Proton radiotherapy for the treatment of patients with central nervous system tumors: an experimental and modeling approach

Topics: Health Physics, Medical Physics, Radiobiology

Project Partners

IJCLab coordinator: Mathilde Badoual, PhD., Prof.

(modeling the migration and behavior of nerve cells, including cancer cells, based on the models and algorithms created)

IFJ PAN coordinator: Justyna Miszczyk, PhD., Assoc. Prof

Research and Development Laboratory

Medical Physics Department, Cyclotron Centre Bronowice

(accumulated physical-biological research on the different application of drugs and proton radiation in cancer therapy)

Research problem

Tumors in the brain and spinal cord are called central nervous system (CNS) tumors

- The most common primary brain tumors are gliomas (classified by WHO into 4 grades, grade IV glioblastoma - GBM), **solid tumors in the pediatric age group**,

- Surgery, postoperative radiotherapy (usually X-ray therapy) are the standard treatments for gliomas, in combination with chemotherapy,

- One of the most aggressive tumors, with high rates of recurrence and very low rates of long-term survival (a median survival of 14 months from the time of initial diagnosis, relative 5-year survival rate of about 5%)*.

Due to the superior physical dose distribution as a consequence of the Bragg peak offered by proton beams over photons,
Proton Beam Therapy (PBT) seems particularly promising and beneficial for gliomas treatment

---

There is a lack of scientific data comparing proton radiotherapy and photon radiotherapy

in the treatment of patients (adults, children) with CNS tumors at different grades (mainly gliomas),

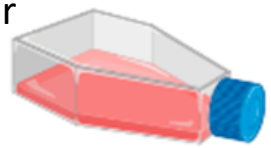
for individual cells (models) in two dimensions (2D) as well as for cells organized in three dimensions (3D)

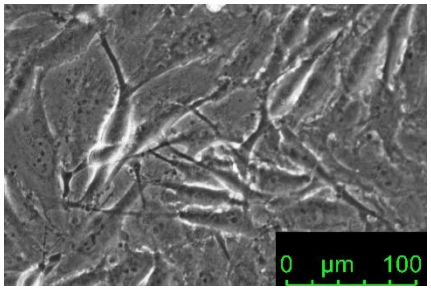
*Vaz-Salgado MA, *Recurrent glioblastoma: A review of the treatment options*. Cancers (Basel), 2023 26;15(17):4279.

Research problem

Cell cultures are widely used models *in vitro* (2D vs. 3D)

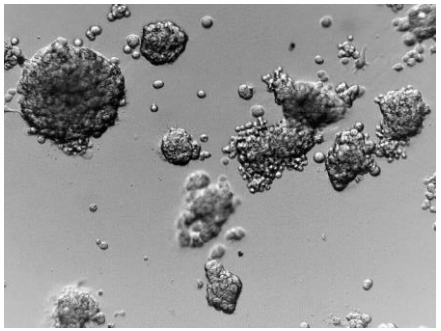
The cultures can be carried out under adherent conditions wherein the cells are attached to a glass or plastic dish





Traditional two-dimensional (2D) lacks the capabilities to replicate important features such as tumor microenvironment

Adherent, fast growing glioblastoma multiforme cells U251 cultured as monolayer in 2D conditions, own materials. Diffusing (infiltrate the surrounding brain tissue), irregular shape cells



These 2D model's disadvantages led to the creation of 3D models - better mimic the complex three-dimensional architecture, cellular interactions, drug efficacy and toxicity testing, offer an alternative model to animal studies

Pancreatic tumor cultured in 3D conditions, https://real-research.com/why-3dcc/

Objectives

The objectives of the project are:

- To quantitatively characterize the growth/morphology/proliferation/distribution/invasiveness/ of 2D and 3D glioma cell populations before and after different doses and modalities of irradiation (PBT), then try to correlate the response to irradiation with the biomarkers and the physical characteristics;

- **To build a mathematical model that could describe the evolution of two and three-dimensional tumor cell populations (mostly for childhood GBM) under the applied type of irradiation**;

- To study whether our model can be predictive of the response of irradiation in the 3D situation, based only on the biomarkers and the 2D response.

