Immunomodulatory Therapy of Solid Tumors

With a Focus on Monoclonal Antibodies

LINDA SANDIN
Cancer, historically considered a genetic disease, is currently acknowledged to affect the whole body. Our immune system is one key player that can elicit a response against malignant cells but can also promote tumorigenesis. Tumors avoid immune recognition by creating a suppressive microenvironment and inducing tolerance. T-cells are regarded a major effector cell type in tumor immunotherapy. An important ”switch” needed for T-cell activation involves so-called costimulatory and coinhibitory receptors. In this thesis, experimental tumor models were used to investigate the potential of immunomodulatory antibodies to stimulate immune cells and subsequently eliminate tumors.

First, systemic antibody blockade of two negative checkpoint regulators (CTLA-4 and PD-1) present on T-cells was evaluated in combination with local CpG therapy or standard BCG treatment. Indeed, this combinatorial therapy with CpG augmented anti-tumor effects with increased levels of tumor-directed T-cells and reduced tumor-infiltrating Tregs.

Secondly, as these immunomodulatory antibodies elicit severe side effects in patients, a local low-dose delivery regimen was explored as an alternative to systemic bolus treatment. Our results demonstrated that an approximately seven times lower dose of aCTLA-4, compared to systemic delivery, could eradicate both primary and distant tumors.

CD40-expressing APCs are another potential target in antibody-mediated cancer therapy. CD40-stimulated dendritic cells (DCs) have the capability to activate tumor-directed T-cells to kill tumor cells. We next sought to investigate agonistic CD40 antibody efficacy and in vivo biodistribution when delivered locally compared to the equivalent systemic dose. Anti-tumor effects were dependent on CD8+ T-cells, host CD40 expression and the presence of tumor antigen at the injection site. CD40 antibodies were cleared from the circulation and accumulated in lymphoid organs, where, upon repeated aCD40 dosing, target APC populations increased in numbers and upregulated their surface CD40 expression.

Lastly, CD40 agonist antibodies were mixed with nanoparticles to enhance their stimulatory properties. B-cells demonstrated increased proliferative capacity and DCs became more activated when exposed to the cocktail. Further, this combination reduced serum levels of pro-inflammatory cytokines compared to plain antibodies.

The results herein advocate further exploratory studies of the delivery of monoclonal antibodies at the tumor site in order to improve anti-tumor effects and reduce toxicity.

Keywords: in situ checkpoint blockade, antibody-mediated tumor immunotherapy, CTLA-4, CD40, monoclonal antibodies, experimental animal model, Fc gamma receptor

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"Accept it and move on"

