Cancer Immunotherapy exploiting engineered antibody fragments against prostate-specific membrane antigen

Abstract

Prostate cancer (PCa) remains a leading cause of male cancer-related mortality, necessitating thus the development of novel therapeutic approaches as conventional treatments have limited efficacy. Prostate-specific membrane antigen (PSMA) is an established biomarker for both imaging and therapy of PCa, as it is highly upregulated in neoplastic PCa tissues and metastatic castration-resistant prostate cancer. Consequently, immunological targeting of PSMA has gained significant attention as a therapeutic platform for the management of the disease.

The thesis is focused on engineering of antibody fragments and fusion proteins derived from the high affinity anti-PSMA 5D3 monoclonal antibody that can be used as immune cell engagers to target and eliminate PSMA-positive cells. To this end, we engineered 5D3 single chain variable fragments (scFv) that were subsequently fused to anti-CD3 scFv and CP33 sequences, creating thus immune cell engagers targeting T-cells (BiTE) and monocytes (5D3-CP33), respectively. The engagers were expressed in insect cells, purified to homogeneity and their biophysical and functional characteristics evaluated using size exclusion chromatography, differential scanning fluorimetry, ELISA and flow cytometry. Ensuing cell-based assays revealed that both BiTE and 5D3-CP33 can eliminate PSMA-positive tumor targets at sub-nanomolar concentrations via cell-type specific cytotoxic pathways. Our data shows that the engagers can be used as platforms for the development of next generation tools for PCa immunotherapy.