EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

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.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Iodine distribution after IUdR infusion in rats bearing F98 glioma: Correlation with the tumor volume and IUdR incorporation (preclinical and in vitro studies).	**Experiment number**: MD660
Beamline: ID17	**Date of experiment**: from: 13/12/2013 to: 15/12/2013	**Date of report**:
Shifts: 9	**Local contact(s)**: Thierry Brochard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*Dr. Jean-Luc Ravanat
*Dr. Hélène Elleaume
* M. Paul Gimenez
***Dr Melanie Flaender**

Aim of the work :

Heavy-atom-enhanced Synchrotron StereotacticRadiation therapy (SSR) involves selective accumulation of high-Z elements in tumors, followed by stereotactic irradiation with x-rays from a synchrotron source. Our team has carried out several in vitro and in vivo experiments at the ESRF, using either iodine as contrast agent or as IUdR (a thymidine analog that incorporates into DNA during cells' replication) and a single x-ray dose fraction 1–4. The in vivo feasibility of IUdR-enhanced SSR was shown on rats bearing F98 gliomas 5, however no long term survivors were obtained in this study. The treatment outcome is expected to be improved by using a fractionated radiation therapy regimen. Doiron et al. have demonstrated that combination of BrdU (another thymidine analog) prolonged infusion and fractionated irradiation enhances response of RIF cells in vivo 6. Preliminary studies with fractionated radiation therapy and IUdR prolonged infusions have been performed. These studies have shown encouraging results with some limitations. To better understand the treatment outcome and improve this promising therapy it is necessary to determine the IUdR volume of distribution and IUdR concentration in the interstitial liquid versus the infusion time.

The aim of the present work was to characterize using synchrotron radiation computed tomography (SRCT) the 5-iodo-2'-deoxyuridine distribution in a rodent glioma model, several days after implantation of mini-osmotic pumps. The second aim of this work was to determine the part of photoelectric effect in cells death after irradiation for cells incubated with either iron nanoparticles or IUdR.

Experimental methods and parameters:

In vitro

Samples preparation / irradiation:

F98 cells were incubated either with IUdR during 48h 2 days before irradiation or with nanoparticles 24h before irradiation. The radiation source energy was tuned to 30, 50 and 80 keV to allow the Sensitive Enhancement Ratio (SER) dependence investigation. In addition, we irradiated the samples at high energy (1.25 MeV – ^{60}Cobalt source – CEA Grenoble). Irradiated samples receive 4Gy whatever the condition. We had several problems with Camz at the beginning of the irradiation. This implied that we had to remake the 30keV condition.

Cell survival measurement:

ATP-lite test was used for measuring cell survival after a treatment (irradiation alone, irradiation, in combination with NPs or IUdR). The toxicity of the drugs alone was also studied.

pH2AX determination:

1hour and 24h after irradiation, cells were fixed with ethanol. We measured by flow cytometry phosphorylation of histone H2AX by labeling cells with a dedicated antibody.

In vivo

Samples preparation:

Seventeen days before beamtime rats were implanted with F98 cells. Thirteen days after implantation, tumor presence was checked by MRI and rats received osmotic pomps containing either IUdR (20mg/mL) or Magnevist (30mg/mL).

Samples imaging

Four days after rats were imaged by MRI to determined tumoral volume and by SRCT using the germanium detector. SRCT imaging was performed at 33,17keV before and after intravenous injection of iomeron.

Selected preliminary results:

Cell survival measurement:

From cell survival measurement we have calculated the SER at 4Gy as the ratio between the survival fraction for cells irradiated without drug and cells irradiated with either NPs or IUdR.

The SER4Gy was found to follow theorical DEF curve, calculated by Monte Carlo simulations with a maximum at 50keV for IUdR and 30keV for Fe-NPs. Determination of SER4Gy at 1.25MeV are currently in progress.

These preliminary results tend to prove the preponderant role of photoelectric effect in cells death after irradiation at low energy.

pH2AX determination:

Phosphorylation of H2AX was for the moment determine only for 50keV condition. We observe a significant increase in pH2AX positive cells 1 hour after irradiation. 24h after irradiation level of pH2AX positive cells return to basal level. No significant difference was observe for IUdR in comparison with control cells.

SRCT imaging

We weren't able to determine IUdR distribution because iodine concentration was at the limit of sensitivity and was only visible on some images. This indicates that the macroscopic concentration of Iodine under these conditions was lower than 1mg/mL. We had problems the fisrt day during data recording just after acquisition which leads to lost of acquired data. Thank to our local contact we modify the set up to allow images correct acquisition and recording and we were able to do almost all we plan to do.

Conclusions:

This experiment has shown the interest of IUdR for enhancing radiosensitivity of tumoral cells with a benefit of a low energy irradiation. We have shown that cell death depend on energy indicating a predominant role of photoelectric effect.

In vivo we found that the macroscopic iodine concentration was lower that 1mg/mL using osmotic pump filled with 20mg/mL of IUdR.

Further experiments using a larger concentration of an iodinated compound could permit to precise the real factor of dilution and thus could help to link in vivo experiment with in vitro experiments.

We are grateful and very satisfied by the help provided to us by the local contact.