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ENGLISH ABSTRACT

A

G protein-coupled receptors (GPCRs) are an important class of transmembrane proteins involved in many biological functions and linked to multiple diseases. For this reason, these transmembrane receptors are considered as a prominent drug target. Approximately 35% of all marketed drugs are directed at GPCRs. The chemokine-chemokine receptor network consists of approximately 24 receptors and 44 chemokine ligands known to date. Chemokine receptors belong to the superfamily of GPCRs and regulate the immune system homeostasis. Typical chemokine receptors act via an activation of G proteins, whereas atypical chemokine receptors (ACKR) do not and activate only β -arrestin proteins instead. CXCR4 and ACKR3 are two types of chemokine receptors that share a common ligand, CXCL12, and regulate a broad range of biological processes. Dysregulation of CXCR4 and ACKR3 function is implicated in many severe and life-threatening disorders, such as cancer, autoimmunity, and HIV progression. In contrast to the superfamily of GPCRs, only a few therapeutics aimed at targeting chemokine receptors are currently approved for clinical use. Monoclonal antibodies and antibody-based molecules represent a potential new class of therapeutics for targeting GPCRs and chemokine receptors in particular. An interesting class of antibody fragments consist of variable domains derived from heavy-chain antibodies naturally occurring in camelids, also known as Nanobodies[®]. Their unique structural features facilitate the development of specific and potent GPCR binding and modulating molecules. Currently, there are no antibodies or nanobodies against CXCR4 or ACKR3 approved for clinical use.

In this thesis, we developed a novel panel of specific and potent nanobodies targeting the extracellular sites of human and murine CXCR4 and ACKR3. These nanobodies were used to give more insight into the membrane organization of CXCR4 and ACKR3 oligomers, as well as to investigate and expand the therapeutic potential of targeting these receptors in cancer. In **Chapter 1**, a broad introduction into the chemokine-chemokine receptor network and nanobodies is given. The current landscape of CXCR4- and ACKR3-targeting monoclonal antibodies, nanobodies and other fragments, their beneficial properties and structural analysis of the binding epitopes are discussed in **Chapter 2**. An efficient methodology for rapid isolation of target-specific nanobodies is described in **Chapter 3**. In **Chapter 4**, novel ligand-blocking CXCR4 targeting nanobodies are characterized for their potencies in blocking CXCR4 signaling and HIV co-receptor activity. Through site-directed fluorescent labelling of the nanobodies, novel versatile and sensitive TR-FRET compatible detection tools have been developed for probing CXCR4 and ACKR3 oligomers in **Chapter 5**. These innovative tools allowed for the first time the detection of endogenous CXCR4 oligomers in cancer cells. In **Chapter 6**, the identified CXCR4 nanobodies were modified as bivalent constructs via a fusion to a functional IgG Fc domain, designated as nanobody-Fc. These novel nanobody-Fc constructs demonstrated superior potencies in inhibiting CXCR4 function and displayed an additional mode of action by killing cancer cells via antibody-mediated effector functions. Furthermore, in **Chapter 7**, the therapeutic potential of similar antagonistic Nb-Fc constructs against murine CXCR4 and ACKR3 were evaluated for their efficacy in an *in vivo* mouse syngeneic colon carcinoma model. Finally, in **Chapter 8** the key findings of this thesis and future perspectives are summarized and discussed.

Overall, this thesis demonstrates the utility of nanobodies and their derivatives for diagnostic and therapeutic applications aimed at the GPCRs CXCR4 and ACKR3. The versatility of nanobodies can be widely used to investigate fundamental research questions regarding functional modulation and structural organization of GPCRs.