MD 237: YEARLY REPORT JANUARY 2008

Status of the LTP project with reference to the applicable milestones

I. LARGE ANIMALS

By decision of the ESRF, the main part of the project, i.e., animal patient studies, has been "frozen" since 2006 II as stated in the official letter of Ms Roselyn Mason, Head of the User Office at the ESRF, to Principal Investigator Pr B. Kaser-Hotz, June 29, 2006:
"I am very pleased to inform you that your project MD 237 (LT) entitled „MRT: Towards Clinical use in Radio-Oncology" …has been reviewed and found to meet the criteria for Long Term Projects at the ESRF. The committee made the following comments:
The reviewers were impressed by the potential of the clinical application even if this concerns animal patients. They support the implementation of the pet experiments, and recommend that ESRF management pursues the proposed actions. The Research Directors have therefore decided to allocate beamtime for one year: 2006/II 33 shifts and 2007/I: 15 shifts initially, since the infrastructure to house the animals does not yet exist. They intend to go ahead with plans for this infrastructure, and advise that you re-submit your request for further beamtime in one year's time, once the support facilities are in place. Kindly note that the Review Committee wishes to see a progress report at the end of the first year…..".

For 2008, Ms Roselyn Mason wrote to Dr. Alberto Bravin on November 2007 (16:58:22):
"I quite agree that the LTP MD-237 (Kaser-Hotz) should continue with the full number of shifts initially allocated, i.e. that the project be "unfrozen" once the new hutch is ready. They would therefore not have to re-apply for beamtime until the end of their total number of allocated shifts. I imagine therefore that you will be in a better position to advise them re reports/continuation request for January 2009."

Milestones, large animal studies: Not applicable

We greatly appreciate that the ESRF facilities to treat large animals, e.g. pet dogs with spontaneous tumors using palliative or, possibly, curative doses of synchrotron-generated X rays in the MRT mode will be ready for use in 2008. In the meantime, to better introduce the concept of clinical applications of MRT to the medical and veterinary clinical communities, we submitted an overview article to a pediatric neurology journal on MRT research also containing our 8-10 year-old data obtained at the ESRF on MRT-like irradiations of comparably large normal piglets along with recent, unpublished data and radiodosimetric analyses of the long-term post-irradiation follow-up. That article, "Prospects for microbeam radiation therapy of children's brain tumours to reduce neurological sequelae" (Laissue et al., 2007), was published in Developmental Medicine & Child Neurology [DMCN], which seems to be the only specialized journal of pediatric neurology among the world's 1500 bioscience journals most highly ranked in impact.

II. MD 237: OTHER EXPERIMENTS

A. MRT, parameter optimization using the Tecomet^{R} Multislit Collimator [TMSC]: hemicephalic irradiation of normal rats.

Present status

To predict the effects of different irradiation parameters (mainly dose fractionation with time), the alpha/beta model is used in conventional radiotherapy. For MRT, analogous predictions for different numerical combinations of essential parameters are not available. Among those MRT parameters are width and height of the array, microbeam width, center-to-center spacing, energy spectrum and dose microdistribution.

To explore these variables with the TMSC, we chose the rat brain of young adult female Sprague-Dawley rats as a model. A parasagittal (i.e., parallel to a median longitudinal vertical plane in the skull), 10 mm-wide array of vertical, 10 mm-high microplanar beams was used to cover almost the entire right hemisphere in the anteroposterior direction, unidirectionally. To monitor the effect of microbeam width variability in the TMSC, identical arrays were produced by translating the rat laterally 52 times through a single 25 micrometer wide slit, although some technical difficulties arose during few of these irradiations. In addition, to compare the TMSC with the former "Archer" collimator, where the beam traverses two 8 mm-thick radiolucent Al plates, several irradiations were carried out to measure the effect of such a 16 mm-thick Al filter in the path of the TMSC, where the collimated beam traverses air, not Al, as in the Archer collimator. Two groups of 45 rats each were followed up "clinically" for 108 and 332 days, respectively, and a third group for 332 days. The brain of every rat was examined and photographed at necropsy, then processed for histopathologic examination.

As end points, for semiquantitative biological „dosimetry", adverse events occurring after irradiation were graded according to the „Common Terminology Criteria for Adverse events" (CTCAE, version 3.0, 2003 (http://ctep.cancer.gov)). An adverse event, as defined at the National Cancer Institute of the U.S.A., is „Any unfavorable symptom, sign, or disease associated with the use of a medical treatment or procedure that may or may not be considered related to or caused by the medical treatment or procedure."
The terminology uses 5 grades, namely: 0 = no adverse event or within normal limits; 1 = mild (e.g., slight depilation), 2 = moderate, 3 = severe adverse event (e.g., cataract); grade 4 = life-threatening or disabling adverse event, and grade 5 signifies death, e.g. due to early delayed brain tissue necrosis, assayed histopathologically. Peak and valley doses were computed (Dr. J. Stepanek, unpublished).

In Table I, the grade of adverse effects is shown versus main irradiation parameters. Several observations emerge from this preliminary table:

I. For identical parameters, **400 µm spacing** (i.e., 400 versus 200 micrometer spacing) resulted in 4 to 5 times lower mean scores and about half the valley dose.

II. **In nine subgroups** (68 rats) **with 400 µm spacing, 100%** of the rats **survived**, with a mean subgroup CTAEC score of 1.1. With 400 µm spacing, an entrance dose of **625 Gy** with a microbeam width of **75 µm** is tolerated (9/9 rats survived for the whole observation period); for entrance doses **> 625 Gy** up to **1250 Gy**, the highest tolerated width is **50 µm.** See also Nr VI below.

III. **Six subgroups** (28 rats) with **400 µm spacing did not achieve 100% survival:**
   a. **1250 Gy / 75 µm, i.e.,** 3 subgroups: **0% of the rats** survived few days after irradiation using the TMSC with Al filter, and **20%** in 2 pooled subgroups (TMSC without Al filter).
   b. **875 Gy / 75 µm: 100% of the rats died** within 27 days after irradiation
   c. **800 Gy / 50 µm (single slit mode): 25% of the rats survived** beyond 272 days after irradiation.
   d. **625 Gy / 75 µm (single slit mode): 40% of the rats survived** beyond 249 days after irradiation.

