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| ESRF | **Experiment title: MICROBEAM RADIATION THERAPY: DEVELOPMENT FOR PEDIATRIC NEURO-ONCOLOGY** | **Experiment number**: LS-2101 |
| **Beamline**: ID 17 | **Date of experiment**: from: November 5, 2001 to: Feb. 11, 2003 | **Date of report**: February 7, 2003 |
| **Shifts:** Total of 90 | Local contact(s)**: Dr. Alberto Bravin (e-mail: bravin@esrf.fr) Mrs Elke Bräuer-Krisch (e-mail. brauer@esrf.fr)** | *Received at ESRF:* |

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**Project LS-2101, Interim Report, February 2003**
Beamline ID 17, total shifts as of February 11, 2003: 90 shifts

**Accomplished experimental program, including a time-table with "milestones" (MS):**
By and large, the "milestones" proposed in the application have been laid down in the period covered by the interim report. In response and adaptation to new problems and perspectives arising in the course of the proposed experiments, some new experimental approaches were taken (see paragraph 4).

## 1. CONTINUATION OF PRECLINICAL TESTS

### 1.1. Pseudotherapeutic irradiation of the brain of suckling rats and of piglets

Experiments were complemented in 2001 and completed in 2002. As microbeam radiation therapy (MRT) might be used for brain tumors in human infants in whom seamless beams of radiations in clinically significant doses is likely to carry unacceptable risks of long-term neurologic disability, it is mandatory to generate data on the tolerance of normal developing tissues to irradiations of the MRT type. The cerebellum of 5 to 6 week-old weanling piglets was used as a surrogate for the radiosensitive human infant hindbrain. We irradiated two litters of 7 and 11 piglets, respectively. A 1.5 cm x 1.5 cm array of 76 equally spaced, 25 µm-wide, upright microbeams was propagated horizontally through a prone piglet's cerebellum from its left side. Skin-entrance doses ranged from 150 Gy to 600 Gy. Absorbed doses in the cerebellum were computed with the PSI version of the Monte Carlo code GEANT. More than one year (first litter), or about half a year (second litter) after irradiation, the irradiated piglets remained indistinguishable from their sham-irradiated littermates in terms of body weight, behavior, neurologic status and cerebellar MRI's. It has been shown (see 1.2) that MRT implemented with similar doses, followed by immunoprophylaxis, is safe and either palliative or curative in young adult rats bearing intracerebral 9L gliosarcomas.
**MS**: Preliminary publication in 2001 (1). 2003: Publication (suckling rats) in preparation (2).

### 1.2. Intracerebral 9 L gliosarcoma in rats: MRT treatment and immunoprophylaxis

Rats with advanced, imminently lethal, ~4 mm diameter, left-sided intracerebral 9L gliosarcomas (9LGS), a well characterized malignant glioma with some similarities to human malignant

astrocytomas, were used as a therapy model fourteen days after intrastriatal implantation of $10^4$ 9LGS cells. If untreated, such tumor-bearing rats die from large (> 100 mg) brain tumors within two weeks (median, 6 days) thereafter. However, if these tumors are exposed on tumor-day fourteen to a single 0.5 cm-wide array of 25 μm-wide quasiparallel microbeams of synchrotron-generated X rays at 210 μm center-to-center intervals, with a 625 Gy peak skin entrance dose (unidirectional MRT), short-term survival is extended 4.6-fold to a median of 32 days, and apparently neurologically normal long-term survival (> 1 year) is increased to ~ 20%. Multiple post-MRT subcutaneous inoculations of radiation-disabled 9LGS cells that had been transfected with granulocyte-macrophage colony stimulating factor (i.e., gene-mediated immunoprophylaxis, GMIMPR) further increased long-term survival to ~ 44%. These results are the first to show that the combination of microbeam radiation therapy and gene-mediated immunoprophylaxis act synergistically for an advanced, imminently lethal rat brain tumor.
**MS**: By 2002, two controlled series of 9GLS-bearing rats have been treated successfully by MRT and immunoprophylaxis as described above. Abstract / manuscript submitted for publication (3, 4).

**2. DOSIMETRY, MODELLING; PSI VERSION OF MONTE-CARLO CODE GEANT**
The aim is the validation of the dosimetric computations for all MRT animal experiments by microdosimetry and appropriate additional methods. Preclinical MRT-related experiments are carried out with ~20-30 μm-wide, ~10 mm-high parallel microbeams of hard, broad-"white"-spectrum X rays (~50-600 keV). Novel physical microdosimetry (implemented with MOSFET chips in the "edge-on" mode) and Monte-Carlo computer-simulated dosimetry have been done for selected points in the peak and valley regions of a microbeam-irradiated tissue phantom, as radiation damage from an array of parallel microbeams tends to correlate with the range of peak-valley dose ratios (PVDR). The dosimetric MOSFET measurements were compared with Monte-Carlo calculations. Peak doses at depths < 22 mm were 18% less than Monte Carlo values, possibly from unaccounted losses within the multislit collimator, whereas those at depths > 22 mm, and valley doses at all depths investigated (2 mm - 62 mm) were within 2 – 13% of the Monte-Carlo values.
**MS:** Salient microdosimetric results, compared with Monte-Carlo computations, have been published or submitted for publication (5, 6, 6a, 7). A self-contained computer program for the MRT-related PSI version of the Monte Carlo-Geant code, developed by Dr. Jiri Stepanek, is currently made available to Mrs. E. Bräuer-Krisch and Dr. Alberto Bravin at the ESRF.

