425 POSTER

Phase I clinical trial with BNCT for patients with glioblastoma (EORTC Protocol 11961)

W. Sauerwein, K. Hideghéty, R. Moss, C. Goetz, P. Pacquis, J. Rassow, F. Grochulla, K. Haselsberger, J. Wolbers, B. Turowski, H. Fankhauser, F. Stecher-Rasmussen, M. de Vries, D. Gabel. *EORTC BNCT Study Group; Strahlenklinik, D-45122 Essen, Germany*

Purpose: Boron neutron capture therapy (BNCT) is based on the high cross section of ^{10}B to capture thermal neutrons leading to the reaction $^{10}B(n, \alpha)^{7}Li$. In a phase I trial the radiation dose is escalated to investigate the healthy tissue tolerance of BNCT using the drug BSH and an epithermal beam at the European High Flux Reactor in Petten (NL).

Methods: BNCT was performed postoperatively in 4 fractions in patients suffering from glioblastoma. The systemic toxicity of BSH, the early radiation and late radiation toxicity were evaluated.

Results: Up to date the treatment of the first cohort of 10 patients has been evaluated. One transient grade 4 leucopenia possibly due to BSH, tolerable acute radiation toxicity (skin erythema, focal hair loss, headache) and one probably treatment related serious late radiation toxicity (cerebral infarction) were detected. The trial is being continued at the scheduled 10% higher dose level.

426 POSTER

Enhanced expression of drug-resistance protein LRP in astrocytic brain tumor cells

S. Spiegl-Kreinecker[1], J. Buchroithner[2], J. Fischer[2], M. Micksche[1], W. Berger[1]. *[1]Inst. of Cancer Research, Applied and Experimental Oncology, Vienna; [2]LNK Wagner-Jauregg, Neurosurgery, Linz, Austria*

Purpose: Lung resistance-associated protein (LRP), considered to be the human major vault protein, may play a role in drug resistance by regulating vesicular and nucleocytoplasmic transport processes. In order to assess the role of LRP in brain tumors, the expression of this protein was investigated in brain tumor cell lines (astrocytic N = 13; non astrocytic: medulloblastoma N = 2, neuroblastoma N = 4) and primary cultures from human glioblastoma multiforme (GB, N = 14).

Methods: LRP expression was studied by means of RT-PCR and immunfluorescence staining (monoclonal antibody LRP-56).

Results: LRP gene expression at the mRNA level was detectable by RT-PCR in 100% of the studied cell lines and primary cultures. High expression levels were detected in all primary cultures of GB, and only 1/13 astrocytic tumor cell line expressed low levels of LRP mRNA. All cell lines derived from non-astrocytic brain tumors expressed detectable LRP mRNA but at a significantly lower level as compared to the asctrocytic ones. Immunostaining for LRP correlated with RT-PCR data. Whereas in most of the primary cultures 100% of the cells scored positively for LRP, different cell subpopulations were evident in astrocytic tumor-derived cell lines. One subpopulation displayed vesicular LRP-reactivity in the cytoplasm, a second one still a vesicular staining pattern but associated to the outer side of the cell membrane. A third subpopulation (from 5 up to 80%) lacked LRP reactivity. In mitotic cells, homogeneously dispersed small vesicles all over the total cytoplasm displayed LRP-reactivity. In all non-astrocytic tumor-derived cell lines no or only very low LRP-immunostaining was detectable.

Conclusion: A high LRP expression seems to be a characteristic of malignant astrocytic cells. The differences between primary cultures and cell lines suggests that expression might be reduced during in vitro cell culture. Further studies are needed to clarify the impact of LRP on drug sensitivity in brain tumors.

427 POSTER

Supratentorial low-grade glioma: Results and prognostic factors following postoperative radiotherapy

G.G. Grabenbauer[1], C.M. Roedel[1], W. Paulus[2], R. Fahlbusch[3], R. Sauer[1]. *[1]Dept. of Radiation Oncology; [2]Pathology; [3]Neurosurgery, University Hospitals of Erlangen, Germany*

Background: To assess treatment outcome and prognostic factors following postperative external radiotherapy (RT) in 77 patients (pts) with low-grade glioma.

