DNA damage and repair are a natural part of cell function. There are many types of damage, but the most dangerous type are double-stranded DNA breaks. Their accumulation is associated with ageing and leads to the development of cancer. We still do not fully understand the mechanisms of repair of this damage.

Many proteins are involved in the repair of double-stranded DNA breaks, whose functions are regulated by a number of post-translational modifications. **The aim of this project will be to investigate whether one of these modifications, ADP-ribosylation, is involved in the cellular response to DNA double-strand breaks**. To date, it has been established that ADP-ribosylation has a key role in the repair of single-stranded DNA breaks - a less severe but more common form of DNA helix breaks. The process of ADP-ribosylation can be inhibited through the use of inhibitors of PARP family proteins, which are responsible for its formation. Although these inhibitors have been used to treat breast cancers with non-functional repair of double-stranded DNA breaks, **it is still unclear whether ADP-ribosylation also arises in response to this type of severe damage. This issue is still a matter of debate among specialists.** Some studies to date suggest its involvement in double-strand break repair, but these data are ambiguous in their interpretation, as they are often based on artificial and simplified models that do not fully mimic the conditions in a living cell.

To test whether ADP-ribosylation is involved in DNA double-strand break repair, we will use **high-resolution microscopy methods** and the **CRISPR-Cas9** (molecular scissors) **system** to induce DNA damage in cells in a controlled and precise manner and follow its repair. Among other things, we will analyse the timing and location of ADP-ribosylation in relation to proteins that are typically involved in the repair of double-stranded DNA breaks. In addition, we will test whether ADP-ribosylation is a key factor for the recruitment of the mentioned repair proteins.

Our research will help to better understand the mechanisms of the cellular response to double-stranded DNA damage, which is crucial for understanding how cancers arise and develop. This knowledge may contribute to the development of new, more effective targeted therapies that will work precisely where DNA repair mechanisms are impaired. Understanding the role of ADP-ribosylation in the response to severe DNA damage may also help predict which patient groups will respond best to PARP protein inhibitor therapies or identify new therapeutic targets for the treatment of resistant cancers. In the long term, the results of these studies may influence the personalisation of cancer treatment and improve its efficacy.