

Breast cancer is the most frequently diagnosed cancer in women. Currently, this is the second most common cause of death for women, with an average of about 5,000 deaths in Poland. Treatment of breast cancer includes surgery, radiation therapy, chemotherapy, hormone therapy and targeted therapy. It is reasonable to examine what type of radiation should be used in radiation therapy to ensure that a sufficiently high dose of radiation is delivered to the cancerous area, while limiting exposure of healthy organs. For women with left-sided breast cancer, proton radiotherapy can reduce the risk of heart and lung complications due to the rapid drop in dose behind the irradiated area.

The main goal of the project is to investigate the differences in biological response within a tumour, proton irradiated (assuming a homogeneous dose of radiation) and to determine the relationship between biological response and molecular factors and tumour microenvironment for the group of patients treated for breast cancer.

Two hypotheses have been defined:

(1) The biological response within the tumour despite irradiation with a homogeneous dose of proton radiation shows a spread of more than 10% due to the overlap of radiation from protons with different energies - at different places in the Bragg curve.

(2) Molecular factors and tumour microenvironment have a significant impact on the tumour's biological response when proton radiation is exposed.

Cancer is a heterogeneous structure consisting of different cells that react individually to damaging factors, even if it has been recognised as the same subtype of breast cancer. Thus, the main goal of the project is to assess the biological *in vitro* response of breast cancer cells to irradiation using a proton beam of ionising radiation depending on individual molecular factors and tumour's microenvironment in selected patients.

The project will be carried out in cooperation with two partners: the National Center for Nuclear Research in Świerk and the United Institute for Nuclear Research in Dubna. Two research packages are planned (WP1 and WP2).

WP1. Differences in biological response in tumour volume due to proton irradiation from different parts of the Bragg curve. It includes the following tasks (a) design and implementation of a phantom simulating the human body; (b) measuring doses at selected points; (c) irradiation of established breast cancer cell lines at different locations along the proton / Bragg curve route and finally (4) a plan to find a correlation between the type and size of the biological response and the tumour of the protons due to the proton path (position in the Bragg curve).

WP2: Correlation of individual biological response with molecular factors and tumour microenvironment in breast cancer. Tumour / biopsy material will be collected from 600 patients treated for breast cancer over a 3-year period. The treatment tumour schedule will not be changed. The collected tissue / cellular material will be transferred to a biological laboratory to extract primary cell lines. tumour samples for which primary cell lines can be derived will be examined by histopathological methods to assess molecular factors and the tumour microenvironment. We plan to obtain at least 50 primary breast cancer lines from 600 samples. These lines will be irradiated with protons (doses 2, 4, 6 Gy). The radiobiological response will include the analysis of the cell death pathway, damages and DNA repair mechanisms by various methods.

The project will help to reduce the risk of local recurrence by eradicating in a better way persistent cancer cells after surgical tumour resection. Understanding the role of molecular factors and the tumour microenvironment - different for each patient - aims to identify possible subgroups with different biological responses to proton radiotherapy, which may in future enable patients to be stratified for appropriate radiotherapy modification for better treatment results.