# Abstract

Millions of people worldwide die of cancer every year. In the last decade, immunotherapy offered new treatment options achieving long-lasting remissions in a number of patients. Several new immunotherapy-based drugs have been approved by Food and Drug Administration. However, majority of patients either do not respond or soon relapse. Combination of therapies as well as exploring new immune checkpoints seems promising.

This thesis focuses on the new immunotherapeutic target CD73. CD73 is membrane ectonucleotidase, widely expressed on the regulatory leukocytes and on cancer cells. The enzymatically active CD73 contributes to the tumour microenvironment by production of immunosuppressive adenosine. This novel immune checkpoint is being intensively studied. This thesis aims on development of new approaches for targeting and inhibition of CD73.

Soluble recombinant CD73 (rhCD73) was prepared in mammalian expression system and transfectants stably expressing membrane-bound CD73 were prepared as well. Inhibitors necessary for both of my goals have been designed based on published inhibitor of CD73.

Development and evaluation of novel antibody mimetic for CD73 characterisation was done. The so-called iBody, HPMA polymer conjugate decorated with CD73 inhibitor for targeting, fluorophore for visualisation and biotin for immobilisation of the polymer conjugate, was developed and validated in different biochemical methods as well as in cell assays.

Furthermore, the development of high-throughput assay (HTS) is described for CD73 inhibitor screening based on DIANA method (DNA-linked Inhibitor Antibody Assay). The conditions of the method were optimised and first inhibitor testing has been performed.

The findings in this thesis allow us to further develop nanochemical tools that are able to target and modulate immunosuppressive components of tumour microenvironment.



Key words: receptor, ligand, recombinant protein, polymer conjugate, HPMA, fluorescence, immunotherapy, CD73, DIANA