malignant gliomas-a preliminary report

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Introduction: We previously reported that one week on / one week off regimen using half daily dose (75mg/m2) of Wick's regimen is not so effective to recurrent malignant gliomas as Wick's result probably because of the lower daily dose. Since Wick et al. reported that grade 4 lymphopenia developed in 12% of patients, we did not increase dose of temozolomide up to as high as Wick's trial (150mg/m2) but added interferon-beta in an alternating manner, instead.
Materials and Methods: Seven patients with recurrent malignant gliomas (5 glioblastomas and 2 anaplastic astrocytomas) whose tumors progressed after one week on / one week off regimen using half daily dose (75mg/m2) of Wick's regimen were subsequently treated with temozolomide and interferon-beta in an alternating weekly regimen (75mg/m2 of temozolomide Days 1 through 7 and 15 through 21 and 3 milliion IU of interferon-beta on Days 8 and 22 every 4 week).
Results: Tumors remained at stable disease in 3 patients (43%, 1 glioblastoma and 2 anaplastic astrocytoma) although the mean and longest follow up period is 3 months and 6 months, respectively, so far. Grade 4 lymphopenia or myelosuppression developed in none of the patients.
Conclusions: Alternating weekly regimen using temozolomide and interferon beta may be an effective and less toxic regimen against recurrent malignant gliomas.

XI-10 Adjuvant chemotherapy with distinct modes of action for temozolomide-refractory high grade glioma

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Introduction: Standard care for newly-diagnosed glioblastoma (GBM) is postoperative chemotherapy with temozolomide (TMZ) concurrently with radiotherapy, followed by adjuvant TMZ. However, there are no standard regimens for recurrent, TMZ-refractory GBM. We investigated efficacy of three different chemotherapeutic regimens having distinct modes of action against recurrent high grade gliomas (HGG).
Material and Methods: Since August 2004, 29 patients with recurrent HGG were treated with one of following three regimens. (1) Procarbazine (PCZ)+TMZ. Procarbazine was used prior to and concurrently with TMZ to augment TMZ activity. PCZ 60mg/m2 for 10 days; TMZ 150mg/m2 for the last 5 days in 9 patients (4 pontine gliomas, 2 GBMs, 2 anaplastic astrocytomas (AA), and 1 anaplastic oligoastrocytoma (AOA)). (2) CBDCA+VP16. Non-alkylating, genotoxic agents CBDCA AUC4, day1; VP16 100mg/m2, day 1-3 in 10 GBM patients. (3) Bevacizumab (Bev). An antiangiogenic agent Bev 10mg/kg was given every other week in 4 patients (2 GBMs, 2 AAs).
Results: (1) PCZ/TMZ: Average age, median KPS, and median number of treatment lines was 30, 60 (40-90), and 3 (2-4), respectively. Response and survival were poor (RR 0%, SD 14.3%; PFS-6m 0%, mPFS 1.1m, MST 3.7m). In 4 cases other than PG, mPFS was 1.2m, and MST 5.3m. (2) CBDCA/VP16: Average age, median KPS, and median number of treatment lines was 45, 70 (30-100), and 3 (2-3), respectively. There were no response, but nearly half cases could stay stable (RR 0%, SD 40%). Survival was short as PFS-6m 0%, mPFS 1.6m, MST 4.4m, and the treatment was highly toxic (grade 3-4 neutropenia 100%, thrombocytopenia 53%). (3) Bev: Average age, median KPS, and median number of treatment lines was 66, 50 (40-70), and 3 (2-3), respectively. The tumors responded well (RR 33%, SD 66%) with symptom relief, but 2 cases experienced recurrence after initial shrinkage of tumors within 3 months. There were no toxic events.
Conclusions: MGMT targeting with PCZ, and non-alkylating genotoxic regimens showed low responses and short survivals in patients with TMZ-refractory recurrent HGG. Anti-VEGF strategy using Bev resulted in rapid responses in such cohort of patients with clinical improvement. At present, Bev may be the most promising agent for salvage treatment.

Session XII
Poster only

XII-1 CD133 – expressing glioma cells: influence of oxygen on biological function

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Introduction: CD133, a 120kDa transmembrane spanning glycoprotein, has been identified as a marker for a subpopulation of neural cancer stem cells; an assumption which has been hotly debated in the neuro-oncology research community. Solid tumours characteristically contain poorly vascularised areas associated with severe hypoxia, usually related to poor patient prognosis. The functional role of CD133, as well as the influence of oxygen tension on various biological parameters including adhesion, migration, invasion and proliferation has, therefore, been assessed in this study.

