EUROPEAN SYNCHROTRON RADIATION FACILITY

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Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

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	Experiment title: Study of the evolution over time of microbeam radiation therapyinduced microcalcifications on rat brains by X-ray phase contrast micro-CT	**Experiment number**: MD1297
Beamline: ID17	**Date of experiment**: from: 20.01.2022 to: 23.01.2022	**Date of report**: 03.03.2022
Shifts: 9	**Local contact(s)**: Liam DAY, Michael Krisch	*Received at ESRF:*

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Report:

- **Objective of the proposal**

The overall objective of this study is to investigate over time the process underlying the formation of Ca and Fe deposits found in the late stages after irradiation with Microbeam Radiation Therapy (MRT) of healthy and glioblastoma-bearing (GBM) rat brains. The hypothesis to be investigated is that MRT, delivered at high doses, causes the transection of vessels producing micro-bleedings which result in small Fe deposits that calcify and extend with time; additionally, we wish to understand whether there is a thereshold dose for this phenomenon. In this proposal, we focussed on the effects on healthy brain tissue, which are the limiting factor in any treatment strategy. The proposed experiment is one of the steps of a broad multi-annual study including a series of synchrotron radiotherapy and analysis experiments, part of which have been already realized; this proposal, and others that will follow, will allow reaching the statistical significance required to the study (approved APAFIS#27887-2020110512501830 v2 ethical form).

- **Scientific and experimental backgroud**

Results from the previous experiments realized within this multi-annual study have been summarized in an article recently published in Cancers (Romano et al. Cancers, 13, 2021). This proof-of-concept work includes some important milestones, but findings need to be confirmed by increasing the (statistical) significance of the results. The effects of radiation on the rat brains were analyzed by using synchrotron X-ray phase contrast computed CT (XPCI-CT), Small & Wide Angle X-ray Scattering (SAXS/WAXS) and X-ray fluorescence (XRF) as well as histology and immunohistochemistry. We demonstrated that XPCI-CT discriminates with high sensitivity the effects of MRT and broad beam (BB) irradiations on both healthy and GBM-bearing brains producing a first-time 3D visualization and morphological analysis of the radio-induced lesions, such as MRT induced tissue ablations, the presence of hyperdense deposits within specific areas of the brain, and tumor evolution or regression. Histology, immunohistochemistry, SAXS/WAXS and XRF allowed identification and classification of these deposits as hydroxyapatite crystals with the coexistence of Ca, P and Fe mineralization, and the multi-technique approach enabled the realization, for the first time, of the map of the differential radiosensitivity of the different brain areas treated with MRT. 3D XPCI-CT datasets enabled also the quantification of tumor volumes and Ca/Fe deposits and their full-organ visualization. The multi-scale and multi-technique approach enabled a detailed visualization and classification in 3D of the radio-induced effects on brain tissues bringing new essential information towards the clinical implementation of the MRT radiation therapy techniques. Building upon these results (based on a limited number of animals) and on the scientific and technical experience gained in the previous experiments, in MD1297 we irradiated healthy rats and sacrificed at different time-points with the purpose of investigating over time the process underlying the formation of Ca and Fe deposits found in the late stages after irradiation with MRT. These microcalcifications, observed in previous MRT experiments as said, have attracted the interest of our collaborators expert in neuropathology. Those deposits are also seen in children undergoing brain radiotherapy. This study will give an overview on their formation and temporal evolution as a function of the irradiation geometry and delivered dose.

- **The experiment**

Animal irradiation

In this experimental session, 115 male Fisher rats were irradiated using different radiotherapy protocols. Rats were anesthetized (following the protocol described in the approved ethical project) and positioned on a stereotactic frame and irradiated with BB or MRT (unidirectionally or bi-directionally) 50 μm wide and a centre-to-centre (c-t-c) inter-microbeam distance of 200 μm. The irradiation field was of 5x8 mm^2 in the antero-posterior configuration and it was centred on the right-side brain using a well-established protocol, which includes the use of a 2D X-ray radiography of the rat kull (see Figure 1). The field of irradiation was vertically centred with the bregma (bregma +4/-4 mm) and laterally centred at bregma +3.5 mm. For the irradiation the conventional MRT spectrum was set. The same spectrum was used for acquiring the radiographies but with a wider wiggler gap (100 mm instead of 24.8 mm). The dosimetry for the experiment was done by the ID17 team/local contacts before the beamtime.

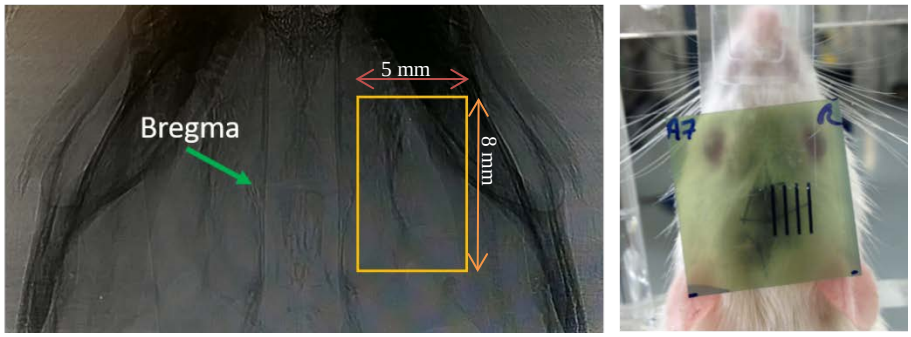

Figure 1

MRT chosen peak doses were 200, 400 and 600 Gy with corresponding valley doses of 7, 15 and 23 Gy; some animals were irradiated with microbeams with a peak dose of 300 Gy from two perpendicular directions. The BB delivered doses were chosen to be equal to the MRT valley doses to obtain a reliable comparison between

BB and MRT treatments. The number of involved animals per group, which is reported in the following table, was intended to reach 9 animals per group, as stated in the approved APAFIS#27887-2020110512501830 v2 ethical form. Animals are (present tense because this part of the experiment is still in progress) sacrificed at 4 different time-points: 14, 28, 63 and 180 days post irradiation. After sacrifice, brains were/will be extracted and fixed in 4% formalin solution. An experimental proposal has been submitted and others will be submitted to analyze by XPCI-CT, SAXS&WAXS and XRF the brains of the rats irradiated in MD1297.
All animals were irradiated and no problems were encountered during the experimental session.

- **Concluding remarks and acknowledgements:**

This irradiation experiment, including both the animal- and the technical-related procedures, went off smoothly without problems.
We are grateful for the help provided to us by the local contacts, the teams of ID17 and the ESRF Biomedical Facility. Technically and experimentally the beamtime was very successful.