IV. With **microbeams spaced at 200 µm, all rats (n=22)** irradiated with the **625 Gy / 25 µm** combination **survived** for the whole observation period.

V. With **microbeams spaced at 200 µm, doses > 625 Gy, or microbeam widths > 25 µm do not allow a 100% survival** and result in **higher mean scores for adverse effects.**

VI. The **single slit technique,** for parameters identical to those produced by the TMSC, yields **similar or lower mean scores** (20% to 65% lower) for adverse reactions.

VII. The mean **scores were not improved** or worsened by the addition of a 16mm-thick **Al filter**.

Table I: Mean score for adverse events (CTCAE) versus irradiation parameters and Valley dose

CTCAE mean score (1 to 5)	N	width (µm)	Spacing (µm)	dose (Gy)	Valley dose Gy, GEANT	TMSC or Single Slit
1	5	26	400	1250	6.1	**TMSC**
0.8	14	50	400	625	5.7	**TMSC**
1	5	50	400	625		**Single Slit**
0.8	5	50	400	800		**TMSC**
1	5	50	400	800		**Single Slit**
2.2	5	50	400	1250	11.3	**TMSC**
1	5	75	400	312	5.5	**TMSC**
1.2	9	75	400	625	11.1	**TMSC**
3.4	5	75	400	625		**Single Slit**
5	5	75	400	875	15.5	**TMSC**
4.8	9	75	400	1250	22.1	**TMSC**
5	5	75	400	1250	22.1	**TMSC & Al**
1.7	9	26	200	625	6	**TMSC**
2.2	5	25	200	625	6	**TMSC & Al**
2.2	6	25	200	625		**Single Slit**
1.6	5	25	200	800		**TMSC**
3	4	25	200	800		**Single Slit**
4.2	5	25	200	1250	12.1	**TMSC**
4.4	5	50	200	625	11.5	**TMSC**
4.2	5	50	200	625	11.5	**TMSC & Al**
5	5	75	200	625	22.7	**TMSC**

Summary: CTCAE scores plotted versus valley doses yield a characteristic dose - effect curve (not shown). Higher valley doses combined with the wider on-center distance of 400 microns yield CTCAE scores similar to those obtained with lower doses combined with on-center distances of 200 microns. The results obtained by using single slits do not appear to differ markedly from the TMSC results, nor do the effects obtained with TMSC + Al filter differ markedly.

Conversely, a direct correlation between peak doses and CTCAE scores was not apparent. The most important parameter that influences normal tissue tolerance appears to be the valley dose.

Addendum: Hemicephalic irradiation of normal mice

An additional experiment was done to study the effects of an MRT-type of irradiation of the head on other than CNS structures. In this study of normal tissues, the left side of the mouse head was irradiated once, unidirectionally, anterioposteriorly, using an array of 49 parallel, ~27 μm-wide, ~50-150 keV synchrotron-wiggler-generated X-ray microbeams at ~210 μm intervals on center, which delivered ~312 or ~625 Gy skin-entrance radiation doses. The mice were euthanized two weeks later, and the whole head carefully decalcified and processed for histopathology. Head structures outside the brain, including the skin, turbinates, skull, skeletal muscle, bone marrow, eyes, and cochleae, were histologically indistinguishable from normal. At the same time, neuropathologic effects were minimal and of no evident neurological consequence. These preliminary results suggest that acute damage to murine tissues outside the brain may be minimal, if discernible at all, following MRT. Additional experiments to study long term extraneural histopathologic effects are in progress (see § III below).

LTP Milestones for "Parameter optimization using the Tecomet^R Multislit Collimator [TMSC]"

Year 2 Completion of the set of unidirectional irradiations. Begin study of microplanar irradiation using ≥ two ports. Follow-up. Histopathologic evaluation.

Year 3: "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." Prepare publication of the data.

Assessment of milestone status

The most important part of this project, the hemicephalic unidirectional irradiation, has been completed. The evaluation of the data will be completed within the time limit set above, as the duration of the follow-up at the animal facility has been restricted to one year after irradiation because of restricted housing capacity and lack of staff at the animal facility of the ESRF. Microplanar irradiation using ≥ two ports could not be initiated mainly for the same reasons.

For the present setup at the ID 17 of the ESRF, a more detailed and refined tabulation of this type should allow the selection of parameters for MRT with regard to the tolerance of normal tissues.

B. MRT of subcutaneously transplanted carcinomas in mice

Among the carcinomas proposed for this project (squamous cell carcinomas (SCCVII), mammary and/or ovarian carcinomas (MCa-4 and OCa-1), we have chosen the most aggressive variant, SCCVII. MRT of subcutaneously transplanted SCCVII in mice yielded median survival times exceeding that achieved by a broad 100 kVp X-ray beam. Those results have been published in the British Journal of Radiology (Miura et al, 2005).

The main results are summarized in Figs. 1 a and b. Briefly, the left tibiofibular thigh of a mouse bearing a subcutaneously [sc] implanted mouse model of aggressive human squamous-cell carcinoma [SCCVII] was irradiated in two orthogonal exposures with or without a 16-mm aluminum filter through a multislit collimator [MSC] by arrays of nearly parallel microbeams spaced 200 μm on center. The peak skin-entrance dose from each exposure was about 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 entrance dose/microbeam width combinations yielded 25-, 29-, 37- and 35-day median survival times [MST] (post-irradiation), respectively, exceeding the 20-day MST from 35 Gy-irradiation of SCCVII with a seamless (broad) 100 kVp X-ray beam.

A **second series of experiments** was done to follow up on the SCCVII experiment described above, to optimize irradiation parameters, to perform hemicephalic irradiation of mouse head for long-term histopathologic studies and to include the use of 1.9 nm gold nanoparticles (Nanogold). The design of the mouse holder was improved to avoid backscatter of radiation to the leg. The array encompassed the whole tumor, but spared the medial part of the normal leg at the level of the tumor, this to deliberately avoid obliteration of the lymphatic vessels draining the foot. The protocol of the experiment is summarized in Table II.

