**Continue and impove the high-energy beams**

**for radiation biology at GANIL**

***Cimap/LARIA, LRCM/IP2L, DLR-Germany, ISTCT/CERVOxy, LRMed/IRSN***

**Introduction**

Since several decades, GANIL provides high-energy beams for radiation biology, with the support and strength of Cimap/CIRIL and Cimap/LARIA platforms.

**Research Topics**

* Improvement of the predictions of simulation models for the calculation of the biological dose –Acquisition of biological data

Effects of energetic heavy ions on cancer cells - as relevant for hadrontherapy

Radioresistance, cancer progression & metastasis:

Molecular mechanisms

Role of reactive oxygen species (ROS)

Role of tumor hypoxia

Role of cancer stem cells

Combined treatments (immunotherapy, nanoparticles, inhibitors...)

* Efficacy and toxicity of Flash therapy

Effects of energetic heavy ions on normal tissue – as relevant for space missions and hadrontherapy

Space radiation effects on different cell types / tissues / organs

Side effects of hadrontherapy on normal tissues, bystander effect

Radioprotection or mitigation of radiation effects:

Elucidation of protective / destructive pathways

Testing of radioprotective substances

**Up-to-date experiments at GANIL: team by team**

***Cimap / LARIA*** Since two decades, the members of the LARIA are in charge of the organization of the beam time for biology, and use this beam time for their own research activity. Our experiments were performed with different ions (Carbon; Neon; Oxygen; Calcium), relevant for radiation biology in the framework of iPAC and France-Hadron. With the help of Cimap engineers, we developed several devices and sample holders to allow the irradiation of new cellular models, with the goal to stay up-to-date with standard and modern experiments in radiation biology. Our cell culture lab, located within the INB area allows us to run fast and efficient experiments, which corresponds to the best conditions that could be expected, and guaranty the quality of our results. We regularly published our research results with GANIL beam lines, with the respect to acknowledge GANIL support.

***LRCM/IP2L*** Since 2003, the members of the Rodriguez-Lafrasse’s lab have regularly performed experiments at GANIL. The ions used were Argon 85 MeV/n; 13Carbon 75 MeV/n; 12Carbon 95 MeV/n. Experiments were performed in the framework of iPAC (more than 15 iPac approved) or FranceHadron. GANIL was originally our main facility for radiobiological studies with carbon ions, which represent more than 50% of our research program. However, as we could not have access to carbon ion beam more than 1 or 2 times per year, we were obliged to find other solutions. Since 2016, a part of the group is going to HIMAC (Japan) to perform experiments. In GANIL, the radiobiological experiments are possible thanks to the presence and the help of LARIA. We collaborate with the researchers of LARIA, they grant us access to their own material as well as platform facilities. They are regularly associated to our publications. The guest-house located on site is very convenient (closed, clean, not expensive).

***DLR*** The Biodiagnostics Group of the DLR Radiobiology Department performed 21 beamtimes at GANIL since 2002, using the following ions: 12C, 13C, 36Ar, 208Pb, 20Ne, 22Ne, 58Ni, 16O. These beamtimes enabled the working group to address fundamental questions of space radiation biology such as the relative biological efficiency of space-relevant heavy ions to activate transcription factors and induce gene expression. The construction of the “Laboratoire de radiobiologie avec des ions accélérés” (LARIA) greatly improved the biological research capabilities and together with the development of the biological sample holders and the control software, the number of samples that can be irradiated per day (24 h) increased from ~100 to several hundreds. Also, the enlargement of the irradiated area allowed using larger cell numbers and different culture vessels suitable for many different biological analyses, such as immunofluorescence staining and microscopy, colony forming ability tests, gene expression and cell cycle analyses. The availability of a hypoxia station and incubators allowing cultivation under defined oxygen concentrations enabled experiments assessing the killing potential of carbon ions under continuous hypoxia for the first time and might result in reformulation of a central radiobiological dogma that hypoxic cells are more radioresistant. The close-by guesthouse is of high importance for the biological experiments, as for many biological endpoints, time kinetics are required, and the short distance allows to perform close meshed kinetics.