Calendar

2024: Focus on experiments in 2D and 3D;

2025: End of the experiments and modeling part;

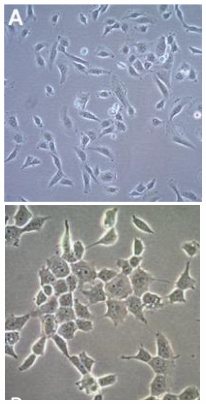
2026: Article preparation and publishing.

Work plan – Radiobiology 2024

Radiobiological part (Poland):

-Selected CNS cell lines of various degrees of malignancy

Ordered in January 2024, received in July, (thawing, passaging, freezing) July-September



Ordered in April 2024…

Additional fibroblast cell line was derived

Cell lines		Grade	Cells provider
SF188 Human Glioblastoma Cell Line (Pediatric model)	Orginating from a temporal lobe tumor of an 8-year-old male patient	GIV	MiliporeCor. (SigmaAldrich)/ Cat. # SCC282
HOG Human Oligodendroglioma	Surgically removed human oligodendroglioma	GI/GII	MiliporeCor. (SigmaAldrich)/ Cat. # SCC282
Primary Dermal Fibroblast (HDFa), PCS-201-012™	Normal skin cells	-	ATCC®

-3D pediatric glioma model will be jointly developed by IFJ PAN and a commercial partner in project entitled **„The importance of proton radiotherapy in the treatment of patients with central nervous system tumors”,** program “Science for Society”, NdS-II/SP/0295/2023/01, granted by the Polish Ministry of Education and Science. **Collaboration with IJCLab included, contract with a Real Research is under preparation.**

SCIENCE FOR THE SOCIETY

REAL RESEARCH

Work plan – Radiobiology 2025

Radiobiological part (Poland) :

- The cells 2D and 3D will be irradiated in the range of: 0.0-8.0 Gy (doses 2 Gy, 4 Gy, 8 Gy) with protons. PBT will be carried out at 4 positions of the broadened Bragg peak

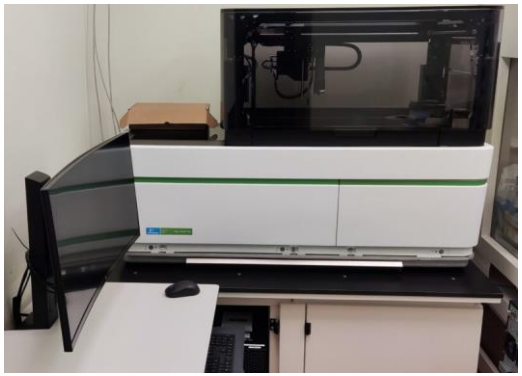
- The cells reaction will be observed using different methods…

the imaging to build a mathematical model that could describe the evolution of two and three-dimensional tumor cell populations under the applied type of irradiation.

Work plan – Radiobiology 2024

First optimizing experiments for imaging

The high throughput automated confocal microscope (Opera Phenix Plus system), purchased for CEPHARES (Center for Development of New Pharmacotherapies of Central Nervous System Disorders) established in
The Maj Institute of Pharmacology (IP PAN) in Krakow will be used for 3D imaging –
meetings with CEPHARES PM and team (Grzegorz Kreiner, PhD., Assoc. Prof/Julita Wesołowska, PhD.)