Preliminary results of the second series

MSTs of 30 and 44 days, respectively (Table II, Fig. 2) were noted for groups 8 and 3 in the current experiment, which used the same parameters used in previous experiments (Miura et al, 2006), i.e., 625 or 884 Gy at 35 μm beam width and 200 μm on center distances. The MST for the 1250 x2 Gy entrance dose at 400 micrometer on center was 20.5 days versus the 30 days for the 625 x2 Gy at 200 micrometer group. Only one mouse of all groups in the current study had to be euthanized because of foot edema. Although several groups had mice that developed such edema, tumor overgrowth rather than foot damage led to euthanasia. In the previous, published study (see above), 8/10 and 3/12 mice had to be euthanized due to foot damage in the 884/35/200 and 625/35/200 groups, respectively. **MRT** using an **on center distances of 400 μm** was **significantly less palliative than that using 200 μm regardless of the dose**, an observation which is in keeping with the lesser normal tissue damage under these conditions (see Table I). **Cross-fired beams** at entrance doses of **1250 Gy** each spaced at **400 μm** on center were less palliative than similar beams at **625 Gy** each, spaced at **200 μm**, all having microbeam widths of **35 μm**.

Table II: MRT – Optimization of irradiation parameters (SCC VII)

Group	Purpose	Nanogold	MRT, Crossfiring, Dose (Gy) per array (exception: groups 9 & 10: unidirectional)	Number of mice	Microplanar width (micrometer)	Center-to-center separation (micrometer)
1	Tumor palliation	No	625	12	70	400
2	Tumor palliation	No	884	11	20	200
3	Tumor palliation	No	884	11	35	200
4	Tumor palliation	No	884	12	35	400
5	Tumor palliation	No	1250	12	35	400
6	Tumor palliation	Yes	625 Gy shortly after Nanogold injection	10	35	200
7	Tumor palliation	Yes	625 Gy ~2h after Nanogold injection	10	35	200
8	Tumor palliation	No	625 Gy No Nanogold	10	35	200
9	Left hemicephalic normal mouse	No	312 Gy unidirectional	4	35	400
10	Left hemicephalic normal mouse	No	625 Gy unidirectional	4	35	400
11	Tumor palliation control	No	None	6 + 4 extra		

The MSTs from **seamless 25- and 35-Gy** X-irradiation on the same tumor model were 14 and 20 days, respectively, which are **lower than MRT**-treated mice where on center distances were 200 µm. Although the MSTs for the MRT mice spaced at 400 µm were comparable to those of the seamless 35-Gy mice, the **normal tissue damage** appeared to be **less**.

Although **Nanogold** was not as effective in enhancing MRT of murine SCCVII leg tumors as it was in enhancing seamless orthovoltage X-ray therapy of much less aggressive murine EMT-6 leg tumors (Hainfeld et al, 2004), one of 10 mice in group 6 showed complete regression of its leg tumour with minimal residual scarring of the overlying skin and apparently normal function of its irradiated leg, whereas complete regressions were not seen in mice of the other groups.

LTP Milestones "MRT of subcutaneously transplanted carcinomas in mice":

Year 2: "Demonstration of a therapeutic index that significantly exceeds that achieved by broad beam irradiation of the same tumors."
Year 3: Manuscript.

Assessment of milestone status

The goal has been attained using the most aggressive tumor model, the SCCVII. The results of the first series of experiments have been published (Miura et al., 2005).

C. Spinal cord experiments (rats)

Methods

Microplanar irradiation: A segment of the spinal cord of young adult male rats was irradiated laterally, from the anatomically right to the left side, by a ~10.6 mm-wide array consisting of 52 microplanar beams ~35 µm-wide, spaced at ~210 µm, 20 mm-high, using the Tecomet multislit collimator at the MRT facility of ID 17. The array covered the 6th and 7th cervical, the first and largely the second thoracic vertebra. The entrance doses were approximately, in gray, 330, 470, 660, and ~940. Eight to 10 rats were irradiated per dose group, 4 rats served as sham-irradiated controls.

Seamless synchrotron X ray beam: Young female rats were irradiated in the same facility, in a similar position, but by a collimated seamless 1.35 mm-wide and 2.5 cm-high X ray beam, with an added 16 mm-thick Al filter. The anterior (cephalad) lateral border of the radiation field was given by a vertical line drawn through a virtual point situated at ~2 cm horizontal posteriorly of the incisura intertragica. The ionization energies imparted to the spinal cord for by this seamless irradiation mode were equal to those delivered by 52 microplanar beams ~26 µm wide. The entrance doses were, in gray, approximately: 80, 160, 200, 250, 310 or 620.

Follow-up: The rats were closely monitored by a person experienced in animal husbandry (Mr. Dominique Dalléry), for impaired function of the forelegs. The rats, held by the tail, were gently drawn with their forelegs across a grid of a large cage cover. Normally, the rats grab a bar of the grid and hold firmly on to it. Five grades of functional disturbance were noted. Failure to immediately grab a bar firmly was termed a grade 1 change, and grade 5 characterized paresis/paralysis of both forelegs with prolonged complete inability to hang on to a bar. Rats displaying grade 5 change were euthanized. The cervical and thoracic spinal cord was carefully removed and processed for histopathology.

Results

The percentage of euthanized rats after microplanar irradiation is shown in Fig. 3, the changes in body weight in Fig. 4. Paralysis was indicated by a loss in body weight in the 660 Gy and 940 Gy dose groups, with all rats euthanized in these two subgroups 66 and 58 days after irradiation, respectively. Conversely, the change in body weight in the 470 Gy dose group was similar to that of control rats. In the latter group, 2 rats had to be euthanized 289 and 311 days (~41 and ~44 weeks) after irradiation, and none in the ~330 Gy group. In the groups irradiated in the seamless mode, no rat survived without paralysis beyond 2, 3, 11 or 156 days (~22 weeks) after entrance doses of ~620, 310, 250 or 200 Gy, respectively, in contrast to 100% survivors in the ~80 and 160 Gy groups.

