To introduce a drug into clinical practice, one must complete several phases of time-consuming clinical studies. Before testing a molecule of interest in a clinic, preclinical examination is required by law. This procedure involves *in vivo* tests, i.e., experiments with animals. The studied substance's activity must be confirmed and toxicity tests positively completed in animals. It is worth mentioning, however, that only 1 of 10 compounds positively verified at the preclinical level passes clinical studies. In other words, 9 of 10 molecules are discarded due to very low or no awaited activity and unacceptable toxicity in humans. This situation led to the suffering and death of a huge amount of laboratory animals (well over 9 million animals comprising horses, dogs, cats, monkeys, and many other species were sacrificed in the European Union between 2015 and 2017).

A possible solution to this ethically ambiguous situation is the employment of 3D cellular cultures stressed by flow, i.e., using devices that simulate real organs. Such microfluidic devices, called Organ-on-a-Chip (OoC), may limit or even eliminate animals from the preclinical studies since OoCs possess most of the characteristics of a real organism because they incorporate important mass-transport limitations and cell–cell signaling pathways inherent to human physiology. Thus, the primary objective of the current application is to verify whether the OoC technology can substitute *in vivo* preclinical studies or at least limit the number of animals sacrificed in those experiments. It is worth mentioning in this context that in December 2022, the President of the USA signed the FDA Modernization Act 2.0 into law. This regulation refutes the Federal Food, Drug, and Cosmetics Act of 1938, which mandated animal testing for every new drug development protocol. While it does not ban the use of animals in scientific research, the law recognizes its limitations and empowers researchers to employ innovative non-animal methods. Recent advancements in science have begun to offer increasingly viable alternatives to animal testing, and the OoC technology along with 3D cell cultures as organoids or spheroids seems to be among the most promising alternative.

To achieve the planned objective, three scientific groups with different specialties were gathered to form a grant research team. Prof. Węgrzyn's group – Molecular Biology Group from the University of Gdańsk (UG) which possesses a long experience in doing in vivo experiments; Prof. Jastrzębska's group from the Warsaw University of Technology whose main interest lies in development and studies of microfluidic devices; and Prof. Rak's group – Biological Sensitizers Group from UG, which for many years works on design, chemical synthesis, radiation chemistry and in vitro studies of radiosensitizers for radiotherapy.

The research program comprises the following tasks: (i) designing and fabricating microfluidic systems for radiation studies on the efficiency of chosen radiosensitizers in spheroids, (ii) optimizing spheroid culturing in microfluidic systems as well as growing mouse xenografts, (iii) designing and testing chemoradiotherapy protocols in microfluidic systems and mouse xenografts, (iv) on-chip and off-chip testing of the efficiency of radiosensitizer-assisted radiotherapy, (v) *in vivo* and *ex vivo* testing of the efficiency of radiosensitizer-assisted radiotherapy in mouse xenografts.

The response to ionizing radiation in the presence of chosen radiosensitizers will be compared in spheroids grown in microfluidic devices and animal models of cancer in the form of xenografts. Cell lines corresponding to the most common cancer in humans treated with radiotherapy, breast and prostate tumors, will be used to form spheroids and xenografts. Four radiosensitizers will be investigated: 5-bromo-2'-deoxyuridine, which possesses strong radiosensitizing properties confirmed by in vitro experiments; cisplatin which besides anticancer activity against a broad spectrum of human cancers, sensitizes cells to X-rays; nimorazole a representative of oxygen mimetics that is currently used as a radiosensitizer against head and neck cancers in Denmark, and tirapazamine a drug specifically targeting hypoxic cells that was investigated in the clinic against head and neck cancers in combination with radiotherapy. All these chemicals are activated by electron attachment, which, as the hydroxyl radicals, is the most abundant product of water radiolysis. Under hypoxia, a trait of solid cancer, hydrated electrons may be utilized by radiosensitizers, leading to the formation of genotoxic radicals. Consequently, solvated electrons non-active toward DNA can be indirectly used to its damage. Hence, the execution of the proposed project should also enable a better understanding of the cellular mechanisms lying behind the action of sensitizers activated by electron attachment, which will allow for a rational design of new, more efficient radiosensitizers of this type in the future.

Because the OoC technology is free of ethical concerns and millions of animals are killed yearly worldwide in preclinical trials, it is difficult to overestimate the impact of positive verification of the hypothesis mentioned above for radiotherapy development.