***LRMed/IRSN*** The IRSN’s LRMed team has long been interested in the role of vascular endothelium in the initiation and the development of tissue damages induced by high doses of gamma-radiation or X-rays in the context of radiotherapy. Two experiments were performed in the framework of iPAC or FranceHadron. Senescence, proliferation, proteins and transcriptional profiles of primary human vascular endothelial cells (HUVECs) exposed to high doses of carbon ions are investigated in comparison to 137Cs and X-rays irradiation. Preliminary results show that transcriptional profiles are markedly different between 137Cs/X-rays and C ions for equivalent doses of radiation. We now need to complete and repeat these experiments to confirm first results and to give insight into the cause of such differences of responses between the two types of radiation.

***ISTCT/CERVOxy*** During the last five years, CERVOxy has confirmed that hypoxia through the transcriptional hypoxia-inducible factors (HIFs) and other hypoxia-related proteins such as erythropoietin (EPO) are involved in resistance to conventional therapies. Interestingly, we have also demonstrated that while carbon ions (provided by GANIL in the framework of iPAC and France-Hadron) are much more efficient to kill tumor cells in hypoxia, the efficacy remains cell line dependent. Moreover, we highlighted that hypoxia-induced radioresistance to carbon irradiation might be reduced by high LET and/or by targeting hypoxia-dependent signaling such as EPO) These results have allowed us to start simulation studies to tune radiotherapy including hadrontherapy dosimetry according to hypoxia *in vivo*. The CERVOxy team has its own cell culture lab, located within CYCERON that allows us to run fast and efficient experiments, which corresponds to the best conditions that could be expected, and guaranty the quality of our results. We publish our research results with GANIL beam lines, with the respect to acknowledge GANIL support.

* **Evolution of GANIL**

1) For radiation biology research

Independent repetitions of the experiments are crucial, therefore, **multiple accesses to short beamtimes (6-8 h) per year** would be more useful than one long beamtime per year. This could greatly fasten the publication of data.

Also, LARIA has already reached the limits of its capacity during beamtimes with several groups, and **enlargement of the cell culture area would help** to improve the quality of the biological experiments.

The hypoxia bench gives unprecedented opportunities for investigation of the role of tumor hypoxia in the response to carbon ion therapy. It has to be considered that one bench will allow the cultivate the cells under one defined oxygen concentration and to compare it to ambient oxygen (21 %) during a beamtime, but in many cases, several oxygen concentrations (e.g. 0.1 %, 0.5 % and 1 %) have to be compared, and such experiments can only be performed sequentially. If **a second hypoxia bench** were available, two hypoxic conditions could be compared to normoxia during one beamtime, or two research groups could perform hypoxia experiments.

2) For space radiation biology

The demand of beamtimes for space radiation biology will remain high as fundamental questions concerning the mechanisms of heavy ion-induced degenerative alterations are still open and the risk assessment is incomplete.

Ions with Z numbers up to 26 are of highest interest. The ions with even Z are more abundant in the galactic cosmic ray spectrum (He, Be, C, O, Ne, Mg, Si, S, Ar, Ca, Ti, Cr, Fe), except for protons, which are most abundant. He ions make up ~ 12 % of the spectrum, followed by carbon and oxygen ions (>2x106 particles/day/m2). A flux peak is observed also for Ne, Mg, Si and Fe ions. For therapy-related research, protons and carbon ions are most important.

It can be expected that a shift from two-dimensional cell culture to three-dimensional culture and organoids will occur and that more complex structures will be irradiated – **adaptations to sample holders and the irradiation field** might be necessary.

Furthermore, experiments with **low dose rates** are of high relevance and might be possible in piggy-back to other experiments. Especially for such experiments, temperature or even CO2 concentration control will be necessary. Tests of radioprotective substances could help to develop space radiation countermeasures, but also reduce normal tissue toxicity during cancer radiotherapy.

3) Beamtime improvement

Short-notice beamtime cancellations or abandonment of a started beamtime have occurred quite often in the past and they always result in high scientific and financial losses, therefore, attempts for **higher robustness and redundancy are very useful**. Furthermore, advanced online dosimetry might reduce the time required for detector calibration based on CR39 plastic detectors.

With the main goal to continue their experiments, radiobiology users have several requests to improve the structure and the capacity of experiments in amount and in quality:

more beam time / year is requested (3-4 times/years with 1 UT / team) ; for the radiobiology experiments, the LARIA cell culture lab is too small for too many groups at the same time, an enlargement is recommended. We would also like to have the possibility: to irradiate small animals (mice); to irradiate with other ions (Proton, Helium, Oxygen); to irradiate at different positions in the Bragg peak for carbon ions; to have flash ion irradiation with proton, helium and carbon ions