EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Long Term Project Report : Interim/Final

Summary Page

1. Beamtime Used

Please give a short summary of progress for each scheduling period for which beamtime has been allocated/used :

Scheduling period	Beamline(s) Used	Shifts Used	Summary of results obtained
2011/II	ID17	3	The establishment of survival curves is in progress. Various concentrations of gadolinium based (AGuIX) nanoparticles and various delays between irradiation and intravenous injection of the nanoparticles were tested. Very interesting results were obtained when irradiation (MRT) were carried out 24 h after the injection of the nanoparticles in comparison to other delays (20 minutes, 1h after injection). The effects of the concentration on the increase in lifespan (ILS) seems depend on the nature of the nanoparticles.
2012/I (23-26/06)	ID17	9	Since the results were very good when irradiation (MRT) were carried out 24 h after the injection of the nanoparticles (*vide supra*), we planned to deposit a similar dose but in several fractions. The 9L gliosarcoma bearing rats were therefore treated by MRT with 3 irradiations performed 24h, 48h and 72h after the intravenous injection of the nanoparticles. As expected, an increase in lifespan (ILS) is observed when MRT was performed after intravenous injection of nanoparticles containing high Z elements in comparison to non-treated rats and to only MRT treated rats. However the MRT seems less efficient when the dose is delivered upon three irradiations. These unexpected results were observed probably because the delivery of a dose in several fractions requires several anaesthesia which weaken the diseased animals. The protocol must be optimized.
2012/II (09-12/02)	ID17	9	Three new classes of gold nanoparticles which were also designed for medical imaging were tested as radiosensitizers. They were administered by intratumoral (IT) injection to melanoma bearing mice. This experiment demonstrates that the decrease of the tumour volume is more important when the MRT is performed after IT injection of these gold nanoparticles. Ninety days after the implantation of the tumour, the volume is four-fold smaller when the animals are treated by MRT after IT injection of gold nanoparticles in comparison to only

			MRT treated animals.
2013/I (20-23/04 and 21-24/06)	ID17	9 + 9	The therapeutic efficacy of the three new classes of gold nanoparticles which was tested in the previous session after intratumoral injection was evaluated in more realistic conditions. They were administered by intravenous injection and their behaviour was compared to the one of the precedent generation of the nanoparticles (Au@DTDTPA). Although all nanoparticles exhibited a similar behaviour when they are administered by intratumoral injection, the increase in lifespan seems to depend to the nature of the nanoparticles when they were intravenously injected. We noticed that one class of the gold nanoparticles provides better results than Au@DTDTPA. This session was devoted to the comparison to SSRT and MRT techniques after intravenous injection of gold nanoparticles and of gadolinium based nanoparticles (AGuIX). Whatever the irradiation mode (SSRT or MRT), an increase in life span was observed. However greater increase was obtained in the case of MRT treatment after intravenous injection of gold nanoparticles and of AGuIX.
2013/II (8-9/02/2014)	ID17	6	This session will be devoted to: * the determination of the lowest gold nanoparticles amount for an efficient radiosensitizing effect in MRT after intratumoral injection, * the effect of the association of chemotherapy (Temodal) and radiosentization (AGuIX + MRT). We observed that efficient control of growth tumor can be obtained with gold nanoparticle suspension containing 1g Au/L (i.e. a dilution by 10 in comparison to the suspension currently used). The association of Temodal and radiosensitization is very promising since an additional increase in life span is observed (in comparison to the group treated by MRT after intravenous injection of AGuIX). In previous experiments we demonstrated the great potential of AGuIX for image-guided radiotherapy. This potential is strengthened by the concomitant use of Temodal and AGuIX administered before MRT.
2014/I (14-19/06/2014)	ID17	12	This session will be focused on: * the influence of PEGylation on the efficiency of gold nanoparticles designed for MRT, * the effect of the dose fractionation in the case of tumor-bearing mice treated by MRT after intratumoral injection. No significative influence of PEGylation was observed. The treatment of melanoma-bearing mice by two sessions (MRT 10 minutes after intratumoral injection) with a delay of 4 days provides interesting

			results. Such a treatment led to a longer-lasting control of the tumor growth.
2014/II (15-17/11/2014) (31/1/2015 – 2/2/2015)	ID17	6 6	Since various gold nanoparticles have been successfully tested, this session was devoted to the comparison in the same conditions of their effect in MRT. For improving the targeting of the brain tumor, an original nanostructure has been developed. Radiosensitizing gold nanoparticles were grafted to biodegradable superparamagnetic iron oxide nanoparticles. Their potential for increasing the effect of MRT will be determined during this session.