**3. MORPHOLOGICAL DEVELOPMENT OF MRT-INDUCED LESIONS IN THE RAT BRAIN**
**MS: The l**ong-term study, including the observation of about 60 irradiated rats up to > 180 days after irradiation, has been completed in 2002 (manuscript in preparation (8)). It includes the painstaking assessment of detailed sequential time- and dose-related quantitative histopathologic changes, cellular events, particularly the immunophenotyping of involved cells versus time after microplanar irradiation parts of the brain of young adult rats.

**4. NEW DEVELOPMENTS: MICROBEAM-TARGETED CELL AND/OR GENE THERAPY**
Four experiments have been done, by i.v. injection of 80 nm-diameter colloidal gold particles stabilized with albumin into mice at various intervals following unidirectional or crossed MRT-irradiation of brain areas. The methodology to retrieve gold particles in tissues (e.g. in the liver) has been developed. Extravasation of gold particles in the irradiated brain areas has not been demonstrable yet. As this extravasation depends largely on the permeabilizationof the blood-brain barrier, the experimental approach has been abandoned temporarily (**MS**) in order to characterize the vascular permeability changes versus times after irradiation (see 5.1. below).

## 5. EXPERIMENTAL APPROACHES ADDED TO THE EXPERIMENTAL PROGRAM

### 5.1. *In vivo* observation of damage and repair in blood vessels from exposure to multiple microbeams of x-rays of hundreds of Gy

The chorio-allantoic membrane (CAM) of the chicken allows direct observation with time of radiation effects on rapidly developing blood vessels. CAMs in a Petri dish have been exposed to arrays of 51 parallel, thin (about 25 µm-wide), closely spaced (5/mm) vertical (10mm-high) microplanar beams of synchrotron x rays along the surface. The entrance doses ranged from 300 Gy to 1200 Gy. All CAMs survived the observation period of 24h. Massive damage, which was not repaired within 24h, was seen after application of entrance doses of 600 or 1200 Gy. For the 300 Gy exposures, the microvasculature between the microbeams was minimally affected and was repaired within 24h. Vascular bridges were observed across areas situated directly in the path of a microbeam.

**MS**: Experimental demonstration that the microbeam effect can also be observed in vivo in the CAM model. At entrance doses of 200 to 300 Gy, the microvascular damage between the beams is absent or minimal. Larger blood vessels survive multiple exposures in their course with 300 Gy microbeams. The damaged microvasculature situated directly in the path of the beams can be repaired, at least partially (9, 10, 11).

**5.2. Extension of preclinical studies: a transplantable squamous cell carcinoma (SCC)** in mice will be used in February as a model for head and neck cancers in humans (see 1.).

### 5.3. Extension of preclinical studies: Cervical spinal cord irradiation in adult rats

The spinal cord is a most important dose-limiting organ in radiation oncology. It is well known that seamless irradiation of several mm-long segments of the spinal cord of rats results in paralysis of extremities at doses < 100 Gy. Similar or higher doses were applied in the MRT and in the seamless mode to young adult rats. The animals of a first series were followed one year after irradiation, animals of the second series now up to 230 days. The preliminary results show that doses of 500 Gy applied to an approximately 11mm-long segment of the cord is tolerated by all rats for up to 12 months when delivered in the MRT mode, whereas 200 Gy, applied in a seamless mode on a very short (approx. 1.4 mm) section of the cord, result in leg paralysis in about two weeks after irradiation. An entrance dose of 624 Gy, applied in the seamless mode, results in foreleg paralysis of all rats within two days. The same entrance dose, applied in the MRT mode, has not (yet?) resulted in paralysis in one-half of the rats now up to 230 days, and in leg paralysis of the other half within 10 weeks after irradiation, respectively. The experiment and its detailed histopathological and statistical analysis of the data are in progress.

## 6. OUTLOOK FOR A CLINICAL APPLICATION OF MRT (PHASE I CLINICAL TRIAL)

Several meetings with representatives of the ESRF, the CHU of Grenoble and the Swiss-based Research Group for MRT (SBRG) were held, and by consensus, first steps were made towards implementing clinical applications of MRT. As one element of the logistic requirements, the SBRG has arranged for the manufacture of a new adjustable multislit microcollimator of a larger size, with more precise specifications than the present model. It should be amenable to commission in about 3 to 4 months.

The relationship between the parameters dose in the microbeam, spacing between microbeams, dose profiles in the valleys and biological response will be further investigated as input into treatment planning for microbeams.

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