Till min familj
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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<tr>
<td>ACT</td>
<td>Adoptive cell transfer</td>
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<tr>
<td>ADCC</td>
<td>Antibody-dependent cell-mediated cytotoxicity</td>
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<td>ADP</td>
<td>Antibody-dependent phagocytosis</td>
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<tr>
<td>BCG</td>
<td>Bacillus Calmette-Guérin</td>
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<tr>
<td>BiTE</td>
<td>Bispecific T-cell engager</td>
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<tr>
<td>CD</td>
<td>Cluster of differentiation</td>
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<tr>
<td>CDC</td>
<td>Complement-dependent cytotoxicity</td>
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<tr>
<td>CpG</td>
<td>Cytosine-phosphate-guanosine</td>
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<tr>
<td>CTL</td>
<td>Cytotoxic T-lymphocyte</td>
</tr>
<tr>
<td>CTLA-4</td>
<td>Cytotoxic T-lymphocyte antigen-4</td>
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<tr>
<td>DAMP</td>
<td>Danger-associated molecular pattern</td>
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<td>DC</td>
<td>Dendritic cell</td>
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<tr>
<td>DLBCL</td>
<td>Diffuse large B-cell lymphoma</td>
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<tr>
<td>ER</td>
<td>Endoplasmic reticulum</td>
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<tr>
<td>FcγR</td>
<td>Fc gamma receptor</td>
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<tr>
<td>GM-CSF</td>
<td>Granulocyte macrophage colony-stimulating factor</td>
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<tr>
<td>Gvax</td>
<td>GM-CSF gene-transfected tumor cell vaccine</td>
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<tr>
<td>HSC</td>
<td>Hematopoietic stem cell</td>
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<tr>
<td>i.t.</td>
<td>Intratumoral</td>
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<tr>
<td>iDC</td>
<td>Immature DC</td>
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<tr>
<td>IDO</td>
<td>Indoleamine 2, 3-dioxygenase</td>
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<tr>
<td>IFN</td>
<td>Interferon</td>
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<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<tr>
<td>irAE</td>
<td>Immune-related adverse event</td>
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<tr>
<td>ITAM</td>
<td>Immunoreceptor tyrosine-based activation motif</td>
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<td>ITIM</td>
<td>Immunoreceptor tyrosine-based inhibition motif</td>
</tr>
<tr>
<td>KO</td>
<td>Knockout</td>
</tr>
<tr>
<td>LN</td>
<td>Lymph node</td>
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<tr>
<td>mAb</td>
<td>Monoclonal antibody</td>
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<tr>
<td>MB49</td>
<td>Mouse bladder-49</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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<tr>
<td>mDC</td>
<td>Mature DC</td>
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<tr>
<td>MDP</td>
<td>Macrophage DC progenitor</td>
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<td>MDSC</td>
<td>Myeloid-derived suppressor cell</td>
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<td>MHC</td>
<td>Major histocompatibility complex</td>
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<tr>
<td>NF-κB</td>
<td>Nuclear factor kappa B</td>
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<tr>
<td>NK-cell</td>
<td>Natural killer cell</td>
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<tr>
<td>PAMP</td>
<td>Pathogen-associated molecular pattern</td>
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<tr>
<td>PD-1</td>
<td>Programmed death-1</td>
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<tr>
<td>pDC</td>
<td>Plasmacytoid DC</td>
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<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
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<tr>
<td>TAA</td>
<td>Tumor-associated antigen</td>
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<tr>
<td>TAM</td>
<td>Tumor-associated macrophage</td>
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<tr>
<td>TCC</td>
<td>Transitional cell carcinoma</td>
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<tr>
<td>TCR</td>
<td>T-cell receptor</td>
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<tr>
<td>TGF</td>
<td>Transforming growth factor</td>
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<tr>
<td>Th-cell</td>
<td>Helper T-cell</td>
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<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
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<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
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<tr>
<td>TNFR</td>
<td>TNF receptor</td>
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<tr>
<td>TRAF</td>
<td>TNFR associated factor</td>
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<tr>
<td>Treg</td>
<td>Regulatory T-cell</td>
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<tr>
<td>UBC</td>
<td>Urinary bladder cancer</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
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1. Introduction

Until recently, cancer research has mainly focused on the cancer cell and on cancer as a genetic disease. A large body of evidence revealed that tumor formation is a multistep process with an accumulation of mutations which, in turn, drive the transformation of normal cells to malignant cells. In 2000, Hanahan and Weinberg proposed six characteristics (hallmarks) that could aid in the definition of a cancerous cell. These hallmarks include the cell’s acquired capability of sustaining proliferative signaling, avoiding anti-growth signals, evading apoptosis, enabling replicative immortality, inducing angiogenesis and tissue invasion and metastasis\(^1\).

A decade later, attention has increased on studying cancer as a systemic disease, leading to the understanding that cancer is not one disease but many. Therefore, to fully understand the nature of cancer, the focus has shifted from the cancer cell to the tumor microenvironment in which the immune system plays a very important part. To this end, a new picture of cancer is evolving, and in 2011 four new emerging hallmarks were proposed. Two of these highlight the dual interplay between cancer and the immune system including, first, the ability of avoiding immune-mediated destruction and subsequently elimination, and secondly, the induction of chronic inflammation that promotes rather than eliminates tumors\(^2\).

The work in this thesis builds on these last two hallmarks of cancer with costimulatory and coinhibitory receptors evolving as an important checkpoint in the balance between immune tolerance and immune activation. By using monoclonal antibodies (mAbs) which block or stimulate these receptors on immune cells, treatment will not be restricted to a specific tumor type and not solely depend on a specific tumor antigen. In this thesis, most work was done in a tumor model of bladder cancer. However, this therapy should probably be applicable to other cancer types as well. Antibodies in cancer therapy are a versatile tool and act in a direct and/or indirect manner. New insights on how different isotypes and Fc gamma receptor (Fc\(\gamma\)R) interactions influence their efficacy have recently opened the door to techniques allowing optimization of antibodies for different purposes. Furthermore, by activating the adaptive immune system to eradicate cancer an immunological memory can develop, which in the end is the ultimate goal for all immunotherapies.
1.1 Basic Immunology

