μESRF	**Microbeam Radiation Therapy (MRT): Development for Radio-Oncology (Final Report)**	**Experiment number**: MD-72
Beamline: ID 17	**Date of experiment**: from: July 1, 2004 to: February 26, 2006	**Date of report**: 2005 12 30
Shifts: 77	**Local contact(s)**: E. Bräuer-Krisch, dipl. Ing.	*Received at ESRF:*

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By and large, the goals stated in the application ("milestones MD 72") were reached. Ten peer-reviewed publications (see list below) have been produced as a result of this beam time (77 shifts, of a total of 96 shifts that were awarded).

1. New Multi-Slit Collimator TECOMET® (TMSC): physical performance, biological effects:

The physical performance of the adjustable TMSC (1) and some novel irradiation geometries enabled by its design have been reported (2).

Biological effects, *"milestone MD 72"*: *"Availability of sufficient computational and experimental data to select the optimal microbeam parameters for delivering palliative/therapeutic doses that are below the threshold for damage to the normal adult rat brain, as a guide for clinical phase I trials in humans...."* Much progress was made toward achieving this goal. Table I indicates the preliminary biological results for normal tissues. Not only do the individual microplanar beam width and beam skin-entrance dose play very important roles, but the spacing of microplanes and the macroscopic dimensions of the array of microbeams do too. The observed imperfect inverse correlation of spacing with the valley dose for a given set of parameters requires further experimental study *in vivo* as well as extended Monte Carlo studies. Histopathologic review of microbeam-irradiated brains is in progress. A systematic investigation of parameters that can be varied to optimize the therapeutic index for tumor palliation was started in November 2005. We used the transplantable murine squamous cell carcinoma SCC VII (3, see § 5A below). These studies will be extended with a goal of being able to predict normal tissue toxicity in the tumor-bearing mouse leg on the basis of MRT parameters - an important, perhaps essential step toward clinical MRT.

2. Dosimetry, modeling; PSI version of Monte-Carlo code GEANT: *"Milestone MD 72": "Validation of dosimetry (in particular for the new MSC and changes in beamline characteristics) at ID 17, published and congruence with computed values....."*: Monte-Carlo calculations of dose deposition for the new setup at ID 17 have been published and validated by physical microdosimetry (4,5).

Mean CTCAE score (1 to 5)	width (µm)	Spacing (µm)	dose (Gy)	Valley dose Gy,	TMSC or MTSS
1	26	400	1250	6.1	TMSC
0.8	50	400	625	5.7	TMSC
0.8	50	400	800		TMSC
1	50	400	800		MTSS
2.2	50	400	1250	11.3	TMSC
1	75	400	312	5.5	TMSC
1.2	75	400	625	11.1	TMSC
3.4	75	400	625		MTSS
5	75	400	875	15.5	TMSC
4.8	75	400	1250	22.1	TMSC
1.7	26	200	625	6	TMSC
2.2	25	200	625		MTSS
1.6	25	200	800		TMSC
4.2	25	200	1250	12.1	TMSC
4.4	50	200	625	11.5	TMSC
5	75	200	625	22.7	TMSC

Table I Microplanar width and spacing, entrance dose ("dose"), valley dose and type of exposure of the **normal rat brain** to synchrotron-generated X rays (TMSC = Tecomet MSC; MTSS = multiply translated single slit to produce a spatially fractionated irradiation field from multiple exposures similar to that produced by a single exposure through the TMSC) that result in a biological score for various organ systems, including the central nervous system, according to the CTCAE criteria (Criteria for Adverse Events, v3.0: http://ctep.cancer.gov//reporting/ctc.html). CTCAE score = 1 denotes a mild Adverse Event of negligible clinical significance; score 5 denotes a most severe Adverse Event (fatality) caused in large part by the irradiation. Valley dose = brain dose midway between the two microbeams at the middle of the array of vertical microbeams, computed using the GEANT Monte-Carlo photon-electron transport program (5).

