

Beam time request

Personal Information

Group leader (First name/Last Name): Mihai Radu

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Proposal details

Lab: 3MV

Co-proposers: Diana Savu (DS), Mihaela Temelie (MT), Mihaela Bacalum (MB) and Nicoleta Moisoi (NM) (collaborator DMU, Leicester, UK - see Memorandum of Understanding (MoU) in the annex

Proposal title: Biological effects of proton irradiation on neuronal stem cells

Start of preferred period: 15.01.2018

End of preferred period: 1.06.2018

Start of undesired period:

End of undesired period:

Local contact (optional): Mihai Straticiuc

Main research area: Biology and biophysics

Other: -

Experimental set-up: 3 MVTandetrone™ external beam

Number of days requested: 20 (if possible only one day/week, Tuesday or Wednesday)

Energy (MeV): 3

Current (nA): as low as possible (fA-pA range)

Sample(s)/target(s) description: Neuronal stem cells cultures

Sample size (please fill in the units): < 1 cm²

Storage requirements (Ambient atmosphere/Vacuum condition): Ambient atmosphere

Requested preparation for samples (only for 1MV Lab proposal): -

Have you performed an experiment at some of our laboratories? Yes, at 3MV

Do you plan to continue the project after this experiment? Yes

Safety aspects

Is there any danger associated with the sample or sample environment? No.

Scientific motivation

The Central Nervous System (CNS) comprises a complex network of multiple cell types that are either differentiated to undertake specific brain functions (i.e. neurons and glia) or in a non-differentiated state (precursor/stem cells). Under physical stress factors like irradiation, brain cells may undergo transformation leading to cellular death or malignant transition, particularly for precursor cells. Most of the data concerning deleterious effects of terrestrial forms of ionizing radiation on the CNS has been derived from radiotherapy patients and from subjects exposed to irradiation during the World War Two atomic bomb explosion and Chernobyl accident. These studies reveal neurological complications such as cognitive impairments, memory, changes in social behaviour, mental disorders (dementia), age-related disease [1, 2]. Recently, radiation has become associated with premature aging and a wide spectrum of age related disorders [3, 4]. The biological effect of irradiation consists of an intrinsic cellular response to the damages inflicted to various cell components, including DNA damage. This activates DNA damage response (DDR), triggering cascades of biochemical reactions resulting in activation of stress pathways to eliminate and recycle damaged cellular components. Several hallmarks of aging including genome instability, epigenetic alteration (DNA methylation and RNA changes), mitochondrial dysfunction, senescence, perturbed intercellular communication present common features in mammalian cells. Dysregulation of DDR and repair is closely associated with human diseases such as aging and neurodegenerative disorders, cancer, cardiovascular diseases [5-10].

In this experiment we plan to use the 3MV Tandetron™ facility and study the response of normal CNS to proton irradiation. This will allow us to shed new light in understanding the relevance of proton irradiation for brain aging as well as for the cellular response to radiobiological applications (radiosensitivity / radioprotection). Preliminary studies were performed during the maintenance period and addressed dosimetry for the external proton beam by using a Markus ionization chamber, RCFs and TLDs. In this stage we also designed and developed the cells irradiation holder prototype.

Design of the experiment

Considering that aging is associated with accumulation of DNA damage, subsequent neuronal cell death and neurodegeneration, we will aim to investigate the molecular networks and pathways (using biomolecular end-points) associated with compromised cognitive function in accelerated aging involved in neurodegenerative diseases in human stem cells exposed to monocomponent proton beams.

Human neural precursor cells will be used either in nondifferentiated conditions or differentiated into neurons or glial cells respectively.

The experiment proposed will be divided in two parts:

- (i) Establish the irradiation conditions (dose response curves);
- (ii) Study of radiation induced biological effects and accelerated aging in normal CNS cells.

Establish the irradiation conditions

The first step will be to perform survival curves and identify the irradiation conditions, which induce 20%, 50% and 80% cell death, respectively. The setting achieving 20% and 50% cell death will be further used to determine signaling mechanisms in brain cells as a model of accelerated aging. The survival curves will be obtained by proton single dose irradiation (dose domain 0-5 Gy). Cell death/survival will be assessed using: i) morphological identification of apoptotic versus of healthy nuclei using specific

fluorescent nuclear markers; ii) MTS survival assay which measures the viability using mitochondrial function abilities iii) clonogenic viability will identify long term survival of the irradiated cells.