The immune system consists of the innate and adaptive immunity arms. During an infection, the innate immune system restrains the spread of microorganisms by physical barriers (e.g., epithelial surfaces), soluble factors (e.g., complement factors) and different effector cell populations (granulocytes, natural killer (NK) cells, macrophages and dendritic cells (DCs)). All these components of the innate immune system respond via pattern recognition receptors to molecules on microbes, so-called pathogen-associated molecular patterns (PAMPs). Further, molecules on and/or secreted by stressed cells, so-called danger-associated molecular patterns (DAMPs), can also induce an innate immune response. The cells of the innate immune system fight the infection and provide signals to the adaptive immunity arm, which constitutes the second line of defense.

The adaptive immune system specifically recognizes and kills invading microbes and transformed cells. B- and T-cells carry receptors that can target a wide variety of antigens (intact macromolecules for B-cells and processed peptides for T-cells) and upon antigen recognition, undergo clonal expansion. Moreover, the cytokines that are produced and secreted by the adaptive immune system are important for further immune cell activation. The major difference between the innate and the adaptive immune system is that the latter aids development of memory cells, which upon a reinfection with the same antigen can eradicate the pathogen/tumor faster and sometimes sub-clinically.

DCs constitute an important link between the innate and the adaptive immunity arm. They engulf cells, debris and pathogens, degrade larger proteins into shorter peptides, and present them in the major histocompatibility complex (MHC) on the cell surface. Here, the peptide in conjunction with the MHC can be recognized by cluster of differentiation (CD)-4+ (helper T-cell; Th-cell) and CD8+ (cytotoxic T-lymphocyte; CTL) T-cells carrying the correct T-cell receptor (TCR). Also, DCs express costimulatory receptors and receptors for recognizing PAMPs and DAMPs, and thus, their degree of activation is a major switch between immune activation and induction of tolerance.

1.1.1 Dendritic Cell Activation

DCs were originally described by Steinman and Cohn in 1973 and constitute a heterogeneous group of cells clearly distinct from macrophages. They originate from hematopoietic stem cells (HSCs) and have earlier been described to be of both myeloid and lymphoid linages. However, due to confusing data of how to relate the different subsets this terminology is no longer used. Most research on DC differentiation has been performed in mice, and even though there are similarities with humans, a major challenge will
be to translate this knowledge into the human system. Therefore, in this text, the murine system is described. HSCs in the bone marrow differentiate to a common myeloid progenitor and further into a macrophage DC progenitor (MDP). MDPs can then differentiate into monocytes and a common DC progenitor, which has the capacity to differentiate into plasmacytoid DCs (pDCs) and pre-DCs. Monocytes and pre-DCs leave the bone-marrow via the blood and migrate to peripheral and lymphoid tissues where they give rise to lymphoid DCs and tissue-resident DCs. HSCs also differentiate into common lymphoid progenitors which have the ability to differentiate into DCs but how this occurs is not very well known.

DCs are classified into conventional and non-conventional DC subsets, where the conventional DCs derive from pre-DC populations and are further divided into lymphoid and migratory DCs. The lymphoid DCs are resident in lymphoid tissue like thymus, spleen and lymph nodes (LNs), lack the ability to migrate, and can be subdivided into CD8α+ or CD8α-. CD8α+ DCs are believed to be the most potent crosspresenters of exogenous antigens to CTLs and therefore important in tumor immunotherapy. The migratory DCs are found in intestine, liver, skin, lung, and kidneys and have the capacity to sample antigens in the periphery and subsequently migrate to LNs where they interact with T-cells.

Non-conventional DCs include pDCs and monocyte-derived DCs which are located in many different peripheral organs. pDCs are found in both lymphoid and non-lymphoid tissues and constitute a distinct DC subset with the unique ability to secrete enormous amounts of type I interferon (IFN) in response to viral infections. Toll-like receptor (TLR) 7 and TLR9, which recognize viral single-stranded RNA and unmethylated cytosine-phosphate-guanosine (CpG) DNA, respectively, are expressed by pDCs. In paper I, CpG DNA is used for therapeutic purposes in combination with blockade of coinhibitory signaling pathways, cytotoxic T-lymphocyte antigen-4 (CTLA-4) and programmed death-1 (PD-1). Monocyte-derived DCs, similar to the migratory DCs, have the ability to acquire antigen in several peripheral tissues and migrate to draining LNs. This subtype was first believed to arise only during inflammatory conditions but has also been described during steady-state.

