EUROPEAN SYNCHROTRON RADIATION FACILITY

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Experiment Report Form

	Experiment title: Comparing the effects of classic microbeam and pencilbeam radiation on DNA integrity, tumour stem cell behaviour and survival in an animal model of malignant brain tumour. --- PRELIMINARY REPORT ---	**Experiment number**: MD 741
Beamline: ID 17	**Date of experiment**: from: June 12 to: June 16, 2013	**Date of report**: July 26 , 2013
Shifts: 12	**Local contact(s)**: Elke Bräuer-Krisch	*Received at ESRF:*

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Report: In the previous beamtime (MD533) we had conducted first *in vivo* studies with pencilbeam irradiation, a modification of the microbeam radiation concept (1). The aim of the beamtime here reported was to assess the suitabily of pencilbeam radiation whole brain radiotherapy (**WBRT**), which would be clinically releveant for extensively infiltrating malignant primary brain tuomurs or multiple brain metastases.

This was the first experiment testing pencilbeam radiation in a small animal model of a highly malignant brain tumour (F98 glioma in athymic mice). The experiment had several components, including:

1. Testing pencilbeam radiation in a small animal model of highly malignant brain tumour to assess the suitability of the technique for whole brain irradiation.
2. Assessing DNA integrity and tumour stem cell behaviour in the acute phase after pencilbeam radiation, and
3. Investigation of radiation-induced bystander effects after pencilbeam irradiation in athymic animals.

To generate the brain tumour, 10,000 F98 glioma cell were implanted in the right cerebral hemisphere of anaesthetized athymic mice. Although the tumour cell line was originally generated in Fischer rats, it grows just as aggressively in athymic mice, keeping its highly invasive character. As a matter of fact, we found that the F98 cells from the ESRF stock unexpectedly behaved much more aggressively than the cell stock in our Freiburg base. With the implantation of 10,000 F98 cells from the ESRF stock we generated, within 1 week after tumour cell implantation, a fairly large, end stage brain tumour. Several animals, treated and untreated tumour-bearing animals, died from their disease on days 8 and 9 after tumour cell implantation.

We irradiated groups of 8 animals each with 200 Gy, 1,000 Gy and 1,500 Gy in pencilbeam mode and with 21.7 Gy (valley dose equivalent to 200 Gy) and 108 Gy (valley dose equivalent to 1,000 Gy) in classic MRT mode. The irradiation experiments were conducted on day 8 after tumour cell implantation at ID 17. Animals for the short-term studies (points 2 and 3) were sacrificed at the end of the experiments at the ESRF. Thirty-nine life animals were transported to Freiburg for further assessment.

RESULTS:

1. WBRT administered unidirectional right-to-left lateral, with square beamlets, 50 µm wide x 50 µm high and a center-to-center distance (ctc) of 400 µm increased survival time even in an end-stage model of malignant brain tumour in all treated groups except for the 200 Gy pencilbeam mode (Figure 1). Limitation of the small animal model: survival is counted in days, not in weeks or months as it would be typical in human patients and therefore the differences in survival gain between groups are not very distinct.
2. There was a stronger incremental gain in mean survival time between the groups treated in pencilbeam mode than between the groups treated in classic MRT mode (Table 1).

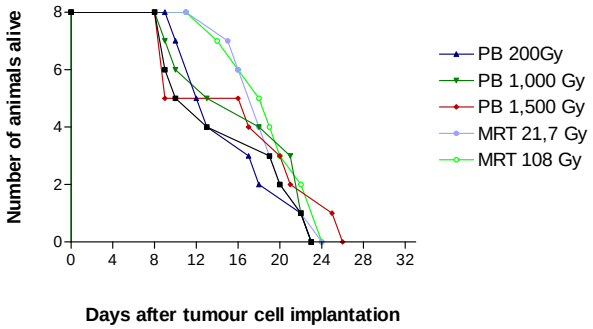

Figure 1:

Survival time in a small animal model of very advanced (end stage) malignant brain tumour.

If the animals that died acutely after irradion are taken into account, the mean survival gain with 1,500 Gy pencilbeam (PB 1,500 = red line) is only 1.4 days (14%), compared to the untreated controls.

If we discount the acutely expired animals in the PB 1,500 Gy group because we could have successfully treated the radiation-induced edema, which has most likely caused the acute fatal increase of intracranial pressure, we obtain **Table 1**: Untreated tumour-bearing animals had 14.6 days mean survival.

	PB 200Gy	PB 1,000Gy	PB 1,500Gy	MRT 21.7Gy	MRT 108Gy
Mean survival days:	13.8 (1,5)	16.2 (2,0)	20.8 (1,6)	18.1 (1,0)	18.6 (1,3)
Survival gain (%):	- 5.5%	+ 11%	+ 42.5%	+ 24%	+ 27%

With this modification, the best survival gain was seen in the PB 1,500 Gy group. We assume that the animals that died acutely after irradiation were those with the most advanced tumours, although they did otherwise appear by no means noticeably more affected by their disease than any of the other animals, neither before nor immediately after irradiation. Thus, had they survived the acute postirradiation phase, the survival gain in this group might not have been quite as impressive because the might (or might have not) died within the first three days after irradiation. However, there is still the fact that the last animals to die were in this experimental group. Therefore it is important to test this assumption in experiments with less advanced tumours, where the loss due to edema is less likely and the overall longer survival times should enhance the differences in survival gain between the groups.

Classic MRT groups show a better survival gain than the groups treated with pencilbeam at the same valley dose. Although there is a visible difference between the survival gain after 200 Gy and 1,000 Gy pencilbeam irradiation, there is no significant difference in survival gain between the MRT groups with equivalent valley doses.The comparison between the untreated tumour-bearing controls and the PB 200 Gy group suggests that about 10% difference could be due to chance. In any case, the survival gain appears larger for the valley dose-equivalent MRT groups. Due to the short overall survival time, no behavioural assessment could be done to investigate the late functional consequences of radiosurgically slicing the entire brain in coronal 350 µm slices (ctc 400 µm, beam width 50 µm). However, given the three-dimensional distribution of X-ray energy deposition in the brain tissue, we would expect that permanent structural and therefore also functional damage would be of lesser extent after pencilbeam irradiation than after classic MRT.

Final preparation and analysis of the material generated with the short-term experiments are in progress. Preliminary results show that bystander effects do occur in immunocompromised (athymic animals).

We wish to thank everybody at ID 17 for helping to make our experiments running as smoothly as they did. Our special thanks go to our local contact, Elke Bräuer-Krisch, to Charlene Caloud and Hélène Bernard.

1) Schültke E, Trippel M, Bräuer-Krisch E, Renier M, Bartzsch S, Requardt H, Döbrössy MD, Nikkhah G. Pencilbeam irradiation technique for whole brain radiotherapy: technical and biological challenges in a small animal model. *PLoS One.* 2013;8(1):e54960.