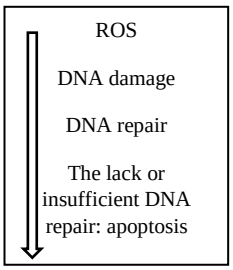
The main objective of this study is to evaluate the **safety level of chondrocytes obtained from stem cells (SCs) as a result of differentiation process.** The investigations will be focused on so far little-known mechanisms activated in response to damages of genetic material in stem-derived cells.

In cells treated with ionizing radiation (IR) and selected chemotherapeutics in accordcance with conventional therapy protocols the detailed analyses will be performed:



1) evaluation of level of reactive oxygen species that reflects the oxidative stress,
2) analysis of damage of genetic material formed throughout direct activity of ionizing radiation and cause by oxidative stress,
3) analysis of activated mechanisms taking part in repair of genetic material,
4) evaluation of level of programmed cell death (apoptosis) in the case of insufficient DNA repair
5) the comparison of cellular response between individual stem cells, chondrocyte-like cells derived from stem cells and mature chondrocytes.

The research material in the project will involve two pluripotent stem cell lines (enabling the differentiation into derivatives of three germ layer): human embyonic and induced pluripotent stem cells as well as mature adult chondrocytes. The differentiated process of stem cells towards chondrogenic lineage, will be caried out in accordance with the most efficient method earlier etablished by principal investigator.

The particular analyses will be conducted on the basis of the following methodology:
Ad.1) the flow cytometry measurement with the usage of green fluorescence dye in the presence of reactive oxygen species,
Ad.2) the flow cytometriy measurement of H2AX histone phosphorylation allowing to visualization of DNA damages,
Ad. 3) the analysis of activated DNA repair mechanisms both on the gene (segment DNA that encodes protein products) and protein level.
Ad. 4) the flow cytometry measurement with the usage of characteristic marker of cell death (apotosis)-labelled anexin V and propidium iodide.

Herein, we assume that differentiation process leading to obtaining of specialized cells cells have a significant effect on mechanisms activated in cells exposed to anticancer agents. Moreover, these mechanisms significantly differ from processess induced in stem cells that give rise to stem-derived cells and fully mature cells obtained from patients.

We believe that it has a great impact on genetic stability of cells derived from stem cells, what has a direct reflection in safety of application of these cells in clinical practice.

Nowadays, anticancer therapies are mainly based on agents causing the damage of genetic material and consequently death of cancer cells: ionizing radiation and chemotherapeutics. However, the influence of ionizing radiation and chemotherapeutics can be observed in all organism's cells. In treated cells, the damages of genetic material very often has a result in the rising of mutations. They cause the induction of secondary tumors. Over last years increasing emphasis on **protection of healthy tissues and critical organs during radiotherapy is observed**. Acute radiation syndrome (ARS) is an undesirable effect that significantly diminishes effectiveness of treament because of negative influence on patient's quality of life. **The proposed investigations perfectly reflect directions determined for last years in radiotherapy**.

The regenerative medicine is a rapidly growing, field within stem cells begin to play crucial role. Currently, pluripotent stem cells that have the capacity to self-renewal and differentiation into specialized cells. Therefore, they holds promise for combating many currently unresponsive diseases. The response of stem-derived cells to treatment with ionizing radiation and chemotherapeutics is a questionable issue, particularly with regard to rise of cancer morbidity in patients over 50 years old. However, **there is still little known about activated mechanisms of cells differentiated from stem cells in response to treatment with ionizing radiation and/or chemotherapeutics.** It is worth mentioning that un- and differentiated cells possess different radiosensitivity. **It is also unknown, whether their DNA damage response mechanisms of stem-derived cells are more similar to those from "parental" stem cells or perhaps those from completely differentiated cells.** Summing up, the results of planned experiments will contribute to the development of regenerative medicine and radiobiology as well as establishment of reliable, effective and first of all **safe hiPS cells-based** approach for clinical cartilage repair and **treatment**.