2. Resources Provided by User team (financial, personnel, technical...):

Two researchers (post-doc) were at the disposal of Dr Géraldine Le Duc for the preparation of the first experiment of LTP MD-606. For the other sessions, Dr Sandrine Dufort (Nano-H and Institut Albert Bonniot) was at the disposal of Dr Géraldine Le Duc.
The selection of the project TheraGuIma (Multifunctional nanoparticles for image-guided radiotherapy, coordinator: Stéphane Roux, October 2011-September 2014) by the French Research Agency (Agence Nationale de la Recherche, ANR) results in a financial support which amounts to 126 777 euro for ESRF.
The value of samples produced for the LTP is estimated to 10 000 euro per session.

3. Technical and Scientific Milestones Achieved (in relation to the milestones identified in the original proposal)**:**

Year 1
No milestone has been achieved during the first year (3 shifts) but the results about the nature of the nanoparticles and the delay between injection of the nanoparticles and the irradiation (MRT) were very encouraging.

Year 2
The radiosensitizing effect of gadolinium based nanoparticles (AGuIX) and of nanoparticles with gold cores (AuNP) was confirmed. In the case of AGuIX, a great ILS is observed when the MRT is performed 24h after the intravenous injection of the nanoparticles. Although the amount of radiosentizing elements (Gd) is very low in the tumor (according to chemical analysis performed by ICP and data collected by MRI), the irradiation by MRT on gliosarcoma bearing rats provides the best results when the animals are treated 24 h after the intravenous injection of the nanoparticles (in comparison to animals treated 20 and 60 minutes after the injection). The mapping of gadolinium in the brain which was determined by experiments at ID21 Beamline (ESRF) shows that the gadolinium containing nanoparticles are only present in the intercellular medium of the tumour (no gadolinium was detected in the surrounding healthy tissue). These results indicate that the most important parameters for treating 9L gliosarcoma bearing rats with a combination of MRT and radiosensitizing nanoparticles (*i.e.* containing high Z elements) is the content of the radiosensitizing elements

in the surrounding healthy tissue which must be as low as possible and the distribution of these element inside the tumor.
Prior to the LTP study, we demonstrated that gold nanoparticles (Au@DTDTPA) designed as contrast agents for medical imaging (MRI, CT, nuclear imaging) exhibit a promising potential for radiosensitization. The experiments carried out at ID17 Biomedical Beamline (ESRF) and at the Institut Curie showed that the irradiation of cells in presence of these gold nanoparticles induces a higher number of DNA strand breaks than in the absence of nanoparticles. Moreover the increase of lifespan of osteosarcoma bearing rats and the decrease of the tumour volume is more important when the irradiation was performed after the intratumoral injection. LTP gave us the opportunity to complete the previous studies by determining the radiosensitizing effect of gold nanoparticles after intravenous injection to 9L gliosarcoma rats. Since these nanoparticles can be followed up by MRI, the most opportune moment for the irradiation (presence of the nanoparticles in the tumour and a low content of the nanoparticles in the surrounding healthy tissues) is comprised between 3 and 7 minutes. We demonstrated that lifespan increases when the rats are treated by MRT 5 minutes after intravenous injection (Small 2014). These results are very encouraging. However we are convinced on the basis of the study with AGuIX nanoparticles (ACS Nano 2011, Angewandte Chemie 2011, Chemistry 2013) that better results could be obtained if we succeed to ensure a more accurate control of the pharmacokinetics of the gold nanoparticles. For this reason, the development of three new classes of gold nanoparticles is under progress in order to shift the irradiation window (i.e. the most opportune moment for the irradiation after intravenous injection) from 3-7 minutes to a few hours. For circumventing the biodistribution issue, preliminary experiments were performed after intratumoral injection of the nanoparticles. This study revealed that these new classes of gold nanoparticles behave as promising radiosensitizers. Therefore MRT experiments after intravenous injection were planned. MRI imaging demonstrates that the accumulation in gliosarcoma is visible after intravenous injection of these gold nanoparticles. However the distribution in the tumour depends on the nature of the ligands present onto the gold core. This probably explains why the ILS depends on the type of gold nanoparticles.

Year 3
Owing to the promising results obtained with the various types of gold nanoparticles (year 2), an extensive comparative study of these gold nanoparticles was performed during the third year of this LTP. The objective of this study, which was performed with 9L gliosarcoma-bearing rats, consists in the evaluation of the effects of the nature of ligands, of the presence of PEG chains, of the conjugation of biotargeting groups, of the delay between injection and irradiation on the survival of the diseased animals. Whatever the type of nanoparticles, great increase in life span (ILS) has been obtained when MRT is performed after intravenous injection of gold nanoparticles. However the optimal conditions for obtaining the highest ILS are not the same for each type of nanoparticles. This difference emphasizes the crucial role of the chemical composition of the surface of the nanoparticles.