Histopathologic findings

The spinal cords of the rats euthanized from 55 to 311 days after microplanar irradiation were examined histopathologically by an observer blinded to the irradiation parameters. The sagittal sections displayed mono- to plurifocal areas of white matter necrosis measuring up to several mm in diameter, situated predominantly in the ventral region (ventral funiculus), in some instances also dorsally, often associated with fibrinoid vascular necrosis, sometimes with fibrinoid thrombi, all within the array. The latter was recognizable in most instances, measuring about 10 mm in length. There was also often focal hemorrhage, with diameters up to a few hundred micrometers, and in very rare instances small amounts of hemosiderin. The stripes were roughly 40 micrometer wide, difficult to measure exactly, as they are clearly visible particularly within the white matter, but only at low magnification, and the on-center distances about 190 micrometer. Only few of our cross-sections were taken within the array. Those cross-sections displayed focal white matter necrosis in the ventral funiculus, sometimes involving parts of the lateral funiculus. Neuronal changes such as vacuolation, chromatolysis, shrinkage, „ghosts“ and, very rarely, nuclear fragmentation were noted predominantly in the array region. The correlation of quantitative and qualitative histologic findings with irradiation parameters is in progress**.**

Discussion

Tentative dose-effect curves are plotted in Fig. 5. A rough visual interpolation yields an ED50 for paralysis of ~530 Gy after microplanar irradiation (MRT), and of ~180 Gy after seamless irradiation of a ~1.4 mm-long segment with synchrotron X rays (SR-X), about three times the value noted after seamless irradiation. So far, considerable histopathologic lesions have been found in the spinal cord of all rats irradiated in the MRT mode and euthanized for clinical signs of paralysis. The presence of marked vascular lesions such as necrosis, hemorrhage / hemosiderosis, involving not only tissue slices irradiated with peak doses, is compatible with the notion of a primarily endothelial pathogenesis of central nervous system (CNS) damage caused by ionizing radiation (Slatkin et al, 1988) that may result in a CNS syndrome, or in white matter necrosis (Coderre et al, 2006). The latency of about 8 weeks for both high dose groups, i.e., 940 and 660 Gy, and of about 10 months in the 470 Gy subgroup, caution against early assessments of radiation damage or absence thereof, particularly in the CNS. Thus, Serduc et al (2006) reported no damage to the brain vasculature within one month days after a 1000 Gy radiation entrance dose applied in the MRT mode. Dilmanian et al (2006) exposed the spinal cord of few rats transaxially to four 400 Gy, 0.68 mm-wide microbeams, spaced 4 mm. None of these rats, observed for 7 months, showed paralysis or behavioral changes. Because of logistic constraints, we will not be able to observe our rats beyond one year after irradiation.

The dose delivered to tissue slices between the microplanar beams, the “valley dose”, is likely to be of prime importance for the extent of tissue damage, as a spatially fractionated array may biologically result in a broad beam effect when the valley dose exceeds threshold values for normal tissue damage. Conceivably, the valley dose might also alter the ED50 of the peak dose. Thus, a macroscopic model of inhomogeneous irradiation of the rat spinal cord has been described by Bijl et al (2006): Twenty millimeters of cervical spinal cord were irradiated with variable subthreshold (= “bath”) doses (4 and 18 Gy) of 150 MeV protons (plateau region of the depth-dose profile). At the center, or at the distal end of the 20-mm segment, a short segment (2 mm or 8 mm in the central, 2 mm in the distal part,) was irradiated with variable single doses („showers“). The spinal cord tolerance of relatively small “shower” volumes was strongly reduced by a “bath” of low-dose irradiation of the adjacent cord. The results of all “bath-and-shower” experiments show the effect of a low bath dose to be highest for a short field of 2 mm, less for 4 mm, and absent for 8 mm.

The reasons for the remarkable tolerance of the CNS for MRT-type irradiations are possibly related to the volume effect and particularly to the presence of a huge interface between highly irradiated tissue slices and nominally unirradiated interjacent tissues. Even few - four - spatially fractionated macroscopic "minibeams", 0.68 mm thick, widely (1.36 mm) spaced, with an entrance dose of 170 Gy, impacting transaxially on a rat spinal cord, may be tolerated for 7 months (Dilmanian et al, 2007). Such findings suggest potential applicability of microbeams – and perhaps also minibeams – for clinical tumor therapy.

Seamless or broad-beam irradiation of an 8 mm-long cervical cord segment with a single dose of conventional X rays (X), resulted in an ED50 ~30 Gy in >30 weeks and, for a 4 mm-long segment, of ~51 Gy (Hopewell et al, 1987). Those differences between the effects of SR-X rays and X rays may relate to the length and the exact location of the irradiated segment of the cord, the ionization energy imparted to that segment, dose-rate effects (Pop et al, 1997) and other factors. The latency to myelopathy depends to some extent on the age of the animals at irradiation (Ruifrok et al, 1994). Regional differences in radiosensitivity within the rat cervical spinal cord, and in attenuation of the microbeams may have played a role, as we used a lateral irradiation to minimize potential damage to esophagus and trachea, theoretically more likely occur when an irradiation is performed in the dorsoventral direction. The lateral white matter of the cervical spinal cord has been shown to be more radiosensitive than the central part, whereas the gray matter appears to be highly resistant , with no lesions observable by light microscopy even after high-precision localized proton irradiation (150 MeV) with a single dose of 80 Gy (Bijl et al, 2005).

LTP Milestones "Spinal cord experiments"

Year 1: Determine the ED_{50} for delayed hind-limb myeloparesis resulting from transverse irradiation of a 2 cm-long segment of the spinal cord, centered on the T2 vertebral spine of rats.
Year 2: Determine the maximum additional MRT array/dose which can be delivered safely, unilaterally, to the same region in the rat cervico-thoracic spinal cord already irradiated seamlessly to its maximum tolerated dose 6 months previously.
Year 3: Manuscript.

Assessment of milestone status

Year 1: Goal largely attained. For publication results from few additional dose groups may be advisable.
Year 2: Due to logistic problems in the US, this experiment could not be done in 2007. Neither would a follow-up of the animals have been possible at the ESRF because of restricted housing capacity in parallel with a lack of staff at the animal facility.

D. Nanogold

See chapter B above.

