Osteosarcoma is the most common type of bone cancer, which mainly affects children and young adults. Relatively low prevalence of the disease in humans and difficulty in running clinical trials in children, resulted in no significant therapeutic advancement over the last 35 years. Thus, there is a need for novel treatment approaches, which are currently hampered by lack of spontaneous models of the disease. Recently, cancer treatment approach was revolutionized by implementation of PD-1/PD-L1 inhibitors. PD-1 (programmed death receptor 1) is expressed by several types of immune cells. Upon activation by its ligand PD-L1 (programmed death ligand 1), PD-1 acts as a brake to the immune system and prevents excessive immune response. While it protects us from autoimmune response, cancer cells express PD-L1 at high levels to escape elimination by the immune system. PD-1 immune checkpoint blockade selectively inhibits PD-1 signalling in immune cells, restoring their cytotoxic function and ability to eliminate cancer cells. Despite strong expression of PD-L1, immune checkpoint blockade has failed to improve survival of patients with osteosarcoma. Our understanding of currently available immunotherapies is based on their mechanism of targeting the PD-1/PD-L1 axis in the immune cells.

Even though PD-1/PD-L1 blocking antibodies have recently shown remarkable success rates in treating some cancer types, there is still a group of patients who do not respond to therapy or even rapidly deteriorate after immunotherapy implementation. Although several hypotheses were proposed, this phenomenon remains unsolved. Surprisingly, recent studies shown that cancer cells not only express PD-L1 but also PD-1 and proposed the tumour intrinsic PD-1 expression as a cause for cancer patient relapse after immune checkpoint blockade, but the scarce reports on this type of signalling provided contradictory results. Strikingly, our recent results revealed that cancer PD-1 intrinsic signalling in human osteosarcoma may be of great importance in cancer cells invasion and metastasis, suggesting that it may be a mechanism of osteosarcoma resistance to immunotherapy.

However, to overcome the limited number of osteosarcoma patients, there is a need to find a spontaneous model of osteosarcoma in order to study the disease. Interestingly, dogs develop osteosarcoma 10 times more frequently compared to humans. Moreover, many reports already demonstrated pathological, morphological and genetic similarities of the disease in both species. Also, dogs have a fully functional immune system, live in a similar environment to humans and any research progress will be beneficial for both species, what makes them a perfect model to study osteosarcoma.

The aim of this project is to use canine osteosarcoma cell lines to determine the role of PD-1 intrinsic signalling in canine osteosarcoma and to establish how closely it resembles human disease. To accomplish our goal, we plan to implement liquid chromatography – mass spectrometry technique to analyse changes in global proteome induced by PD-1 expression and carefully compare the data between species. Moreover, we will conduct additional studies to determine how PD-1 expression by both human and canine osteosarcoma cells correlates to cancer cell invasion and metastasis *in vivo*. Additionally, we will implement PD-1 blocking antibodies to mimic a potential response of cancer cells to immune checkpoint blockade and compare the result between species.

Overall, our studies will support basic research on PD-1 intrinsic mechanism in cancer, which is crucial to understand in order to ensure safety of immunotherapy in tumours expressing PD-1 receptor. Also, revealing resistance mechanisms to immunotherapy may help to identify new druggable targets in the future in both species.