Study of radiation induced biological effects and accelerated aging in normal CNS cells

First will be investigated DNA damage response and signaling mechanisms in CNS cells. DNA double strand breaks will be investigated using gamma-H2AX/53BP1 foci immunostaining. The assay will be performed at specific time intervals and will allow the assessment of both the damage inflicted by irradiation and the DNA repair processes (starting from the moment of DNA damage induction, were possible, until one division time hours after).

We will study the effect of irradiation genotoxic treatment on gene expression. This will be performed under irradiation that only induces 20% cell death to identify signaling mechanisms triggered by the irradiation. Genes that will be identified as significantly upregulated or downregulated will be further validated by real time quantitative PCR (q RT-PCR) and Western blotting (WB).

We will monitor a) mitochondrial function (relative ATP levels-luciferase assay), mitochondrial respiration b) oxidative stress and ROS accumulation (fluorescent indicators DCF and MitoSOX. We expect to identify changes induced by irradiation on mitochondrial activity and metabolism, which will indicate potential age related dysfunctions.

IFIN-HH together with the **DMU, Leicester UK** (collaborator-see MoU) have prior experience in working together with these techniques and aging/neurodegenerative cell models [11-14]. DMU collaborator will bring in this project its expertise in studies of mitochondrial function cellular differentiation and transcriptomic analysis [15-17].

The current research strategy of the IFIN-HH favours the employment of its state of the art nuclear facilities (3MV TANDEM Accelerator, 9MV TANDEM Accelerator completely modernized – pelletron 2007-2014, new TR19 cyclotron (ACSI, Canada) - <http://www.nipne.ro/facilities/facilities>, ELI-NP facilities - <http://www.eli-np.ro/>) **for bioscience and biomedical studies. We aim to gain expertise in exploiting these nuclear facilities in order to develop a new research domain towards biological effects of irradiation by protons/accelerated ions with future development into risk estimation for solar systems travel (galactic cosmic rays) and proton therapy.** This new direction will contribute to the development of biological studies by **using nuclear technology** which is a priority in the research strategy of IFIN-HH. Moreover, using the existing facilities in IFIN-HH such as to provide the appropriate conditions for irradiation with proton beams in the range of doses and dose-rate useful for radiobiology/radiobiophysics research will open a completely new domain of research not only in IFIN-HH but also at the national level. Thus, other national and international research scientists will be attracted and this will enhance **the network** of capabilities for improving the present study and for discovering new avenues for other applications.

We mention that this study was included in a proposal submitted to 2017 ELI-RO national call and also to ESA AO-2017-IBER [Announcement of Opportunity for Investigations into Biological Effects of Radiation Using the GSI Accelerator Facility (AO-2017-IBER)] and also in the already national funded projects (PN 16 42 02 03).

The relevant equipment necessary to perform *in-vitro* experiments is located at DFVM (Department of Life and Environmental Physics), IFIN-HH and includes: A fully equipped cell culture laboratory, LightCycler® 96 Real-Time PCR, Cell LabQuanta SC flow cytometer, BioSpectrum Imaging

System 810 Western Blot, Mithras LB 940 plate reader. Inverted fluorescence microscope Olympus IX71 equipped with Till Photonics Polychrome V illuminator, AndoriXon EM camera, Prior motorized stage, Sutter filter wheel, complete set of filter cubes and a thermostated holder for imaging living cell samples. AndoriQ 1.8 software is used to control the set-up.

Beam time estimation

The beam time estimated is 20 days, one day/week (if possible Tuesday or Wednesday), which is justified below.

To gather sufficient data in the experiments proposed, we need at least 6 samples per irradiation point. To have a good dose-response curve and all the parameters we proposed to follow, at least 5 different doses need to be applied. If we consider an average time needed to change the samples and perform the irradiation around 10-15 min, also the time to stabilize the accelerator for the working parameters, the time estimated to perform the experiments will be around 6-7h.

Also, to have a good statistic of the results, the same conditions need to be replicate at least 3 times, in different days. The days during which the irradiation will be performed can't be successive, but at least one week apart. This requirement comes from the working protocols involving biological cells. We need at least one day before the irradiation to grow the cells and depending on the test performed after, 1-3 days to obtain the results.

Beam parameters

The experiments will be performed at the 3 MV Tandetron™ accelerator with the following parameters:

- the 3 MV Tandetron™ accelerator external beam setup (IBA beamline)
- the current used must be as low as possible (from tens of fA to pA) and very stable
- XYZ automated stage will be used to hold and move the irradiation chamber.