Milestone: Demonstrate an enhanced therapeutic index using gold nanoparticles using SCCVII test tumors in mice.
Assessment of milestone status: The experimental results are encouraging (one long-term survivor), but not sufficient to demonstrate an enhanced therapeutic index using gold nanoparticles using SCCVII test tumors in mice.

E. a. Transplanted brain stem tumors in rats – MRT

The application of a standard method (Wu et al, 2002) has yielded encouraging results. In a pilot experiment, the brainstem of 10 rats was inoculated with 10,000 9LGS cells suspended in 2 microliters) on January 19, 2005. Tumors of various sizes were found in all rats after their spontaneous death – or euthanasia - about 3 weeks after tumor implantation. The tumors were located in the pons, involving pontine nuclei, often bilaterally, and in the cerebellum. The mostly nodular tumors with diameters up to 8 mm consisted of densely packed cells with dark nuclei typical for 9LGS. There were no conspicuous necrotic zones. Quite often, the tumor periphery was markedly infiltrating, with small tumor islands detached from the main tumor mass. The tumor showed also a remarkable tendency to infiltrate along Virchow-Robin spaces of many intracerebral blood vessels, as well as leptomeningeal infiltration. It also formed tumor nodules within the leptomeninges and infiltrated underlying brain tissue. In several instances, the tumor also penetrated into the fourth ventricle and/or compressed the cerebellum.

Altogether, 9LGS tumors implanted in the brains stem clearly have the very aggressive histopathologic aspect of a malignant intracerebral tumor and should be a very good model for posterior fossa neoplasms.

Milestone

Demonstration of a therapeutic index for any technically defined variety of MRT for a rat brain stem tumor that significantly exceeds the index achieved by broad beam irradiation of the same tumor in the same posterior fossa location.

Assessment, present status

The continuation of irradiation experiments using this model were foreseen only if sufficient beam time **and optimal logistics at the animal facility became available**. Thus, in 2008 II, additional pilot experiments are tentatively envisaged, with the following goals: 1. Feasibility of imaging of the developing tumors ~10 days after implantation (~15 rats) (probably at the MRI unit of the CHU, possibly with contrast media): Does the tumor size permit visualization in a high percentage of cases? If possible, the size of an irradiation field will be determined according to those results. 2. Palliative lateral irradiation ~10 days after tumor implantation, 3 groups of rats: a) MRT mode, ~50 µm-wide beams spaced 200 µm, entrance dose 400 Gy. b) Idem, but entrance dose 500 Gy. c) Lateral broad beam irradiation, conventional X rays, single exposure, 25 Gy. Follow-up of the rats at the animal facility of the ESRF; determination of the

MST; necropsy. If a significant palliation together with a good neurologic condition of the rats can be achieved by MRT, the treatment will be optimized by use of a combination of MRT with radiation-effect enhancing compounds (tentatively: 2009 I).

E. b. Transplanted glial brain tumors in mice: The characterization of this novel tumor model has been published (Smilowitz et al. 2007).

III. COLLABORATIVE PROJECTS IN PROGRESS

The following publications based on results of collaborative projects mentioned in the LTP have been published or are in submission, with one exception: Bystander effect in MRT, collaboration with N. Foray and A. Joubert:

- Regnard P, Le Duc G, Bräuer-Krisch E, Troprès I, Siegbahn EA, Kusak A, Clair C, Bernard H, Dallery D, Laissue JA , Bravin A: Irradiation of intracerebral 9L gliosarcoma by a single array of microplanar X-ray beams from a synchrotron: balance between curing and sparing. Phys Med Biol (2007), in print.
- Schültke E, Juurlink B, Ataelmannan K, Laissue J, Blattmann H, Bräuer-Krisch E, Bravin A, Minczewska J, Crosbie J, Taherian H, Wysokinsky T, Chapman D, Griebel R, Fourney D: Memory and Survival after Microbeam Radiation Therapy. Submitted (2007).
- Serduc R, Vérant P, Vial J-C, Farion R, Rocas L, Rémy C, Fadlallah T, Bräuer E, Bravin A, Laissue J, Blattmann H, van der Sanden B: In vivo two-photon microscopy study of short term effects of microbeam irradiation on normal mouse brain microvasculature. Int J Radiat Oncol Biol Phys (2006); 64 (5):1519-1527.
- Serduc R, van de Looij Y, Francony G,Verdonck O, van der Sanden B, Laissue J, Farion R, Bräuer-Krisch E, Siegbahn EA, Bravin A, Segebarth C, Rémy C, Lahrech H : Characterization and quantification of cerebral edema induced in mice by synchrotron x-ray microbeam radiation therapy. Submitted to Phys Med Biol (2007).

Evaluation of the Swiss Membership in the ESRF by the Swiss State Secretariat for Education and Research

According to the report of the Swiss State Secretariat for Education and Research, Federal Department of Home Affairs (2007), the quality of research after 10 years of participation at the ESRF was deemed to be very good; technical innovations have resulted from frequent collaborations of Swiss scientists with the ESRF staff. Medicine was deemed to be the most “popular” discipline.

New collaborators:

Bley Tim, D.V.M., Department of Clinical Veterinary Medicine, Division of Clinical Neurology, University of Bern, Laenggassstr. 128 CH-3012 - Bern, Switzerland

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IV. BEAM TIME REQUEST (Table III)

2008 II and 2009 I: Total of 84 shifts. Beamtime, important constraints

In contrast to the usual experiments, the treatment of animal patients imposes constraints on the flexibility of access to the beamline, as it is compulsory that treatment follows the diagnosis of a tumor as soon as possible - if and when the owners of suitable animal patients agree. On the other hand, it is not possible to predict when such animal patients will be recruited. As proposed in the LTP, a practical solution to this problem, already agreed to by Dr. Alberto Bravin and confirmed by him in December 2007, is for our group to be able - on short notice - to use the ID 17 MRT beamline for large animal irradiations for 2 to 3 days each month, (including 39 shifts for setup at the beginning of the protocol, to be reduced to 6 after the first 6-month period). It should be realized that variable numbers of suitable patients may be expected at irregular intervals. Thus, timing and total numbers of shifts should be flexible.

The feasibility of experiments on rodents depends on the availability of enough housing capacities and staff at the animal facility of the ESRF.

