| ESRF | **Experiment title: Nanoscintillator-photosensitizer conjugate for X-ray induced photodynamic therapy : *in vitro* studies** | **Experiment number**: MD1260 |
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| **Beamline**: ID16A-ID17 | **Date of experiment**: **ID17:** from 30/06/2021 at 8 am to 03/07/2021 at 8 am **ID16A:** from 20/07/2021 at 8 am to 25/07/2021 at 8 am | **Date of report**: 22/09/2021 |
| **Shifts:** 9 (ID17) + 15 (ID16A) | **Local contact(s)**: **ID17:** Herwig REQUARDT **ID16A:** Sylvain BOHIC | *Received at ESRF:* |


**Experimentalists who contributed to the experiments:**

- Dr BULIN Anne-Laure, STROBE, Grenoble Alpes University (UGA) - INSERM
- Dr ELLEAUME Hélène, STROBE, UGA - INSERM
- Dr FULBERT Clémentine, STROBE, UGA - INSERM
- JACQUET Thibault, STROBE, UGA – INSERM
- NOMEZINE Anthony, STROBE, UGA - INSERM
- STELSE-MASSON Sarah, STROBE, UGA - INSERM
- ABU EL RUB Anamarija, STROBE, UGA – INSERM
- Dr BROEKGAARDEN Mans, Institute for Advanced Biosciences, UGA – INSERM – CNRS


**Report:**

The aim of this project was to study the ability of radioluminescent nanoparticles, also called nanoscintillators to potentiate synchrotron radiotherapy by inducing deep-tissue photodynamic therapy (PDT) and radiation dose-enhancement (RDE). While the RDE effect results from an excess of energy deposited by photo- and Auger electrons that are created when X-rays interact with heavy-elements, PDT relies on photosensitizers that generate cytotoxic species upon light activation. As light penetrates weakly in tissues, PDT is limited to superficial tumors. To reach deep tumors, nanoscintillators can be used to locally convert X-rays into visible light and remotely induce PDT. In this project, we investigated the ability of a nanoscintillator ($Lu_3Al_5O_{12}$:Pr, LuAG) conjugated to a clinically used photosensitizer (protoporphyrin IX, PpIX) to induce a PDT and RDE effect *in vitro* in 2D and in state of the art 3D cultures of pancreatic tumors.

In this project we investigated two aspects:

- The intracellular localisation of the nanoparticles/nanoparticles conjugated to the photosensitizer (experiments performed on ID16A).
- The therapeutic efficacy of the nanoparticles/nanoparticles conjugated to the photosensitizer to enhance the efficacy of synchrotron radiation therapy as well as the role of the X-ray energy and delivered radiation dose (experiments performed on ID17).

**Experiments performed on ID16:**

We were able to successfully image PANC-1 cells that were previously incubated with the different investigated samples. The cancer cells were grown on $Si_3N_4$ membranes. They were exposed to 0.1 mg/mL of nanoscintillators or nanoconjugates for 24 hours. After these 24 hours, the medium was refreshed and the cells were cryofixed in liquid nitrogen chilled ethane (- 180°C). Images were acquired on ID16A upon an X-ray irradiation sets up at 17 keV with a resolution of 100 nm per pixel.

These experiments allowed us to identify were and how the nanoparticles were distributed regarding individual cells. This information is crucial to understand the results we obtained when investigating the treatment efficacy achieved with every type of nanoparticles.

**Experiments performed on ID17:**
We investigated the effect of both the dose and the beam energy on the therapeutic efficacy.

**Clonogenic assay :**
PANC-1 and MIA PaCa-2cells grown in culture flasks were incubated (or not) with nanoparticles (LuAG) for 24 hours after which the medium was refreshed and the flasks were irradiated. Cells were collected and seeded at various concentrations in 6-well plates for clonogenic assay. Cells were seeded with 3 different concentrations (3 replicates/condition). 10 days after seeding, the colonies were fixed and counted.

**Experiments performed on 3Dmodels of pancreatic tumors:**
We worked with 3D pancreatic tumor models grown as adherent microtumors on a hydrogel. These cultures are developed in 24-well plates. Nanoparticles were added 5 days after initiation of the cultures and washed away before irradiation. Two radiation dose escalation experiments were performed; doses ranging from 0 to 8 Gy were delivered at two distinct energies:

- 62.31 keV (1 keV below the Lu K-edge)
- 64.31 keV (1 keV above the Lu K-edge)

After irradiation, the cultures were maintained in the incubator for an additional 6 days and a viability assay was performed. To isolate the contribution of the dose enhancement and the PDT effect, these experiments were performed with the nanoscintillators alone (dose enhancement only) and the nanoconjugates (dose-enhancement + PDT).

Data analysis is in progress.