CEPHARES

Maj Institute of Pharmacology
Polish Academy of Sciences

https://www.cephares.pl/en/news-en/opera-phenix-plus-2/

Work plan – Radiobiology 2024

First optimizing experiments for imaging

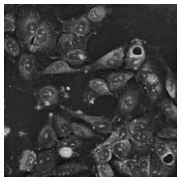
Modelling the effect of dose and cell density both in bright field and the red fluorescence

I. **The images/movies were captured by a scanning confocal microscope (Leica DMi8) at 10 × magnification, every 1,5 h till 72 h**
June 2024; Julita Wesołowska, PhD.; IF PAN (waiting for the results)

Seeding (250-7500 cells/well), different glioma cell types

II. A holotomographic 3D cell explorer (Nanolive, Tolochenaz, CH) microscope, allows to show changes with high-resolution (200 nm), in-situ, real-time occurring even for 12 hours by every 5 minutes
July 2024; Joanna Depciuch-Czarny, PhD. hab. Eng.; DFN (NZ32)

H4, 1000 cells



H4, 2000 cells

We should follow imaging longer than 12 h, counting better after fluorescent transfection

Work plan – Summary 2024

Project is carried out according with research plan and timetable

2D models and protocols are established

3D pediatric GBM model – contract is under preparation

Data for different methods are collected

First imaging experiments were performed…

Fluorescence videoimaging to visualize the nucleus ---- transfection protocoles using a red fluorescent protein specifically targeting the nuclear compartment for
SF188 and HOG must be tested and optimize – not all the cell lines can be transfected!

(cells and protocols ⟶ France, Nov 2024)

Work plan – Theoretical part

Theoretical part (France, 2025):

The different steps are planned:

- Data analysis from images in 2D: we will either count cells (if possible) or measure the confluence (the total area occupied by the cells) as a function of time under different modalities of irradiation. We will develop our codes or we will use existing Python functions;

- Data analysis from images in 3D: the radius of spheroids in 3D will be measured as a function of time, in the same conditions as in 2D;

- Development of a model and fitting of the biological data;

- Studying if it is possible to predict the efficacy of PRT.

Outcomes

Article in the high impact journal

2 and 3D glioma models and protocols (transfections)

A mathematical model that could describe the response of 2 and 3D cel population after protons

Open database and data exchange

New collaborations

Training the new generation of students at different university degree

Promotion *via* social media and movie (possible with selected company)

Budget

IFJ PAN

Type of expenses	Amount
Personnel	3600 EUR
Equipment	-
Consumables	3000 EUR
Travel & subsistence	1000 EUR
Total	7600 EUR

IFJ PAN: 3000 EUR will needed for consumables like cell culture media, laboratory plastics, and cellular and molecular tests (CellTiter-Blue® test, Ki-67 expression, X-Gal). We ask for 3600 EUR for team training and salaries. We are also asking for 1000 euros for traveling to France for presentations, consultations, and paperwork.

IJCLab

Type of expenses	Amount
Personnel	3600 EUR (6-month internship)
Equipment	-
Consumables	-
Travel & subsistence	1000 EUR
Total	4600 EUR

IJCLab: we are asking for an internship of 6 months for a master's student, in 2025. We also ask for 2000 for a good laptop. We are also asking for 1000 euros for missions.

Teams

IFJ PAN

First name / Family name	Function (Researcher, Engineer etc)	Role in the pre-project	% of participation
Justyna Miszczyk	Researcher CCB	Radiobiology experiments	30%
Anna Zając-Grabiec/ Beata Biesaga	Researchers CCB	Radiobiology experiments/ 2D/3D cells visualization	10%
Monika Krzyżowska	Technical person CCB	Laboratory procedures and cell culturing	10%
Joanna Depciuch-Czarny	Researcher DFN (NZ32)	2D/3D visualization of cells	10%

IJCLab

First name / Family name	Function (Researcher, Engineer etc)	Role in the pre-project	% of participation
Mathilde Badoual	Researcher	Mathematical modeling	30%
Stéphane Plaszczynski	Researcher	Data analysis, numerical simulations	10%

Filip Michałkiewicz

Dominik Wiśniewski

JAGIELLONIAN UNIVERSITY IN KRAKOW

Wrocław University of Science and Technology

THE HENRYK NIEWODNICZAŃSKI INSTITUTE OF NUCLEAR PHYSICS POLISH ACADEMY OF SCIENCES

Acknowledgements

Department for Functional Nanomaterials (NZ32)

Maj Institute of Pharmacology Polish Academy of Sciences

CEPHARES

REAL RESEARCH

Excited about our future endeavors !