Table III: Beam Time Request

Experiment	2008 I				2008 II				2009 I			
	# of exp	shifts per exp	shifts set up	shifts sum	# of exp	shifts per exp	shifts set up	shifts sum	# of exp	shifts per exp	shifts set up	shifts sum
Large animal irradiation	~~2~~	~~3~~	~~9~~	~~24~~	3	3	6	**27**	3	3	6	**27**
TECOMET-MSC, parameters	~~1~~	~~3~~	~~3~~	~~6~~	0				1	3	3	**6**
Transplant. Carcinomas, mice	~~1~~	~~6~~	~~3~~	~~9~~	0				~~1~~	~~6~~	~~3~~	~~9~~
Spinal cord irradiation	~~1~~	~~6~~	~~9~~	~~15~~	1	3	9	**12**	1	3	9	**12**
Nanogold ME-MRT	~~1~~	~~3~~	~~3~~	~~6~~	~~0~~			-	~~1~~	~~3~~	~~3~~	~~6~~
brain stem tumors, rats	0			-	1	3	3	**6**	1	3	3	**6**
~~**Glial brain tumors in mice**~~	~~0~~	-	-	=	~~1~~	~~3~~	~~6~~	~~9~~	~~0~~	-	-	=
Sum per scheduled period				~~84~~ **0**				~~51~~ **45**				~~69~~ **45**

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VII . LEGEND TO FIGURES AND FIGURES

Fig. 1 a. (top) Survival graphs of mice bearing SCCVII carcinomas treated with seamless 25 or 35 Gy skin-entrance doses of X rays in comparison with unirradiated controls (Miura et al, 2006).

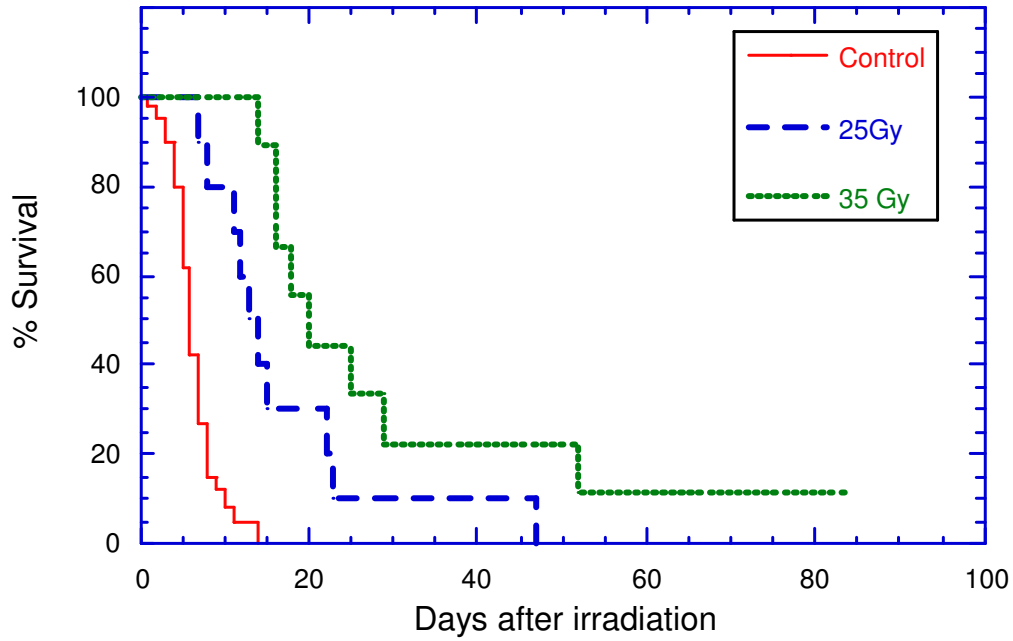
Fig. 1.b. (bottom) Survival graphs of similar mice in MRT groups (1 – 6) with skin entrance doses of 442, 625, and 884 Gy at 35 μm and 442 at 70 μm beam width. "Al" designates a 16 mm-thick aluminum filter placed upstream from the collimator (Miura et al, 2006).

Fig. 2. Survival graph of SCCVII carcinoma-bearing mice treated with MRT at various skin entrance doses, with microbeam widths of 35 or 70 μm and 200 μm on-center distances, compared with untreated control mice (unpublished).

Fig. 3. Rats surviving a microplanar irradiation of the cervical spinal cord. All sham-irradiated control rats survived (unpublished).

Fig. 4. Changes in median body weight of rats after microplanar irradiation of the cervical spinal cord. Paralysis was indicated by a loss in body weight in the ~660 Gy and 940 Gy dose groups, with all rats euthanized in both subgroups 66 and 58 days after irradiation, respectively. Conversely, the change in body weight in the 470 Gy dose group was similar to that of control rats. In the latter group, 2 rats had to be euthanized 289 and 311 days (~41 and ~44 weeks) after irradiation, and none in the ~330 Gy group (unpublished).

Fig. 5. Dose-effect curve: Paralysis after seamless (broad beam) of microplanar irradiation of the cervical spinal cord. 3. A rough visual interpolation yields an ED50 for paralysis of ~530 Gy after microplanar irradiation (MRT)(unpublished), and of ~180 Gy after seamless irradiation of a 1.35 mm-long segment with synchrotron X rays (SR-X)(unpublished), about three times the value noted after seamless irradiation. Seamless or broad-beam irradiation of an 8 mm-long cervical cord segment with a single dose of conventional X rays (X), resulted in an ED50 ~30 Gy in >30 weeks and, for a 4 mm-long segment, of ~51 Gy (Hopewell et al, 1987).



