A key step in cancer development is obtaining by cells the ability to continue unlimited cells divisions, mainly due to the restoration of telomerase activity. It was shown that telomerase is active in about 90% of cancer cells, however its activity is not observed in most of somatic cells. Due to the multidimensionality of carcinogenesis process and due to participation of many factors in control of tumor growth, the possibility of using shRNA against telomerase (as one of these factors) will be evaluated. Combination of that approach with administration of cytostatics and/or radiation therapy may lead to reducing of drugs doses and radiation necessary to induce cell death. The strategy of effect on cancer cells including telomerase regulation is currently widely used (antisense nucleotides, ribozymes, vitamin D, G-quadruplex stabilizers, adenoviral vectors). Many studies described regulation of telomerase were published, but this process still remains unclear. The varied mechanisms of cell death, including autophagy, mitotic catastrophe and necrosis, have many common areas in the context of anticancer therapy. Some of the connections are difficult to identify due to the complexity of these processes, as well as the possibility of existence an independent of telomerase an Alternative Telomere Lenghtening mechanism (ALT). The consequence is difficulty in evaluating cause-and-effect relationships and further opportunities to use this knowledge against cancer cells.  is difficulty in evaluating cause-and-effect relationships and further opportunities to use this knowledge against cancer cells. Understanding this mechanism will give the base to application of a highly specific therapy based on RNA interference. It was shown that up-regulation of telomerase expression in tumor cells correlates with higher drug resistance, hence in the project will be examined the antitumor efficacy of the RNA interference concomitant with chemo- and radiotherapy.

Telomerase complex is consist of two main subunits: hTERT – reverse transcriptase activity, and hTR – template for telomere synthesis. Furthermore, in order to function properly, requires the presence of shelterin complex proteins, and among others, hsp90 or dyskerin. In the studies performed so far it was demonstrated that RNAi targeting telomerase expression inhibits cell proliferation and increases apoptosis in tumor cells.

Only few reports reveal a possible application of TERT silencing by siRNA in head and neck oncology. However, the knowledge concerning lentiviral stable telomerase knockdown of head and neck cancer cells is very unclear. Proposed approach would allow to provide a long term effects knowledge after efficient telomerase knockdown. In particular the interesting phenomenon is development the resistance to chemotherapy by cancer cells. As demonstrated, the increase in telomerase expression and activity correlates with cancer cells resistance to chemotherapy. However, in cells with downregulated TERT expression increased susceptibility to cisplatin and paclitaxel was indicated. Nevertheless, recent studies do not explain the molecular effect of cytostatics, particularly after telomerase silencing. It is suggested that the cytostatics effect on the activation of apoptosis in cancer cells, but the effect of autophagy is also investigated extensively.

We will also examine the impact of ionizing radiation on cancer cells with telomerase depletion. So far, studies with usage of adenovirus OBP-301 indicate an increased sensitivity to ionizing radiation by inhibiting of DNA damage repair mechanisms. According to the authors of the project, increased radiosensitivity is associated with higher production of double-strand DNA breaks as a result of a genome instability after a telomerase silencing. Further studies will explain the complexity of cell death mechanism after telomerase knockdown concomitantly with chemo- and radiotherapy. Such an approach has not been used in research on head and neck cancer. Thus, the complexity of carcinogenesis, telomerase expression/activity regulation but also senescence response requires further detailed studies performed at various levels which constitutes the subject of the project.

In this project we intend to analyze the efficiency and potency of lentiviral RNA interference system, directed against the TERT telomerase subunit in order to eliminate cancer cells. The unique properties of RNAi give hope for specific and efficient technology development, particularly when combined with chemo- and radiotherapy. Due to complexity of telomerase regulation and its contribution to carcinogenesis or senescence it is hard to say, that stopping the telomerase might be sufficient to induce cell death. Moreover, knowledge is too unclear to know if this cell death mechanism is apoptosis, autophagy, or includes both mechanisms. Thus, by identification of telomerase inhibition results we might develop a method of individual cell death induction and also develop an universal strategy against all the cancers revealing telomerase expression and activity.

Results of this project will be presented at international conferences as both oral and poster presentations. Due to the innovative nature of the project the results will be published in international journals with high impact factor. That allow to extension of global knowledge about the regulation of telomerase, anticancer activity of telomerase inhibition, its interaction with radiation therapy and chemotherapeutic agents commonly used in the treatment of patients with head and neck region squamous cell carcinoma, and the mechanisms of cell death.