Bibliography

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MEMORANDUM OF UNDERSTANDING

BETWEEN:

(1) DE MONTFORT UNIVERSITY of Trinity House, The Gateway, Leicester LE1 9BH
("DMU"); and

**(2) "Horia Hulubei" NATIONAL INSTITUTE FOR RESEARCH AND
DEVELOPMENT IN PHYSICS AND NUCLEAR ENGINEERING** of 30 Reactorului
St., Magurele, jud. Ilfov, P.O.B. MG-6, RO-077125, ROMANIA ("NIPNE");

(Each a "Party" and together the "Parties")

Background:

(A) DMU is a higher education corporation established in accordance with the Education Reform Act 1988.

(B) NIPNE is an autonomous institution engaged in non-profit research activities, in the coordination of the Romanian Ministry of National Education and Scientific Research.

Purpose:

1. The purpose of this Memorandum of Understanding ("Memorandum") is to formally record the mutual interest of the Parties in strengthening and promoting research collaboration between the Parties based upon the principles of reciprocity, mutual respect, best effort and frequent interactions.

Aims:

2. The Parties will undertake research collaboration with the aim to:
 - promote institutional exchange of scientific and technical information; and
 - use their best efforts to encourage joint publication of research outcomes arising from investigations of mutual scientific benefit proposed by either Party;

in the field of 'Combining radiobiology, cell biology and pharmacological approaches in studies of life models of neurodegeneration and aging'.

Preliminary discussions:

3. The Parties have discussed their intention to explore areas of activity which may include:
 - Joint research activities and programs
 - Joint performance
 - Joint experiments
 - Exchange of organisation (faculty/institute) members and staff
 - Mentoring of early career researchers (postgraduate and post-doctoral researchers)

Next steps:

4. The Parties will work together to develop their relationship further and explore opportunities to develop specific collaborative agreements.
5. Detail of the implementation of any particular activities resulting from this Memorandum shall be negotiated between the Parties as such specific cases arise.

Exchange of public information:

6. The Parties hereby agree that exchanges of scientific and technical information pursuant to this Memorandum will be made based upon public information, and that either Party will not disclose any confidential information to the other Party at any time during the Term of this Memorandum without entering into a confidentiality agreement prior to the disclosure of such confidential information.

Term:

7. This Memorandum is valid for the period of three (3) years from the date of last signature ("Term").

Termination:

8. Either Party may terminate this Memorandum during the Term by giving two (2) months' written notice.

Amendment:

9. This Memorandum may be amended or renewed by written agreement between the Parties.

Notices:

10. Any notice or other communication required to be given under this Agreement, shall be in writing and shall be delivered personally, or sent by pre-paid first class post or recorded delivery or by commercial courier, to each Party required to receive the notice or communication at its address as set out below:

De Montfort University: Head Legal Services, Trinity House, The Gateway, Leicester, LE1 9BH
E-mail: legal.services@dmu.ac.uk

Scientific Director Dr. Livius Trache, National Institute of Physics and Nuclear Engineering
30 Reactorului Street, P.O. Box MG-6, R0-077135 Bucharest-Magurele ROMANIA
E-mail: dirst@nipne.ro or as otherwise specified by the relevant Party by notice in writing to each other Party.

At the time of signing, the two institutions delegate the responsibilities for the scientific program to dr. Nicoleta Mosoi (DMU) and dr. Diana Savu (NIPNE).

Effect of this Memorandum:

11. While the Parties wish by this Memorandum to make clear their enthusiasm to promote cooperative academic and educational links, the Memorandum is not intended to create any legally binding relationship between the Parties.
12. Both Parties look forward to a further period of a long and mutually beneficial relationship.

The Parties hereby establish this Memorandum by duly signing it as of the respective dates below:

Signed for and on behalf of
**NATIONAL INSTITUTE FOR
RESEARCH AND DEVELOPMENT
IN PHYSICS AND NUCLEAR ENGINEERING**

Signed for and on behalf of
DE MONTFORT UNIVERSITY

by NIPNE General Director

by

acad. Nicolae Victor ZAMFIR






Role: Director

Role: *DEPUTY VICE-CHANCELLOR*

Date:

Date: *16 DECEMBER 2016*

MoU Responsible
Diana Iulia SAVU

Savud

MoU Responsible
Nicoleta MOISOI

Nicoleta Mosoi

Date

Date