Paediatric high-grade gliomas (pHGGs) that occur in the brainstem are one of the leading causes of cancer-induced mortality in children. A significant problem in the treatment of these tumours is the exclusion of surgery, as it would pose a great risk of disturbing the structures of the brainstem and potential loss of basic vital functions in patients. The main method of treatment is radiotherapy, i.e. precise ionizing radiation to the affected brain/tumour area, which damages the rapidly dividing cancer cells. However, radiotherapy only temporarily stops the disease, and in most cases, it recurs after a few months. Therefore, there is a great need to aid radiation therapy by developing drugs that would allow the complete elimination of cancer cells with minimal side effects for the patient.

Thanks to the latest technological advances emerging in the last decade, which provided the possibilities to study the genetic information at the resolution of single tumour cells, it was found that most cases of pHGG have a specific mutation in the genes encoding a protein called histone H3. Histone H3 is important in the normal functioning of our organisms because it is one of several proteins around which our DNA strand is wrapped in each cell nucleus. The additional molecules temporarily attached to histone H3 as a result of various signals transmitted in the cells (so-called epigenetic modifications of the histones) determine how tightly the DNA strand is packed and which genes are activated at any given time to enable specific cell functions. In the case of pHGG cells, it turned out that the mutation detected in H3 (called the H3K27M mutation) significantly disrupts the epigenetic modification of H3 and thus globally changes the packaging of DNA, which causes erroneous gene activation and influences tumour growth. Such childhood pHGGs with the H3K27M are currently classified as H3K27-altered diffusive midline gliomas (DMGs).

At the stage of diagnosis, DMGs are already very big, and have extensive regions with low oxygen levels, which are called hypoxia. Based on the research on other tumour types, we know that hypoxia has a huge impact on the epigenetic modification of histones and, ultimately, the packaging of DNA as well as the activation of individual genes. It is very important to understand the cellular responses to hypoxia in detail, as clinical data show that the greater the hypoxia of a tumour, the smaller are chances of the patient's survival. The presence of hypoxia is also of particular importance in tumours treated with radiotherapy, as the lack of oxygen significantly reduces the harmfulness of ionizing radiation for cancer cells, which overall reduces the effectiveness of the therapy. Importantly, the role of hypoxia in tumorigenesis and therapy response has not been extensively studied in DMGs.

In our project, we will focus on identifying the weak points in DMGs that depend on the presence of H3K27M oncohistone and are additionally important for the glioma cells in hypoxia. For this purpose, we will analyse the effect of hypoxia on epigenetic changes in histones, in DNA packaging and in the activation of gene expression in DMG cells with H3K27M. We expect that the results of our research may indicate new important ideas on how to kill DMG cells, and particularly how to target glioma cells in hypoxia which are resistant to radiotherapy. Understanding such differences will allow us to select and test the appropriate available tools/inhibitors/drugs that will increase the death rate of cells in hypoxia and thus potentially increase the effectiveness of radiotherapy. Using specialized equipment to create hypoxic conditions and irradiation unit, we will test such ideas first in killing glioma cells and the we will verify our funding in tumours in mice. Some of our ideas we will also test in biopsies from DMG patients.

In a separate aim, we will investigate the possibility of removing the mutant H3K27M protein from tumour cells. Our preliminary data show that using a group of drugs that increase the epigenetic modification of histones results in the temporary elimination of the mutant histone protein H3K27M from the cancer cells. In this project, we will examine in detail the removal mechanism for mutant H3K27M. First, we will test specific compounds that could block the function of potential enzymes that we have selected as candidates to "cut" mutant histone. In the next step, we will use a mass spectrometric method to identify proteins in the vicinity of H3K27M undergoing degradation. The reduction of H3K27M levels in cells as a result of epigenetic therapy is an original discovery and exploring the mechanism of this process may indicate new directions in the treatment of gliomas with the H3K27M mutation.