A new cancer treatment modality using synchrotron microbeam radiotherapy and chemotherapy in human glioma xenografts in mice

Introduction

Glioblastoma multiforme (GBM) is the most prevalent and malignant primary brain tumor in adults (with a median survival less than one year from the time of diagnosis). Even in the most favorable situations, the majority of patients die within two years (Buckner, Factors influencing survival in high-grade gliomas. Semin Oncol (2003) 30: Suppl 19:10-4 2003; Curran et al., Recursive partitioning analysis of prognostic factors in three Radiation Therapy Oncology Group malignant glioma trials. J Natl Cancer Inst (1993) 85:704-10, 1993; DeAngelis, Brain tumors. N Engl J Med (2001) 344:114-23 2001).

Gliomas, which are characterized by a high proliferation rate, marked neovascularization, central necrosis and extensive local invasion into normal brain parenchyma, have developed resistance to traditional radiation and chemotherapy agents (Veeravagu et al., 2008). Till a few years ago there was no treatment modality that could significantly increase the patient survival without degradation in quality of life.

Only recently, radiotherapy plus concomitant and adjuvant treatment with temozolomide in newly diagnosed glioblastomas was shown to bring small but significant improvement in survival (Stupp et al., Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352: 987-996, 2005), with a median increase of 2.5 months, compared to radiotherapy alone, without degradation in quality of life. Temozolomide, an oral alkylating agent, has demonstrated antitumor activity as a single agent in the treatment of recurrent glioma.

Nevertheless, the general efficacy of chemotherapy in gliomas remains poor and most glioblastoma patients develop recurrence or progression after current standard treatment (consisting in radiotherapy plus concomitant and adjuvant chemotherapy) (Butowski et al., Diagnosis and treatment of recurrent high-grade astrocytoma. J. Clin. Oncol. (2006) 24: 1273–1280, 2006).

The goal of this project is to investigate a new modality for glioblastoma treatment, using a particular temporal combination of synchrotron microbeam radiotherapy and cisplatin in human glioma xenografts in mice. In particular, we want to investigate the effects of synchrotron microbeam irradiation on the permeability of glioblastoma vasculature and therefore on its sensibility to chemotherapy.

Recently it has been reported that synchrotron microbeam irradiation slows the growth of rat gliosarcoma (intracranially implanted in nude mice) and prolongs the survival time (Serduc et al., Brain tumor vessel response to synchrotron microbeam radiation therapy: a short-term in vivo study. Phys. Med. Biol. (2008) 53: 3609-3622, 2008).

Using the results of a previous study on short-term effects of microbeam irradiation on normal mouse brain microvasculature (Serduc et al., In vivo two-photon microscopy study of short-term effects of microbeam irradiation on normal mouse brain microvasculature. Int J. Radiation Oncology Biol. Phys. (2006) 64: 1519-1527, 2006), we want to test a novel approach in temporal combination of the mentioned radiation modality (it causes specific and rather short time-window of increased vascular permeability) and chemotherapy. By this

experiment, we anticipate us to create (improve) treatment protocols, which can be later used in clinical practice.

Materials and methods

All experimental procedures were performed in accordance with the Swiss Federal legislation and the French Government guidelines for the care and use of laboratory animals (authorisation no. FR 210/08 and no. 380988 respectively).

Tumor cells inoculation

Nineteen Balb/c nude mice, males, aged 8-12 weeks, were injected s.c. (left flank) with 10^6 U-87 (human glioblastoma) cells, suspended in 100 µl PBS.

Radiation source and tumor treatment

The microbeam radiation therapy (MRT) was applied at days 16 (11 mice), 26 (4 mice) and 34 (4 mice) after tumor cells inoculation, with tumor diameters ranging from ≤ 5 mm to about 10 mm.

The MRT-x rays were emitted from the ID 17 wiggler (ESRF), with the following parameters:

- unidirectional beam
- multislit collimator
- entrance dose: 850 Gy
- 25 µm FMHM
- 200 c-t-c spacing
- field size: 12 x 12 mm

Fig. 1. The position of the anesthetised mouse before irradiation. A special film (blue rectangle) indicates the irradiation field.

In the post-irradiation period, each mouse was administered (i.v., tail vein) 0.4 ml of cisplatin solution. We choose cisplatin instead of temozolomide because of its longer $t_{1/2}$ (at least 5 days, for the platinum-albumin complexes, vs 1.8 hours of temozolomide).