Material and methods: Between 1977 and 1996, 45 pts with astrocytoma, 14 with oligodenroglioma and 18 with mixed glioma received postoperative RT with a median total dose 52 Gy (range 40–61 Gy). Sixty-seven pts were treated immedialety following surgery, ten pts with tumor progression. The influence of various factors (histology, gender, age, seizures, duration of symptoms (≤6 weeks vs. >6 weeks), CT pattern (enhancement vs. no enhancement), type of surgery, total RT dose, timing of RT, proliferation and apoptosis) on relapse-free survival and overall survival was investigated.

Results: The median overall survival time was 81 months, while the 5- and 10-year-overal survival (OS) rates were 54% and 31%, respectively. The median time to progression was 56 months, while the 5- and 10-year-progression-free survival (PFS) rates were 45% and 24%. Univariate analyses identified the total RT-dose (p = 0.01), duration of symptoms (p = 0.05), seizures (p = 0.04), patients age (p = 0.03) and the CT pattern (p = 0.005) as significant prognostic factors for OS. PFS rates were influenced by the identical factors. On multivariate analysis, only the age at diagnosis and the CT pattern remaind independent prognostic factors for both PFS and OS.

Conclusion: A minimum total dose of 52 Gy is recommended for the postoperative RT in low-grade glioma. Tumors with CT enhancement seem to need further intensification of treatment.

428 POSTER

A brain cancer clinical assessment tool (BC CAT) for monitoring disease status in high-grade gliomas

D. Osoba[1], B. Zee[2], M. Brada[3], W.K.A. Yung[4], C.S.W. Weng[5], D. MacDonald. *[1]QOL Consulting, Vancouver; [2]Clinical Trials Group, National Cancer Institute of Canada, Kingston, Canada; [3]Institute of Cancer Research and Royal Marsden NHS Trust, Sutton, United Kingdom; [4]MD Anderson Cancer Center, Houston; [5]Schering-Plough Co., Kenilworth, Canada*

Purpose: To develop a practical clinical tool for monitoring the clinical progress of patients with glioblastoma multiforme (GBM) and anaplastic astrocytoma (AA) who are at high risk for progression of disease.

Methods: A recursive partitioning method was applied to data from health-related quality of life assessments (EORTC QLQ-C30 plus Brain Cancer Module 20) and neurological examination obtained in 364 patients (pts) with recurrent GBM and 163 pts with recurrent AA. The data came from 3 studies involving the use of a new chemotherapy agent (temozolomide), in which HRQL data was obtained every 4 wk and enhanced MRI/CT scan data was obtained every 8 wk. The data was used to construct a clinical decision tool applicable in practice.

Results: For pts with GBM, a simple tool with 3 variables (speech, appetite, physical functioning) had a specificity (true negative rate) of 87% and sensitivity (true positive rate) of 34%. In AA, a tool with 2 variables (role functioning, neurological motor status) has a specificity of 90% and a sensitivity of 33%.

Conclusion: A BC CAT composed of HRQL and neurological examination data has high specificity (accuracy in diagnosing nonprogression), but its low sensitivity (accuracy in predicting progressive disease) requires further work.

(Supported by Schering-Plough.)

429 POSTER

Comparison of radiotherapy dose distribution and FDG-PET after irradiation in patients with malignant glioma

F. Paulsen, M. Müller-Berg[1], G. Becker, M. Reimold[1], P. Hipp[1], T. Hehr, M. Buchgeister[2], W. Müller-Schauenburg[1], R. Bares[1], M. Bamberg. *Depts. Radiother., [1]Nuclear Med.; [2]Med. Phys., University of Tübingen, Germany*

Purpose: Despite multidisciplinary therapeutic approaches local relapses are often seen in patients with malignant gliomas. Informations derived from positron emission tomography (PET) are seldom taken in account for treatment planning in radiotherapy (RT) or follow-up. We correlated dose distribution of radiotherapy and posttherapeutic FDG-PET-imaging.

Patients and methods: 11 patients with malignant gliomas (glioblastoma (n = 6), mixed glioma III° (1), oligodendroglioma II° (1) and III° (1), astrocytoma II° (1) and III° (1)) were examined in suspicion of recurrence with FDG-PET 3–14 months after radiotherapy (54–60 Gy). In 1 patient with in-field-relapse after RT (60 Gy) a second irradiation with 20 Gy was performed before PET. FDG-PET activity was compared with dose distribution in treatment planning-CT.

Results: In 10/11 cases hypermetabolic areas were found: 4 in the RT-target volume (3 histologically proven (pr) recurrences), 4 at the field edge in the central beam (1 pr non malignant reaction, 2 pr recurrence), 1 at the caudal field edge (pr recurrence), in the patient with re-RT at the caudal field edge of the re-RT-field (pr recurrence). The lesions had different levels of (hyper-)metabolic activity in comparison to the gray matter.

