Thesis outline

Treatment strategies to eliminate tumours have been changing rapidly in the past decade. For many cancer types the principal treatment is surgical removal of the solid primary tumours. Additionally, patients are often treated with chemotherapy and/or radiotherapy[1-3]. However, in the last decade also therapies that stimulate anti-tumour immune responses have gained a prominent place in the arsenal of cancer treatments.

It has become clear that a tumour does not only consist of malignant cells. Due to an increasing demand for nutrients and oxygen, which are necessary for expansion, tumours interact with their surrounding tissues. This induces infiltration of various stromal cell types such as fibroblasts and endothelial cells, which are necessary for vascularization of the tumour[4,5]. Additionally, many immune cells can be found in the stroma of the tumour. The great variety of cells infiltrating the tumour includes a high amount of immune regulatory cells such as myeloid derived suppressor cells (MDSC), macrophages, neutrophils, regulatory T cells (Treg) and T helper (Th) cells[6-9]. These immune cells can be attracted to the tumour by cytokines and chemokines that are secreted by the tumour itself. Several of these cells can have either a beneficial or a disadvantageous role in regulating the tumour micro-milieu. MDSCs and Tregs are suppressors of immune effector cells such as macrophages, cytotoxic CD8^{+} T-lymphocytes, and natural killer (NK) cells. Macrophages can have cytotoxic abilities, but have also been identified as one of the most dominant immune suppressors cells in the tumour microenvironment[10-13]. Additionally, they can promote tumour growth by secretion of angiogenic factors, proteases, growth factors, and other cytokines and chemokines.

Therefore, rapidly emerging strategies aim to target immune cells, preferentially within the tumour[6,14,15]. The majority of the studies try to target cells of the adaptive immune system, either by directly activating T cells or alternatively by targeting dendritic cells (DCs) via vaccination strategies to induce tumour specific adaptive immune responses[16-18].

However, also innate immune cells, such as NK cells, macrophages and neutrophils have potent tumouricidal activity, especially when tumour cells are opsonized with specific antibodies[19,20]. Particularly macrophages are good candidates for new therapeutic strategies, as they often represent a vast majority in the tumour microenvironment. Therefore, in this thesis I focus on the activation of effector cells via monoclonal antibodies (mAbs), either to target solid tumours or for the removal of circulating tumour cells (CTC).

Monoclonal antibodies have been used in cancer therapies for almost two decades with rituximab being the first approved antibody[14,21]. Rituximab is a chimeric immunoglobulin G1 (IgG1) targeting CD20 expressed on B-cell lymphoma[22]. This antibody has been shown to activate the complement cascade and immune effector cells that express IgG Fc receptors (Fcγ receptors). In ***chapter 1*** I discuss the role of myeloid cells, which are the primary Fcγ receptor expressing cells, during monoclonal antibody therapy with the focus on macrophages and neutrophils[20]. Moreover, I compare techniques that are used to investigate the role of macrophages during antibody therapy.

Using a mouse B16F10-gp75 metastasis model we investigated if we could improve tumour cell clearance by modifying tumour targeting TA99 antibodies. We increased the binding capacity of humanized TA99 IgG1 by producing them without core-fucose

on the glycan in the Fc domain. As a result, human macrophages and NK cells showed improved tumour cell killing *in vitro* (**chapter 2**). More importantly, in a mouse peritoneal metastasis model the afucosylated TA99 hIgG1 was better in preventing metastasis outgrowth compared to the non-modified TA99 hIgG1. Therapeutic efficacy of antibody therapy was dependent on mouse FcγRIV, which is the homologues of human FcγRIIIa.

Additionally, we generated a TA99 mAb with a human IgG3 Fc domain. IgG3 has been demonstrated to induce better killing of pathogens compared to IgG1, and is better in activating the complement pathway. However, hIgG3 has a shorter half-life in humans and mice because IgG1 is preferentially recycled by cells expressing the neonatal Fc receptor (FcRn). Therefore, we mutated one amino acid in the IgG3 Fc domain so that the binding site for the FcRn is similar to the IgG1 (**chapter 3**). Nonetheless, we did not observe any improved tumour clearance *in vitro* and *in vivo*, suggesting that IgG3 may not be a suitable antibody isotype for anti-cancer therapeutic antibodies.

Our group as well as others demonstrated that mAb therapy is not very effective to target solid tumours, including metastases[23–26]. In the studies described in this thesis I used *in vitro* and *in vivo* assays with tumour cells as single cells in co-cultures, which mimics tumour cells released from the primary tumour or CTCs.

In these conditions mAb therapy proved very effective, post-operative mAb therapy eliminated CTCs and prevented metastasis formation after surgery in animal models[27]. Therefore, we studied whether we could use mAbs to eliminate circulating tumour cells around surgical removal of the primary tumour and, as a result, prevent metastasis formation. We therefore investigated if anti-epidermal growth factor receptor (EGFR) mAbs are suitable for pre-operative antibody therapy of colorectal cancer patients (**chapter 4**). Currently – when they have advanced late stage disease with colorectal metastases – patients can be treated with cetuximab or panitumumab, which are EGFR antagonizing mAbs of the human IgG1 or IgG2 subclass. We hypothesised that CTCs may be cleared by effector cells such as liver macrophages by treating patients with anti-EGFR mAbs shortly before surgical removal of the primary tumour, which may prevent the development of metastases. In **chapter 4** we investigated the potential of the anti-EGFR antibodies cetuximab, panitumumab and zalutumumab for this purpose.

In solid tumours macrophages can be present in high amounts where they have a dominant role in the micro-milieu. They are potent regulators of a microenvironment in both inflammation as well as wound healing and immunosuppression. As a consequence, tumour associated macrophages have a big impact on the development of the tumour by suppressing or supporting growth, which I review in **chapter 5**[28]. We also investigated why macrophages may have these different roles within the tumour microenvironment. All cells secrete mediators, such as chemokines and cytokines, to communicate with their environment. Therefore, we compared the secreted proteins of cell lines that originated from mamma carcinoma, where high macrophage presence is associated with a worse prognosis, versus colorectal carcinoma in which high macrophage infiltration correlates with a good prognosis (**chapter 6**)[29]. We identified several proteins that might be involved in manipulation of monocyte/macrophage polarisation resulting in an improved or worsened prognosis. The most promising candidate was versican. By knocking down versican in colorectal carcinoma cell lines we managed to change monocyte maturation into a less inflammatory state. This suggested that versican expression has a positive effect on macrophage polarisation and patient prognosis. Two other candidates, Fam3C and Fat1, did not show a significant effect on monocyte maturation (**chapter 7**). Finally, in **chapter**

7 I summarize the potential applications of my findings in cancer treatments and propose novel combination therapies to fully exploit the potential of macrophages as effector cells in cancer.

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