3. Permeabilization of the blood-brain barrier by MRT

Sub-radiotherapeutic doses of broad-beam photon radiation can promote the diffusion of some agents from the blood to tissues, and increase the toxicity of these agents in their therapeutic targets. The purpose was to develop microbeam-targeted cytotherapy to prevent or palliate acute and delayed neuroradiotoxicity after high-dose, potentially curative radiosurgery. Efforts to achieve this goal comprise experiments with Nanogold and a collaborative study in mice. Serduc and co-workers (6) irradiated parts of the cortex of a brain hemisphere in nude mice anteroposteriorly with an array of vertical, parallel microplanar beams (width ~ 25 µm, spacing ~210 µm, entrance dose 312 or 1000 Gy). The vascular volume in the irradiated portion of the brain was estimated in vivo. The vascular permeability was detected as extravasation of sulforhodamine B (577 Da) in the irradiated microplanar tissue slices. For all time intervals after MRT and after either 312 or 1000 Gy, FITC-dextran (70 kDa) remained in the functional vessels. No significant change in vascular volume was observed. After exposure to 1000 Gy, diffusion of sulforhodamine B in microbeam stripes was observed from 12h until 12 days after MRT, but no diffusion was detected 1 month after MRT. A permeability of the BBB for small molecules occurs between 12h and 12 days and fades between 12 and 30 days after irradiation. In the light of these observations made in vivo, it is understandable in retrospect that intravenously injected Nanogold particles with diameters of ~80 nm (i.e., ~10 x the diameter of human albumin in solution) were not detected in histologic sections of the brain at various intervals after MRT-crossfired irradiation of a mouse left cerebral white matter volume of ~4 mm^3 using entrance doses up to 1250 Gy. At necropsy, the gold nanoparticles were detected in Kupffer cells of the livers of these mice.

4. Chorio-allantoic membrane of chicken (CAM): *"Milestone: Demonstration of the conditions under which the microbeam effect can be observed in the CAM model."*

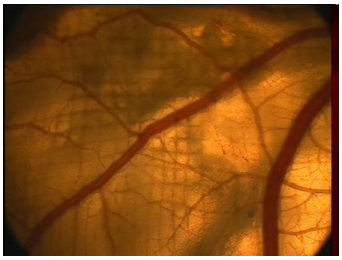

Fig. 1. 12 d old CAM 2 h after entrance doses of 300 Gy; orthogonally crossfired arrays, each comprised of 25 µm-wide microplanes, spacing 207 µm. The trace of the crossfired arrays is clearly visible *in vivo* - without optical enhancement by vital staining - as a grid in the left half of the photograph.

Salient results for the CAM have been published (7, 8, 9). A single, 25 µm-wide microplanar beam (entrance dose 2500-5000 Gy) causes minimal capillary damage. Multiple microplanar beams (width 27 µm, spacing 207 µm) cause capillary damage in the tracks of the beams. 24 h after application of entrance doses of 300 Gy, some repair has taken place, and the perfusion is being maintained. Entrance doses of 600 Gy lead to marked microvascular damage and perfusion loss. For broad beam irradiation, the threshold dose for microvascular damage/perfusion loss is ~10 Gy.

5. Transplantable glial brain tumors in mice: A new orthotopic, syngeneic, transplantable mouse brain tumor model using cell lines Tu-9648 and Tu-2449 previously isolated from tumors that arose spontaneously in GFAP-v-src transgenic mice has now been developed (10). The high rate of engraftment of this murine model, its similarity to the malignant glioma of origin, and its rapid locally invasive growth may make it useful and extraordinarily economical for preliminary assessments of novel therapies for human malignant gliomas. A manuscript describing this murine glioma model has been prepared (11). The use of this tumor for testing MRT has been postponed as it appears prudent to first gain

experience with more common experimental murine tumors outside the CNS, such as the squamous cell carcinoma VII implanted subcutaneously in the thigh (SCC VII, see 5 A. below).

5.A. Extension of the LTP: a transplantable squamous cell carcinoma in mice: The left tibiofibular thigh of a mouse bearing a subcutaneously [sc] implanted mouse model of aggressive human squamous-cell carcinoma [SCC VII] was irradiated in two orthogonal exposures through a multislit collimator [MSC] by arrays of nearly parallel microbeams spaced 200 μm on center [oc]. The peak skin-entrance dose from each exposure was 442, 625, or 884 Gy from 35 μm-wide beams or 442 Gy from 70 μm-wide beams. The 442/35, 625/35, 884/35 and 442/70 MRTs yielded 25-, 29-, 37- and 35-day median survival times [MST] (post-irradiation), respectively, exceeding the 20-day MST from 35 Gy-irradiation of SCC VIIs with a seamless 100 kVp X-ray beam (3). In a follow-up study (Nov. 2005), a new setup to reduce radiation backscatter**,** as well as 200 μm spacing oc, entrance doses of 625 or 884 Gy (35 μm-wide beams), and 884 Gy (20 μm-wide beams), and spacing of 400 m oc with entrance doses of 884 Gy or 1250 Gy (35 μm-wide beams), and 625 Gy (20 μm-wide beams), was used. Spacing of 400 μm oc rather than 200 μm oc greatly reduced the efficacy of tumor palliation. Increasing the entrance doses or widening the microbeams while maintaining the 400μm on-center beam separation did not compensate for that reduction. A related long-term histopathologic study is in progress (12).