1 normal FDG-activity (pr recurrence) occurred. In 4 patient we found a hypometabolism in the region of dose deposition.

Conclusion: These preliminary results with 5/11 recurrences at the field edge seem to justify a further examination of the usefulness of pretreatment image fusion with PET to delineate an improved treatment volume.

430 POSTER

Establishment of a nitrosourea-resistant in vivo brain-tumor model: For evaluation of different appraoches to conquer the resistance

Y. Manome[1], M. Watanabe[1], T. Kunieda[2], T. Ohno[1]. [1]*Jikei University School of Medicine, Department of Microbiology, Tokyo;* [2]*Jikei University School of Medicine, Department of Medicine II, Tokyo, Japan*

Purpose: Chloroethyl-nitrosourea (ACNU in Japan) is one of the most potent chemotherapeutic agents for brain tumors. However, acquired resistance to this drug has become a serious problem for treatment of patients with these tumors. The main mechanism of resistance is a recruitment of the O6-methylguanine-DNA methyltransferase (MGMT) in the tumor cells. Many approaches including decoy, antisense, and ribozyme, have been reported to overcome the resistance. To evaluate these strategies properly, we designed a syngenic rat brain-tumor model resistant to ACNU.

Methods: The 9L rat gliosarcoma cells were retrovirally transfected with MGMT cDNA. After selected with geneticin, cells expressing MGMT (9L-MGMT) were isolated and compared to the parental (9L-WT) or control cells (9L-Neo). Next, we stereotactically injected these cells into the brain parenchyma of syngenic rats and scored the survivals. Using this model, we also evaluated an RNA antisense approach.

Results: The 9L-MGMT cells, were significantly resistant to ACNU (IC50 = 330 ug/ml, vs. 9L-WT = 48 or 9L-Neo = 40). Further transduction of MGMT gene under CMV promoter did not confer the additional resistance. When cells were implanted into the cerebrum, all rats died within 15 days without treatment (median survival = 13 days). When treated with ACNU, rats with 9L-MGMT died earlier than the other control groups (median survival = 15 days vs. both controls = 23 days, $p < 0.0001$). Transduction of an RNA antisense did not alter the sensitivity to the drug.

Conclusions: Because of the limited intracranial spaces, animals presented a dependable survival curve in this model. Since these survivals were highly reproducible, our system may have a great roll for evaluation of the treatments of drug-resistant brain-tumors.

431 POSTER

Hypermethylation of the hMLH1 promoter region in high-grade gliomas

E. Andión, J. García-Foncillas, E. Salgado, E. Jiménez, M. Huarriz, M. Guzmán, I. Okroujnov, V. Vanaclocha[1], G. Villares[1], A. Brugarolas. *Laboratory of Molecular Oncology, Department of Oncology,* [1]*Department of Neurosurgery, University Clinic of Navarra, Pamplona, Spain*

Purpose: To analyse the methylation status of the 5'CpG islands of the mismatch-repair (MMR) gene hMLH1 promoter in 26 patients with high-grade gliomas (22 glioblastoma multiforme and 4 anaplastic astrocytomas). We evaluated the relation of hypermethylation at hMLH1 promoter and survival and prognosis.

Methods: MSP (methylation-specific PCR) was performed, a technique that permits distinguishing methylated and unmethylated alleles based on sequence changes produced by treatment of DNA with sodium bisulfite, which converts unmethylated cytosines into uracils, whereas methylated cytosines remain unmodified.

Results: The rate of hypermethylation at hMLH1 promoter gene detected in our series was 4/26 (15.38%). All tumors with hypermethylation at MLH1 promoter gene showed relapse of neoplastic disease. In this way, 2/19 (10.5%) high-grade gliomas with histology of glioblastoma multiforme and harboring normal methylation status remained without evidence of disease after 19 and 24 months from the end of therapy. However, overall survival revealed no significant difference between patients with hypermethylation and normal methylation status.

Conclusion: Hypermethylation at hMLH1 promoter is not a frequent event in advanced gliomas. All patients with hypermethylation showed worse outcome within a period shorter than 24 months.