Follow-up after double treatment

At d1 after double treatment, eight mice were brought back to the animal facility in Fribourg (quarantine): five of them were double treated, three of them only underwent irradiation. In all of these mice tumor size measurements were performed each second day following double treatment or MRT only treatment. They were sacrificed (tumor harvested) at day 32 after treatments. To notice that three out of the nineteen experimental mice were lost few hours after double treatment.

Imaging (fpVCT)

At d2 after double treatment eight mice were transported to the Göttingen Universitaetsklinikum Zentrum Innere Medizin Abteilung Haematologie/Onkologie for tumor imaging studies, by means of fpVCT (flat planel volumetric computed tomography).

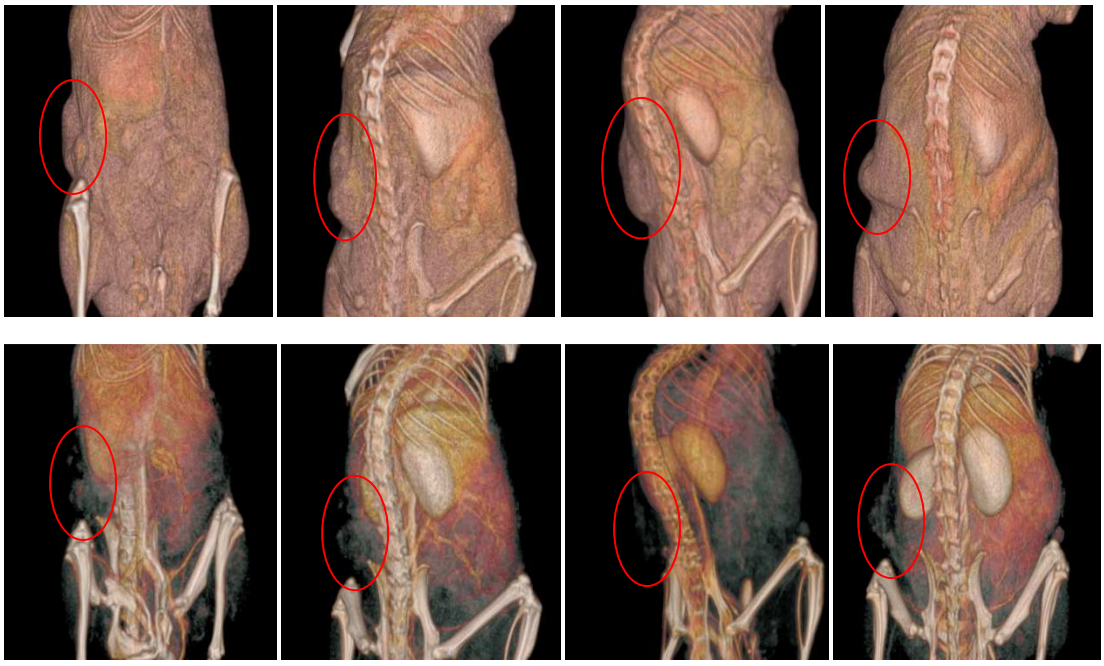
Animals were imaged with a non-clinical flat panel based volume computed tomography prototype, the fpVCT (GE Global Research, Niskayuna NY, US). They were anesthetized with vaporized isoflurane at 0.8 – 1% concentration throughout the imaging session and placed face-down perpendicular to the fpVCT gantry axis of rotation. The nonionic isoosmolar small-molecular iodinated contrast agent, Isovist 300 (Bayer-Schering, Berlin, Germany) is routinely used for scanning. It is administered intravenously via the tail vein using an insuline syringe at 30 g x ½'' (Braun, Melsungen, Germany) at 150 µl 30 seconds before each scan. All fpVCT data sets are acquired with the following protocol: 500 views per rotation, 4 seconds rotation time, 360 used detector rows, tube voltage of 80 kVp and current of 100 mA. A modified Feldkamp algorithm is used for image reconstruction resulting in isotropic high-resolution volume data sets (512 x 512 matrix with an isotropic voxel size of about 100 µm to prevent the creation of resampling artefacts). Tumors are first detectable from a size of 0.001 cm^3 which corresponds to 10 voxels. For tumor segmentation and volume estimation data sets are analyzed with voxtools 3.0.64 Advantage Workstation 4.2 (GE Healthcare, UK).

