Mechanism of potentiating effect of low-dose fractionated radiation (LDFRT) on paclitaxel and carboplatin in head and neck cancer cells. The role of nucleoshuttling of the ATM protein.

The basis of conventionally fractionated radiotherapy is dose per fraction of 2 Gy given once a day for five days a week continuing for 3-7 weeks. Until recently, doses per fraction <1 Gy were not used in radiotherapy of malignant tumours because they were presumed to be ineffective. The clinical significance of low doses changed when the phenomenon of low-dose hyper-radiosensitivity (HRS) was discovered. The phenomenon of HRS is an effect in which cells die from excessive sensitivity to low doses (< 0.5 Gy) of ionizing radiation, but become more resistant (induced radioresistance, IRR) to larger doses. Compared to conventional radiation doses (2 Gy), low doses are thought to be more effective (per unit dose) because they do not activate cellular repair mechanisms, resulting in all damaged cells being killed. Since HRS was reported, there has been a considerable interest in exploiting HRS in radiotherapy of cancer patients. One possibility to benefit in the clinic from HRS effect is by using low-dose fractionated radiation (LDFRT) as an enhancer of systemic chemotherapy. The rationale for this treatment strategy came from *in vitro* studies that reported that cancer cells are more sensitive to taxanes and cisplatin combined with low-dose fractionated radiation (4 x 0.5 Gy) than with single dose of 2 Gy. To date, eight phase II clinical trials have been conducted to assess the benefit of combining LDFRT with drugs such as taxanes and cisplatin. Although, all these trials confirmed effectiveness and acceptable toxicity of such treatment in patients with locally advanced head and neck, breast, lung, uterine cervix cancer or brain tumours, randomized clinical trials have not been initiated. The reason might be the fact that the exact molecular mechanism underlying the process of chemopotentiation by LDFRT remains unclear and the data come from one research group only.

The aim of the proposed project is to verify the hypothesis that, unlike a single dose of 2 Gy, low-dose fractionated radiation (4x0.5 Gy) does not activate ATM kinase (resulting in no DNA repair) and pro-survival pathway (resulting in cell killing). Recently, a new theory has been proposed, according to which the response to low and high doses of ionizing radiation depends on the transport of the ATM protein (responsible for recognition and repair of DNA damage) from the cytoplasm to the nucleus (RIANS - radiation-induced ATM nucleoshuttling). Therefore, the goal of the proposed project is also to determine (for the first time) the role of radiation-induced ATM nucleoshuttling in the chemopotentiation by LDFRT.

In this study, the potentiating effects of LDFRT (4x0.5 Gy) *versus* single dose radiation (2 Gy) on paclitaxel and carboplatin will be compared in four human head and neck cancer cell lines. The following methods will be used: flow cytometry-based clonogenic assay (to assess cell survival), fluorescence pATM and γH2AX foci assays (to determine kinetics of recognition and repair of DNA damage), TUNEL test (to measure induction of apoptosis) and western blot (to asses expression of proteins associated with the apoptosis pathway).

As a result of the project, new knowledge about the molecular mechanism underlying the process of chemopotentiation by LDFRT will be added. This knowledge, in turn, should contribute to further exploit low-dose fractionated radiation in the treatment of cancer.