Steady-state DCs are immature (iDCs) and characterized by high endocytic capacity and low T-cell activation properties. To become immunogenic, DCs need to mature (mDCs), which they do upon encounter of an antigen association with danger signals (e.g., TLR agonists or endogenous alarm signals, like heat shock proteins, IFNα and interleukin (IL)-1β, from injured cells). This stimulation leads to upregulation of costimulatory molecules (e.g., CD80, CD86, CD40), secretion of proinflammatory cytokines and homing to T-cell areas of lymphoid organs. During maturation, engulfed antigens are processed by proteasomal degradation and loaded onto the MHC molecule. Extracellular antigens are presented in conjunction with
MHCII to Th-cells while intracellular antigens are loaded on MHCI to be presented to CTLs. However, as described earlier, some DCs have the remarkable property of presenting extracellular antigens, such as tumor debris, to CTLs, a pathway known as crosspresentation\(^{10}\).

Both MHCI and II molecules are assembled in the endoplasmic reticulum (ER). For MHCI antigen presentation, cytosolic proteins are degraded to peptides by the proteasome and translocated to the ER by transporter associated with antigen presentation. In the ER, a peptide is loaded into the peptide-binding groove of MHCI, which will stabilize the complex, before being transported via the Golgi and presented on the cell surface\(^{21}\). Extracellular antigens, on the other hand, are internalized and enter the endosomal pathway. The first stop is the early endosome or phagosome, which has a near neutral pH (pH 7.5). During endosomal maturation, the phagosome is fused with a late compartment giving rise to the late endosome, characterized by a pH of 5.5. The last maturation step involves fusion of the late endosome with the lysosome, where acidic pH (pH 4.7) together with proteases and hydrolyses fully degrade all luminal content\(^{22}\).

In the ER, MHCII forms a complex with the invariant chain before being transported through the Golgi to a late endosomal compartment called MHCII compartment, either directly and/or via the plasma membrane. Here, the invariant chain is degraded and replaced by a processed peptide before being transported to the cell surface\(^{21}\).

For crosspresentation, two main routes are described in the literature: the cytosolic and vacuolar pathway. In the cytosolic model, antigens are transported from the phagosome to the cytosol, where they are processed by the proteasome. The processed antigens can then, either recycle back to the phagosome and be loaded onto MHCI, or enter the conventional pathway and be loaded on the MHCI in the ER\(^{23}\). In the vacuolar route, crosspresentation can occur in stable early or recycling endosomes that do not mature into late endosomes, in which antigens are loaded onto MHCII\(^{24,25}\). DCs have the unique ability to prevent the rapid maturation-induced acidification of endosomal compartments, thereby allowing protein fragments to remain intact for a longer time, facilitating crosspresentation\(^{22}\). These distinct organelles contain the MHCI-loading machinery and proteases needed for generation of peptides, and are sufficient to perform crosspresentation\(^{24,25}\).

Further, DC-derived IL12 activates both NK-cells and T-cells of which the latter will be skewed into IFN\(_\gamma\)-producing Th1-cells\(^{26}\). Activated antigen-specific Th1-cells can stimulate CTLs via the production of IL2 and “license” DCs for crosspriming through CD40L-CD40 interactions, which further improves the capacity of DCs to crosspresent\(^{27-29}\). “Help-less” CTLs, which are activated by unlicensed DCs, lack expression of anti-apoptotic factors, have a short life-span and are unable to perform their cytotoxic effector functions\(^{30,31}\). All these different levels of activation signals required for programming CTLs for cytotoxic functions are believed to be a safety
mechanism to avoid unwanted tissue destruction. Different subpopulations of DCs may also be regulatory in nature and actively terminate an immune response (see section “Immune Escape Mechanisms”).

1.1.2 Costimulation and Coinhibition

As mentioned above, DCs are considered to be the professional APC for activating naïve T-cells as they provide all the signals needed for full activation. Firstly, the APC antigen-MHC complex needs to interact with the correct TCR, which is referred to as “signal 1”. Further, activated DCs express a range of costimulatory receptors, which engage counter-receptors on the naïve antigen-specific T-cells delivering signals important for proliferation and survival (signal 2). Finally, activated DCs produce cytokines (e.g., IL12) needed to promote differentiation into an effector phenotype (signal 3). Signal 1 in the absence of signal 2 was demonstrated to be insufficient for activation32,33 and will instead drive the naïve T-cell into an inactive state, either through anergy, deletion or conversion into a regulatory cell34. Thus, all three signals are required to properly activate T-cells and induce their migration to the site of infection where they pursue different effector mechanisms.