Methods: CD133-positive and negative cell fractions were isolated from a paediatric GBM by magnetic bead immuno-cell segregation (AutoMACS™) and fluorescence activated cells sorting (FACS™). The cells were cultured with stem cell defined growth medium supplemented with the appropriate growth factor concentrations and maintained under hypoxic conditions at 3% oxygen and 5% carbon dioxide. The positive and negative cell fractions were characterised using flow cytometry and immunocytochemistry with the CD133/1 (AC133) monoclonal antibody. Migration and invasion was studied using transwell™ Boyden chambers plus the various ECM substrates. Proliferation analysis was conducted using bromodeoxyuridine (BrdU), proliferatiing cell nuclear antigen (PCNA) and the monoclonal antibody Ki-67. A differentiation adhesion assay with various extracellular matrices, including vitronectin and fibronectin, was also used. Human neural stem cell differentiation arrays were used to assess the progenitors of the positive and negative cells fractions.
Results: A reduction in the levels of oxygen was seen to dramatically affect proliferation rates, cell migration and invasion rates, as well as adhesion. The positive fractions, although displaying an increased proliferation index in comparison to the negative fraction, showed a reduced invasion and migration rate as opposed to the negative CD133 cells.

Conclusions: The in vitro hypoxic microenvironment dictates the behaviour of cultured neoplastic glia as well as dramatically influencing the CD133 phenotype. Distinct biological differences are apparent between the CD133-positive and CD133-negative cell populations. Whether or not CD133 accurately defines a cancer stem cell within glioma remains to be determined but CD133 expression does relate to oxygen environment and biological properties including proliferation, adhesion and invasion.

XII-2 Hyaluronic acid and polio virus receptors in glioma invasions

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Introduction: Increased expression levels of CD44 (Hyaluronic acid/lymphocyte homing receptor) and CD155 (Poliovirus receptor) have been reported on human glioma cells. While CD44 has long been associated with tumour invasion, CD155 has more recently attracted interests in a similar context. Methods: Immunocytochemical and flow cytometric expression of CD44 and CD155 was established on established cell lines and early passage cultures of biopsy-derived glioma. Total Internal Reflected Fluorescence (TIRF) microscopy enabled high signal/low noise imaging of double labelled cells. The effects of monoclonal antibody blocking and siRNA silencing on CD44 and CD155, both individually and together, were assessed using Transwell assays and live cell imaging for invasion, motility and velocity of cell movement. BrdU cell proliferation assays were used to assess the proliferative in siRNA knockdown cells. Interaction and localisation of CD44 and CD155 with F-actin and integrins (ß1, avß1 and avß3) were shown by confocal microscopy.
Results: CD44 was expressed evenly across the cell surface while CD155 sometimes accumulated in "zones" over the cell surface and at the leading edge of invadopodia. TIRF microscopy revealed close proximity between the two epitopes, albeit at distinct sites on the cell surface. CD44 blocking and silencing resulted in a higher level of inhibition of invasion than for CD155; such interference with combined CD44/CD155 resulted in 100% inhibition of invasion within the time frame of the studies. Live cell imaging showed a reduced motility and velocity in cell movement of knockdown cells. Higher proliferative rates were seen in siRNA / CD44 and siRNA/ CD155 cells. Confocal microscopy showed distinct overlapping of CD155 and integrins on filopodia.
Conclusions: Monoclonal Antibody blocking and siRNA knockdown of CD44 and CD155, both singularly and in concert, reduced invasion and increased proliferation in glioma cells. Joint CD44/CD155 approaches may merit further study in targeting infiltrating glioma cells in therapeutic protocols.

XII-3 In vitro models of the blood-brain barrier for metastasis studies

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Introduction: Around 25% of cancers will spread to the brain by passing through the blood-brain barrier (B-BB) and thereby worsen prognosis. In addition the B-BB has proven to be a serious obstacle to therapeutic delivery. In vitro models of the B-BB generally utilise mixed species, non-human cells and can be difficult to extrapolate to the human in vivo situation. The Electric cell-substrate impedance sensing system (ECIS™) and dynamic in-vitro-blood brain barrier (DIV-BBB) models are novel techniques which can be used to monitor the B-BB components more effectively than traditional Transwell® models.
Methods: The B-BB model was comprised of human astrocytes (CC-2565 and SC-1810) with human cerebral microvascular endothelial cells (hCMEC/D3) under human serum supplementation in a Transwell® system. Cells were characterised with appropriate immuno-markers using flow cytometry and immunocytochemistry. ECIS™ was investigated to monitor the hCMEC/D3 grown on a range of extracellular matrices (ECMs), with conditioned media (CM), and cancer cell invasion. DIV-BBB was used to monitor the difference media flow had on hCMEC/D3.
Results: Growth on Transwell® systems and trans-endothelial electrical resistance (TEER) measurements were established. ECIS™ demonstrated the potential of hCMEC/D3 to form a tight barrier. Astrocytes were shown to have no positive effect when added either as cells under the hCMEC/D3 monolayer, or as astrocyte-derived ECM but did increase TEER values when added as conditioned medium. Malignant cells placed upon hCMEC/D3 monolayers were shown to have metastatic potential through the hCMEC/D3 monolayers and recordings were taken on ECIS™. The DIV-BBB model incorporating hCMEC/D3 cells recorded high TEER values.
Conclusion: An all-human in vitro B-BB model has been developed using a Transwell® co-culture system and monitored with the use of ECIS™. The DIV-BBB model has shown the potential to become a novel B-BB model. Work has been achieved with the support of funds from The Lord Dowding Fund for Humane Research, the Isle of Man Anticancer Association and The Institute of Biomedical and Biomolecular Sciences at the University of Portsmouth.

NOTES

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