In four mice the subcutaneous tumors were quite small (diameter of about 4-5 mm). In the other four the tumors' diameters were of about 10 mm. The monitoring was performed at d2, d7, d11 and d15 after double treatment. The parameters to evaluate were the tumor size and the perfusion state. At d15, the mice were sacrificed and tumors harvested.

Results

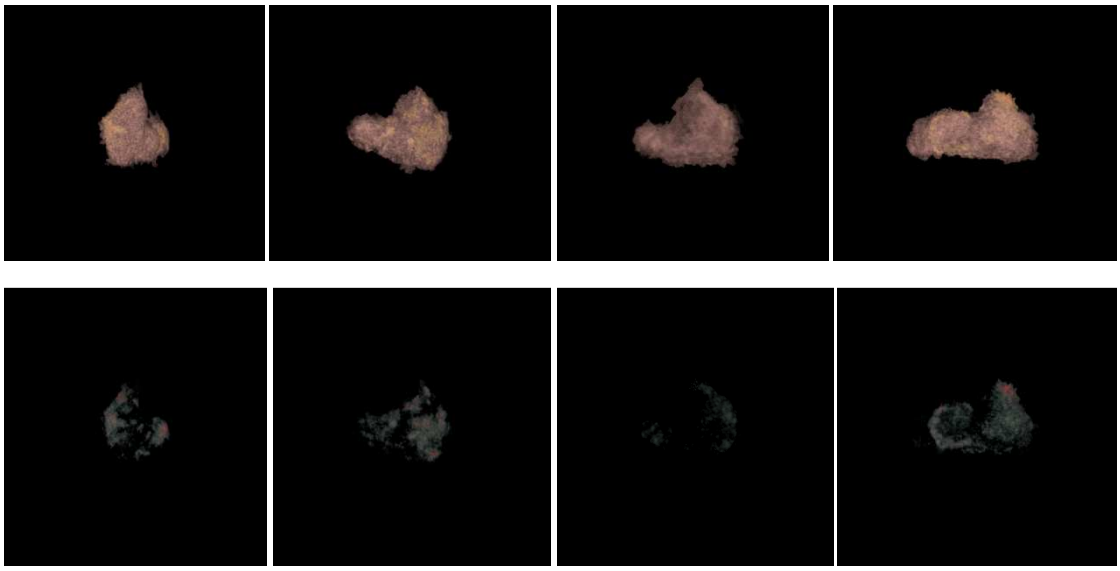
There were differences between the two groups (small and big tumors) of imaged mice concerning tumor growth. The smaller tumors decreased in size, almost disappearing, while the volume of the larger tumors slowly increased.

In both groups the vessel density was decreased after the first scan (except for mouse 2 which was only partially irradiated), but afterwards it increased again.