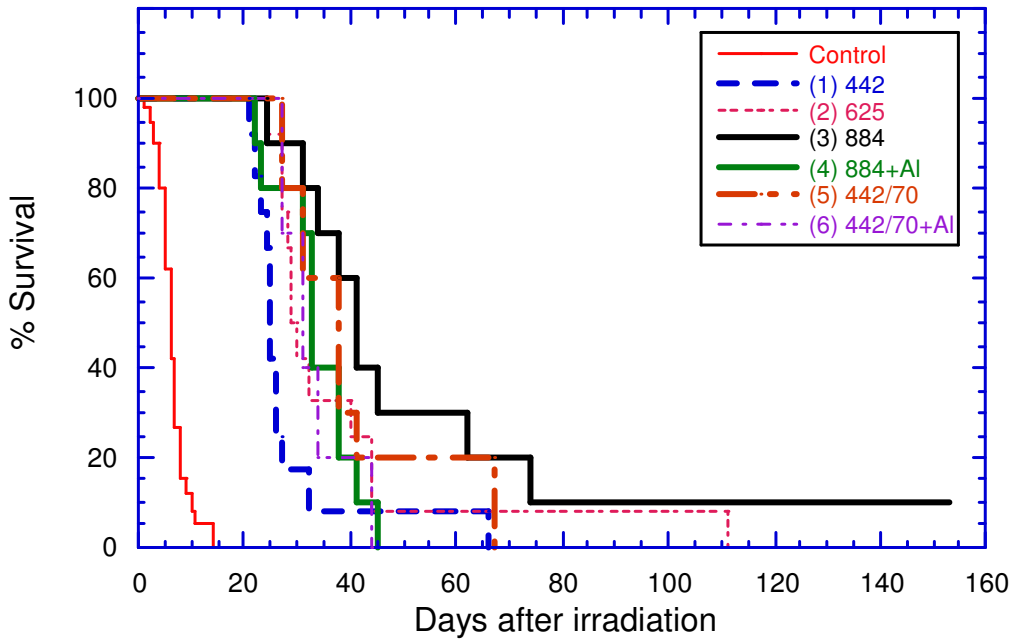


Fig. 1 a (top) and Fig. 1 b (bottom)

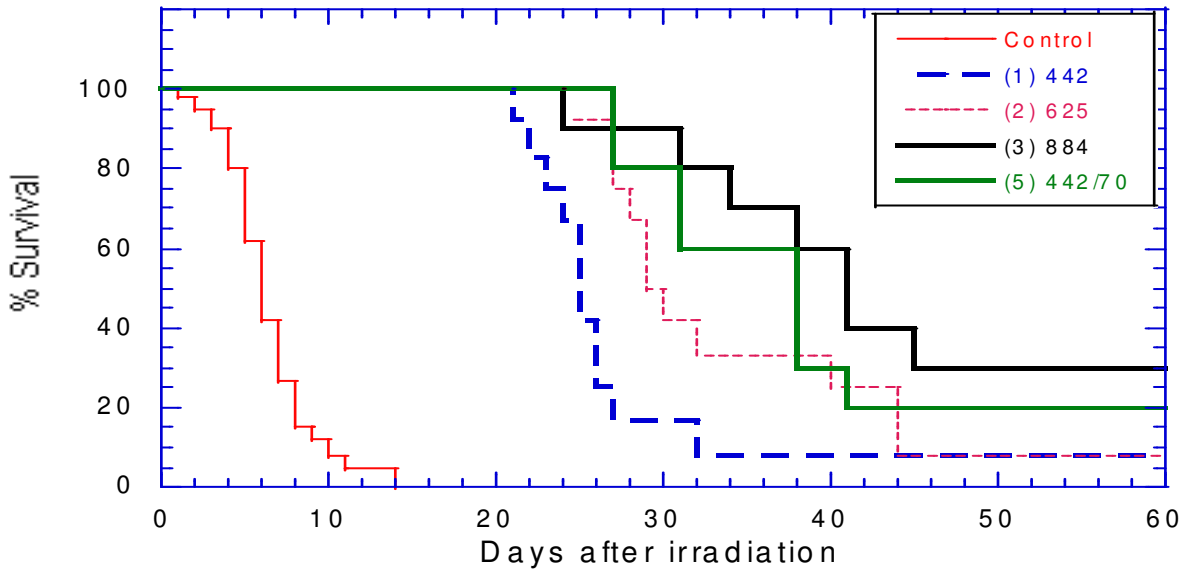

% Survival
100
80
60
40
20
0
0
10
20
30
40
50
60
Days after irradiation
Control
(1) 442
(2) 625
(3) 884
(5) 442/70

Fig. 2

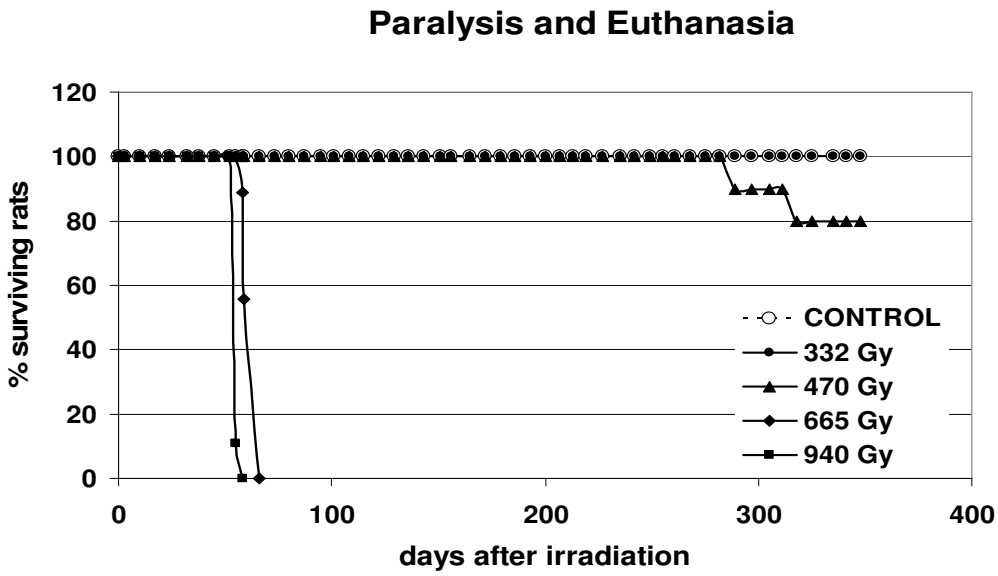

Paralysis and Euthanasia
% surviving rats
120
100
80
60
40
20
0
0
100
200
300
400
days after irradiation
CONTROL
332 Gy
470 Gy
665 Gy
940 Gy