432 POSTER

Linear accelerator based stereotactic radiosurgery (SRS) as an initial treatment for brain metastases: We can control the tumors 2 cm or less with SRS alone

N. Hayashi[1], T. Uchida[1], T. Okimoto[1], K. Minami[1], M. Hayashida[1], M. Morikawa[1], M. Ochi[1], K. Hayashi[1], S. Shibata[2], S. Honda[3]. [1]*Nagasaki University School of medicine, Radiology, Nagasaki;* [2]*Nagasaki University School of medicine, Neurosurgery, Nagasaki;* [3]*Nagasaki University School of medicine, Radiation Epidemiology Atomic-Bomb Disease Institute, Nagasaki, Japan*

Purpose: To evaluate the local effects of linear accelerator based SRS for brain metastases.

Methods and Materials: From April 1993 to March 1998, we treated 146 brain metastases in 67 patients with SRS. Of them, 57 tumors in 38 patients treated with SRS alone as an initial treatment were analyzed. All 38 patients in this study were followed for a minimum of 12 months or to death. The median survival time of these patients was 9.3 months and the follow-up periods with magnetic resonance or computed tomographic imaging were 0.8–61.5 (median 5.2) months. The major primary sites were lung (19 patients) and breast (8 patients). The volume and equivalent spherical diameter of 57 tumors was 0.1–18.3 (median 1.67) ml and 6.0–34.6 (median 17.2) mm respectively. In SRS, all tumors were enclosed by the 31.0–80.0 (median 54.0) % isodose line with 1–3 isocenters and prescribed 15.0–30.0 (median 25.0) Gy on the margin, 18.8–80.6 (median 45.0) Gy at the hot spot. We defined the condition without tumor regrowth nor radiation necrosis after SRS as 'locally controlled'.

Results: The overall local control rate was 84.2% (48/57 lesions). The actuarial local control rate using the Kaplan-Meier method was 86.9% at 6 months and 68.4% at 12 and 24 months for all tumors. Tumor size (<20 mm vs. >20 mm), marginal dose (<20 Gy vs. >20 Gy), maximum dose (<45 Gy vs. >45 Gy) and ratio of marginal/maximum dose (<60% vs. >60%) were evaluated as factors which might influence the local effects. Although univariate analysis revealed tumor size (<20 mm, $p < 0.01$) and marginal dose (>20 Gy, $p < 0.03$) as significantly favorable factors for local control, tumor size is an only significant factor in multivariate analysis ($p < 0.05$). For the small tumors (<20 mm), the actuarial local control rate was 97.1% at 6 months and 84.9% at 12 and 24 months.

Conclusion: Linear accelerator based SRS is an effective treatment modality for brain metastases. Especially for small tumors (<20 mm), excellent local control can be achieved with SRS alone.

433 POSTER

Increased expression of heterodisperse Alu-containing transcripts in glioblastoma multiforme

V. Kavsan[1], V. Dmitrenko[1], K. Shostak[1], O. Garifulin[1], O. Homenko[2], Yu. Zozulya[2]. [1]*Institute of Molecular Biology and Genetics;* [2]*A.P. Romodanov Institute of Neurosurgery, Kiev, Ukraine*

Purpose: Characterization of genes which are activated or repressed in astrocytomas should provide new data for understanding of the mechanisms of their arising and progression, may have potential implication for the prognosis and therapy.

Methods: Differential hybridization of high density cDNA filter arrays of human fetal brain cDNA library was used to identify genes with expression significantly changed in tumor cells as compared to normal brain.

Results: Among clones which displayed increased hybridization signals with glioblastoma multiforme cDNA, four clones contained Alu-repetitive sequences. Using these tumor-enhanced cDNAs as individual probes in Northern analysis, we detected no discrete bands in RNA from either normal human brain or astrocytic tumors; instead, hybridization to RNAs of all sizes (from 0.3 to 7.0 kb) was detected. The hybridization signals to glioblastoma multiforme RNA were much more intensive as compared to RNAs of anaplastic astrocytoma, normal adult and fetal brain, as well as some other fetal tissues. No changes in expression level of corresponding Alu-containing genes were revealed, when non-repeated adjacent sequences of cDNAs were used as hybridization probes. Further investigations showed the polymorphism of Alu-containing gene expression. If some samples of glioblastoma multiforme RNA included an abundant amounts of repetitive sequences, other ones did not display the increase of their contents.

Conclusion: The accumulation of high levels of heterodisperse RNAs with Alu-repetitive elements may suggest the abnormality of synthesis, degradation or processing of RNA in tumor cells. The deregulation of these processes may contribute to the progression of astrocytomas to glioblastoma multiforme.