The most commonly described costimulatory molecule is CD28 on the T-cell, which is constitutively expressed and belongs to the CD28:B7 immunoglobulin (Ig) superfamily and interacts with CD80 and CD86 (also called B7.1 and B7.2) on the APC. Upon engagement with homodimeric CD28, signaling pathways involved in protein synthesis, cellular metabolism and cell survival are induced35,36. Cosignaling through CD28 also stabilizes cytokine mRNA37 and remodels the actin cytoskeleton38. The effects of coligation are activation of naïve T-cells, production of IL2, upregulation of pro-survival factors and initiation of the cell cycle. Therefore, CD28 is acknowledged as the most potent costimulatory receptor.

In tumor immunotherapy, recent focus has turned to the coinhibitory molecules, which, in contrast to the costimulatory receptors, participate in maintenance of peripheral tolerance and termination of immune activation. CTLA-4 (described further below) is one such important immune regulator. CTLA-4 also interacts with CD80 and CD86 but does so with higher affinity and avidity than CD28. Another receptor-ligand pair of this superfamily frequently exploited in anti-cancer treatment is the PD-1:PD-L1/PD-L2 receptor pair interaction. The latest members of the CD28:B7 Ig-family are B7-H3, B7-H4 and VISTA, whose ligands still are under investigation. B7-H3 has presented both negative and stimulatory properties and a proposed ligand for the costimulatory effect is the TLT-2 receptor (triggering receptor expressed by myeloid cell-like transcript 2)39. B7-H4 are believed to take part in negative signaling and the function of VISTA is still being explored40. Lately, this superfamily has expanded with emerging new members and/or proposed new interactions. For example, the earlier suggested recep-
tor:ligand pair TIM3:Gal-9 is now being questioned\textsuperscript{41}. Further, a recent discovery revealed that PD-L1 not only interacted with PD-1, but also with CD80\textsuperscript{42}.

Another major T-cell costimulatory group of receptors is the tumor necrosis factor receptor (TNFR) superfamily. Twenty-seven ligands have been identified for these receptors representing many diverse functions in the immune system. Members of this family are important for T-cell survival after initial CD28:B7 interaction\textsuperscript{43}. Four receptors are considered especially prominent as agonist targets in cancer immunotherapy: CD40, 41BB (also called CD137), CD27 and OX40. The TNFR member with most attention in the clinic is foremost CD40 (described further below) and now recently, immunostimulatory mAbs have been generated for the latter three molecules which are currently under early clinical testing. 41BB can function independently of CD28 and an agonist (Urelumab, BMS) has been investigated in patients and demonstrated responses against a range of tumors, but may cause reversible hepatic toxicity\textsuperscript{44}. The OX40 specific mAb examined is of murine origin, which may limit its use, but humanized products are under development\textsuperscript{45}. A first-in-human phase I dosing study has been performed and combinatorial trials with radiotherapy (metastatic breast cancer) and chemoradiotherapy (metastatic prostate cancer) are currently recruiting patients. A fully human αCD27 has been generated and has just entered phase I study\textsuperscript{44}. Furthermore, the ligand of CD27, CD70, is documented to be expressed by many hematological and solid tumors. Therefore, a humanized αCD70 has been generated and is currently undergoing preclinical evaluation for the treatment of CD70+ tumors\textsuperscript{46}. Figure 1 summarizes representative costimulatory and coinhibitory members located in the immunological synapse.
Figure 1. Schematic illustration of representative costimulatory and coinhibitory receptor:ligand pairs of the CD28:B7 Ig, TNFR and TIM families. LAG3, lymphocyte activation gene 3; GAL-9, galectin-9, TIM3, T-cell Ig mucin-3; HVEM, herpes virus entry mediator; BTLA, B- and T-lymphocyte attenuator; LIGHT, lymphotoksin-like, exhibits inducible expression, and competes with herpes simplex virus for HVEM, a receptor expressed by T-lymphocytes; LTβR, lymphotoksin β receptor; DcR3, decoy receptor 3; ICOS/L, inducible costimulator/ligand; VISTA, V-domain Ig suppressor of T-cell activation, GITR/L, glucocorticoid-induced TNFR-related protein/ligand
1.2 Cancer Immunoediting