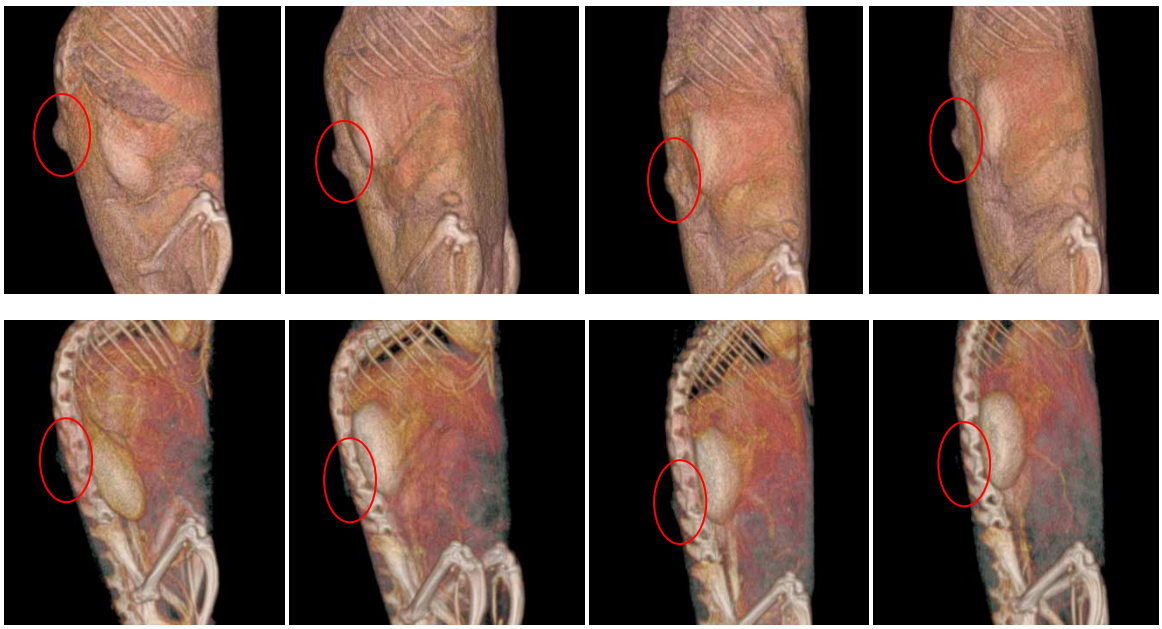
d2 d7 d11 d15

Fig. 2. Big tumors bearing mice: fpVCT images. Upper and lower panel are the same mouse in the same position, but with different visualization protocols. Circles show the tumor position. The different visualization protocols enable to see the size and the position of the tumor as well as to get an impression about the vessels. Size of each picture (mouse and tumor): edge length = 3.5 cm.



d2 d7 d11 d15

Fig. 3. Isolated big tumors: fpVCT images of tumor vessels: perfusion state.



d2 d7 d11 d15

Fig. 4. Small tumors' bearing mice: fpVCT images. Upper and lower panel are the same mouse in the same position, but with different visualization protocols. Circles show the tumor position. The different visualization protocols enable to see the size and the position of the tumor as well as to get an impression about the vessels. Size of each picture (mouse and tumor): edge length = 3.5 cm.

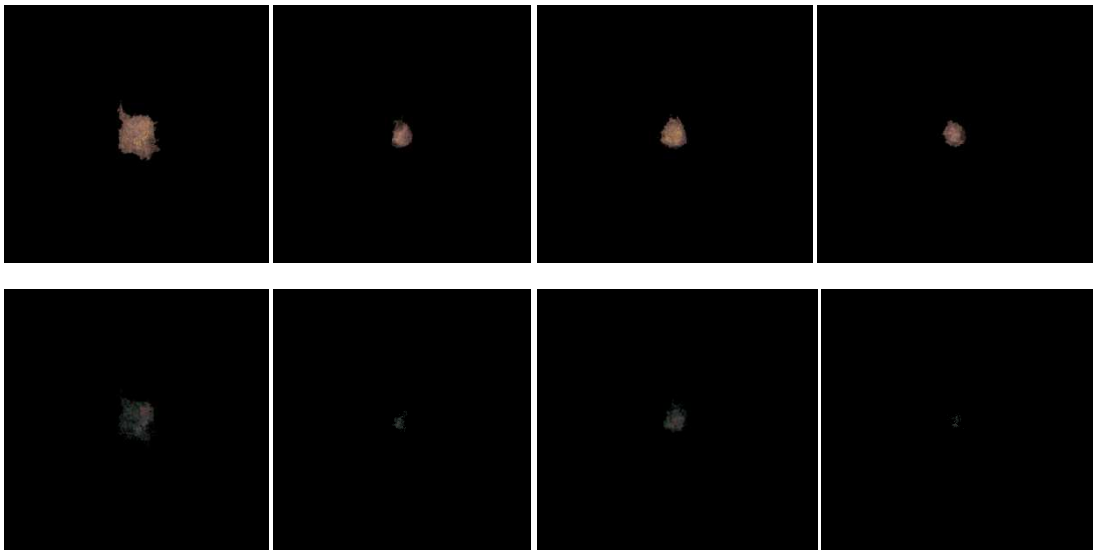


Fig. 5. Isolated small tumors: fpVCT images of tumor vessels: perfusion state.