5.B. Synergy of Gene-Mediated Immunoprophylaxis and Microbeam Radiation Therapy (MRT) for Advanced Intracerebral Rat 9L Gliosarcomas. This report (13) is the first demonstration that gene-mediated immunotherapy (GMIMPR) enhances the efficacy of MRT for advanced 9L GSs. Male Fischer 344 rats were implanted intracerebrally (ic) with 10^4 9L GS cells on day (d) 0. Each of the 14 untreated (control) rats died from a large (>100 mg) ic tumor before d29 (median, d21). On d14, the remaining 62 rats were given deliberately suboptimal microbeam radiation therapy (MRT) by a single lateral exposure of the tumor-bearing zone of the head that delivered 625 Gy peak skin doses at ~211 μm oc intervals in ~300 ms either without additional treatments (MRT-only, 25 rats), with post-MRT GMIMPR (=multiple sc injections of irradiated (clonogenically-disabled) GM-CSF gene-transfected 9L GS cells), or with post-MRT IMPR (multiple sc injections of irradiated (clonogenically-disabled) 9L GS cells. The median post-implantation survivals of rats in the MRT-only, MRT+GMIMPR and MRT+IMPR groups were over twice that of controls; further, ~20% of rats in MRT-only and MRT+IMPR groups survived >1 yr with no obvious disabilities. In addition, over 40% of MRT+GMIMPR rats survived >1 yr with no obvious disabilities, a significant ($p < .04$) increase over the MRT-only and MRT+IMPR groups. These data suggest that a MRT+GMIMPR combination might be better than MRT alone to palliate a unifocal glioma.

6. MRT of spontaneous tumors in large animal patients (dogs, cats)

Status: The first preliminary study is scheduled for February 2006. Veterinarian and ethical safeguards have been met. Our collaborator Prof. Barbara Kaser-Hotz, DVM, Veterinary Hospital, University of Zurich, a veterinary oncologist experienced in treating spontaneous canine and feline neoplasms (14), obtained Swiss authorization Nr. 160/2005 on October 31, 2005 to perform MRT for canine and feline brain tumors. The authorization, valid until November 30, 2007, has been issued by the Department of Public Health, Canton of Zurich, Veterinary Office, by the Cantonal Veterinarian, Regula Vogel, DVM. However, corresponding authorizations from the ESRF and, in particular, technical specifications for radiation and biohazard safety during and after large-animal MRT, have not yet been issued by the ESRF. Thus, in February, we plan to conduct extensive tests with canine/feline irradiation phantoms, including anesthesia, positioning, dosimetry etc. The project, recently presented at internal reviews at the ESRF and at a scientific meeting (15) has been rated as important and experts have recommended that such spontaneous tumors in large mammals be studied before clinical trials in humans are implemented.

Publications: ***1.*** Bräuer-Krisch et al, Review Sci Instrum **76**, 1-7, 064303 (2005) ***2.*** Bräuer-Krisch et al, Nucl Instrum Meth **A 548** (2005) 69-71) ***3.*** Miura et al, Brit J Radiol **78** (2005), 1-5 ***4.*** Siegbahn et al, Nucl Instrum Meth **A 548** (2005) 54-58 ***5.*** Stepanek et al, (2004 - 2005, unpublished) ***6.*** Serduc et al, Int J Radiat Oncol Biol Phys (2005, in press) ***7.*** Blattmann et al, Radiother Oncol (2002) **64** (Suppl 1), 219 ***8.*** Blattmann et al, Strahlenther Onkol 2002) **178**: 118-118 Suppl 1 ***9.*** Blattmann et al, Nucl Instrum Meth A **548** (2005) 17-22 ***10.*** Smilowitz et al, Swiss med Forum (2004) **4** (suppl 21) 512 ***11.*** Smilowitz et al, submitted, 2005 ***12.*** Gebbers et al, (2005, submitted) ***13.*** Smilowitz et al (J Neuro-Oncology, 2006, in press) ***14.*** Kaser-Hotz et al Vet Radiol and Ultrasound (2002) **42**, 480-486 ***15.*** Laissue JA et al, Atelier "Médecine et Lumière Synchrotron", December 6-7, 2005, Institut Curie, Orsay, France: http://www.synchrotron-soleil.fr/workshops/2005/Colloque_medecine_sante/index.html