The other important result of this third year is the improvement of MRT effect on melanoma-bearing mice (sc A375 melanoma). The treatment was based on two sessions (MRT 10

minutes after intratumoral injection of gold nanoparticles) with a delay of 4 days provides interesting results. The preliminary experiments of dose fractionation (please see 2012/I) which was slightly disappointing permit us to determine more suited parameters. Such a treatment led to a longer-lasting control of the tumor growth when MRT was performed in each session after intratumoral injection of the gold nanoparticles.

4. List of publications directly resulting from beamtime used for this Long Term Project:

Previous experiments performed at the ESRF by our consortium provided important data. LTP MD606 was conceived from these results which were published in two renowned journals:

Le Duc, G.; Miladi, I.; Alric, C.; Mowat, P.; Bräuer-Krisch, E.; Bouchet, A.; Khalil, E.; Billotey, C.; Janier, M.; Lux, F.; Epicier, T.; Perriat, P.; Roux, S.*; Tillement, O. "Toward an Image-Guided Microbeam Radiation Therapy Using Gadolinium-Based Nanoparticles" *ACS Nano* **2011**, *5*, 9566-9574.

Lux, F.; Mignot, A.; Mowat, P.; Louis, C.; Dufort, S.; Bernhard, C.; Denat, F.; Boschetti, F.; Brunet, C.; Antoine, R.; Dugourd, P.; Laurent, S.; Vander Elst, L.; Muller, R.; Sancey, L.; Josserand, V.; Coll, J.-L.; Stupar, V.; Barbier, E.; Rémy, C.; Broisat, A.; Ghezzi, C.; Le Duc, G.; Roux, S.; Perriat, P.*; Tillement, O. "Ultrasmall Rigid Particles as Multimodal Probes for Medical Applications" *Angewandte Chemie International Edition* **2011**, *50*, 12299-12303.

From the results obtained during this LTP, four papers have been published, one manuscript is under submission (focused on the increase of lifespan when the MRT is performed 24 hours after the injection of AGUIX nanoparticles although the radiosensitizer content is very low in the tumor) and two other ones are under preparation (focused on the combination of Temodal and radiosensitization and on the new classes of gold nanoparticles).

Mignot, A.; Truillet, C.; Lux, F.*; Sancey, L.; Louis, C.; Denat, F.; Boschetti, F.; Bocher, L.; Gloter, A.; Stéphan, O.; Antoine, R.; Dugourd, P.; Luneau, D.; Novitchi, G.; Figueiredo, L. C.; Cesar de Morais, P.; Bonneviot, L; Albela, B.; Ribot, F.; Van Lokeren, L.; Déchamps-Olivier, I.; Chuburu, F.; Lemercier, G.; Villiers, C.; Marche, P. N.; Le Duc, G.; Roux, S.; Tillement, O.; Perriat, P. "A Top-Down Synthesis Route to Ultrasmall Multifunctional Gd-Based Silica Nanoparticles for Theranostic Applications" *Chemistry - A European Journal* **2013**, *19*, 6122-6136.

Miladi, I.; Alric, C.; Dufort, S.; Mowat, P.; Dutour, A.; Mandon, C.; Laurent, G.; Bräuer-Krisch, E.; Herath, N.; Coll, J.-L.; Dutreix, M.; Lux, F.; Bazzi, R.; Billotey, C.; Janier, M.; Perriat, P.; Le Duc, G.*; Roux,S.*; Tillement, O. "The *In Vivo* Radiosensitizing Effect of Gold Nanoparticles Based MRI Contrast Agents" *Small* **2014**, 10, 1116-1124.

Le Duc, G. Roux, S.; Paruta-Tuarez, A.; Dufort, S.; Bräuer-Krish, E.; Marais, A.; Truillet, C.; Sancey, L.; Perriat, P.; Lux, F.; Tillement, O. "Advantages of gadolinium based ultrasmall nanoparticles vs molecular gadolinium chelates for radiotherapy guided by MRI for glioma treatment" *Cancer Nanotechnology* **2014**, 5:4 (open access)

Sancey, L.; Lux, F.; Kotb, S.; Roux, S.; Dufort, S.; Bianchi, A.; Crémilleux, Y.; Fries, P.; Coll, J.-L.; Rodriguez-Lafrasse, C.; Janier, M.; Dutreix, M.; Barberi-Heyob, M.; Boschetti, F.; Denat, F.; Louis, C.; Porcel, E.; Lacombe, S.; Le Duc, G.; Deutsch, E.; Perfettini, J.-L.; Detappe, A.; Verry, C.; Berbeco, R.; Butterworth, K. T.; McMahon, S. J.; Prise, K. M.; Perriat, P.; Tillement, O. "The use of theranostic gadolinium-based nanoprobes to improve radiotherapy efficacy" *Br. J. Radiol.* **2014**, *87*, 20140134.