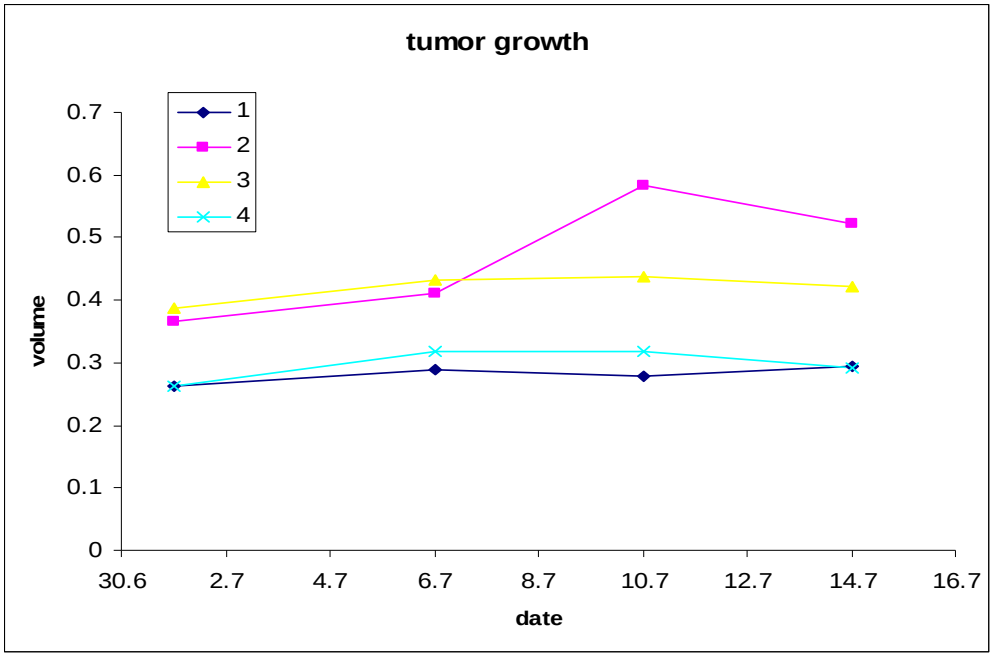


Fig. 6. Growth profiles of the imaged big tumors. To notice that T2 was only partially irradiated.

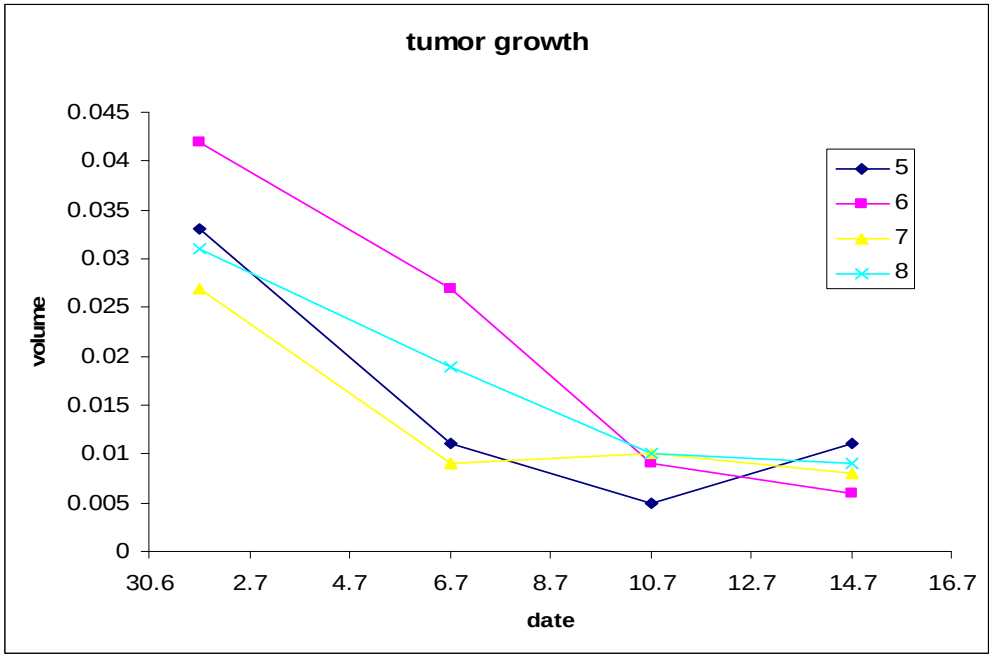


Fig. 7. Growth profiles of the imaged small tumors.

Conclusions

MRT (Microbeam Radiation Therapy) may induce some changes in tumor vasculature in terms of increased permeability, which may improve the delivery/effects of chemotherapeutic agents at tumor site. The small tumors are more sensitive to the double treatment (MRT + chemotherapy) probably because of the more immature vessels, compared to bigger tumors.

Further experiments

We need a comparable control group (time 0 of the experiment). Moreover, morphology and molecular biology analyses have to be performed in all the following groups: 1) control, 2) chemotherapy only, 3) MRT only, 4) double treatment (MRT+ chemotherapy).