Fig. 3

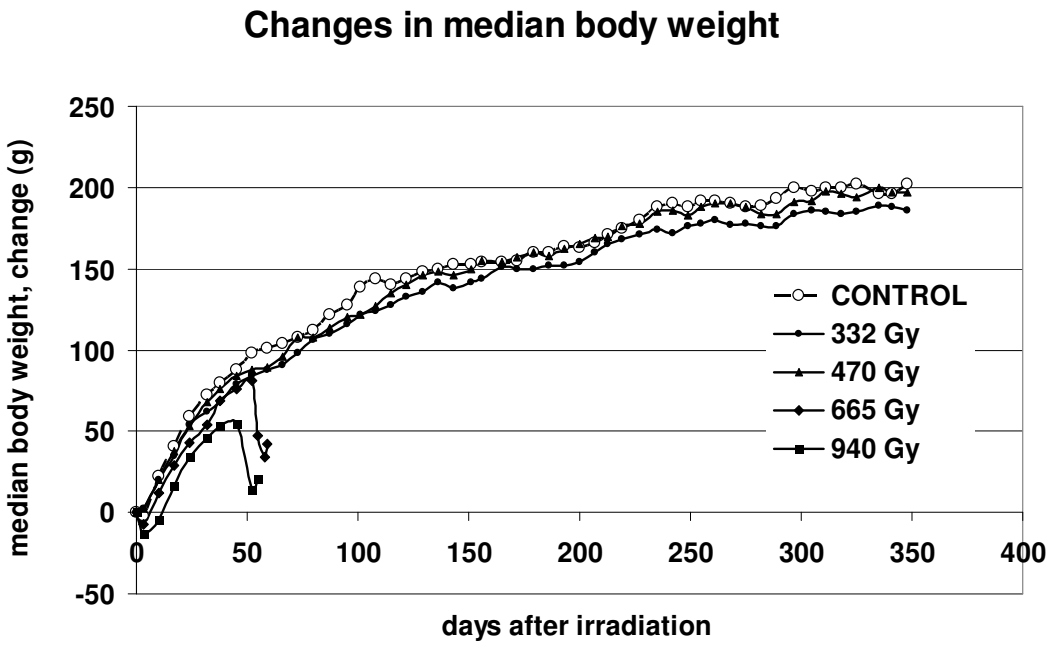

Changes in median body weight
median body weight, change (g)
250
200
150
100
50
0
-50
0
50
100
150
200
250
300
350
400
days after irradiation
CONTROL
332 Gy
470 Gy
665 Gy
940 Gy

Fig. 4

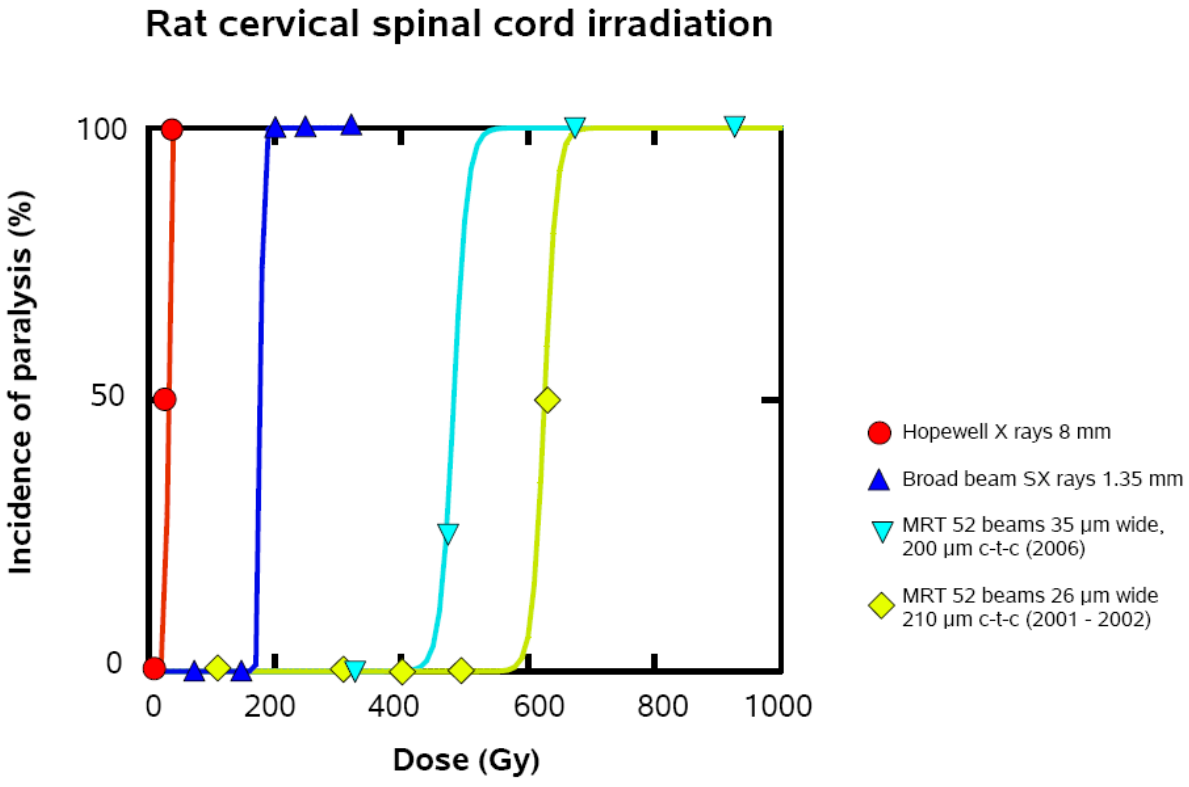

Rat cervical spinal cord irradiation
Incidence of paralysis (%)
100
50
0
0
200
400
600
800
1000
Dose (Gy)
Hopewell X rays 8 mm
Broad beam SX rays 1.35 mm
MRT 52 beams 35 µm wide, 200 µm c-t-c (2006)
MRT 52 beams 26 µm wide 210 µm c-t-c (2001 - 2002)

Fig. 5