One of the most controversial questions in immunology over the last century has been if the immune system can recognize and eliminate tumors. The trend has shifted back and forth depending on the tools available and hypothesis at the time. Already in the eighteenth century, the first indication of the immune system’s role in the protection against cancer was noted in cancer patients with feverish infections who occasionally experienced remission. This idea was adopted by Coley, who in the 1890s seriously started to investigate the phenomenon and injected a mixture of streptococcal cultures, under the name “Coley’s toxin”, in 900 patients with inoperable sarcomas with a cure rate of about 10%. Also during this era, Ehrlich proposed in 1909 a primitive form of immunosurveillance, where he postulated that the immune system may control cancer. Coley and Ehrlich’s ideas were however not widely accepted by their peers, possibly due to the induction of severe fever in patients treated with Coley’s toxin. Also, the observed immunity against transplantable tumors in mice, at the time, was discovered to be due to alloreactivity in the outbred animals.

Later during the 1950s, the skepticism for the immunosurveillance theory became even more profound when Burnet proposed the theory of acquired immunological tolerance (central tolerance). This concept was experimentally verified by Medawar and colleagues, suggesting that tumors cannot be rejected as they are considered as self. Nevertheless, several studies during this time demonstrated that animals can be immunized against syngeneic transplantable tumors and the idea of tumor-associated antigens (TAAs) began to evolve. Ironically, during the 1960s the same Burnet played an important role in the change of attitudes when he, and at the same time Thomas, proposed the immunosurveillance theory, where lymphocytes constantly patrol tissues eradicating malignant cells.

However, this positive attitude was not long-lived. From 1970-85 experimental evidence started to accumulate questioning the theory. These included athymic mice with normal incidence of tumors, evidence saying that all auto-reactive T-cells are deleted during thymic selection and the notion that the anti-tumor immunity observed was actually directed against endogenous viruses. Again, in the mid-1980s, the opinion shifted in part due to evidence of auto-reactive T-cells escaping the thymic selection process and that tumors are genetically unstable, possibly capable of generating many unique TAAs. From the early 1990s, the hypothesis of immunosurveillance was widely accepted with studies demonstrating that mice deficient in important immunological factors (e.g., STAT1, perforin, IFNγ and IFNγR) had higher incidence of carcinogen-induced tumors, and in some cases, spontaneous carcinomas. It was also concluded that the immune system not only triggers an immune activation against the tumor but may also
shape its immunogenicity. Thus, the term immunosurveillance was replaced with cancer immunoediting.

Cancer immunoediting has been divided into three phases; elimination, equilibrium and escape of the tumor. In the elimination phase, immune cells are activated and will remove newly transformed cells (i.e., immunosurveillance). However, if not all malignant cells are destroyed they can, together with the immune system, enter a dynamic equilibrium phase. Here, the adaptive immunity arm faces a constant battle to keep the cancer in check, which is constantly sculpted by different immune editors to produce new tumor variants with reduced immunogenicity to avoid an immune attack. The equilibrium phase can continue subclinically for a very long time until these new tumor variants find a successful way to evade the immune system. Tumor clones that escape the equilibrium phase have usually undergone major genetic and epigenetic changes, and the immune system can no longer restrain tumor growth. These clones can therefore expand in an uncontrolled manner and become clinically detectable in the escape phase. Tumors have developed many different escape mechanisms to avoid immune recognition and some of them are described below.

1.2.1 Immune Escape Mechanisms
The maintenance of peripheral tolerance is essential for protection against fatal lymphoproliferative disease and autoimmunity. However, a major problem in tumor immunotherapy is that the growing tumor may not always induce the danger signals needed for optimal DC activation and subsequent T-cell activation. Although tumor-directed T-cells are frequently detected in cancer patients these may be too few or they are suppressed in different ways resulting in persistent tumor progression.

Cancer cells have evolved many different ways to avoid immune recognition such as secretion of inhibitory factors, attraction of suppressive cell types and tampering with the antigen-processing machinery. All of these mechanisms will reduce the ability of T-cells to recognize cancer cells. Some cells secrete factors that either directly or indirectly downregulate immune responses. Indirect downregulation may occur via induction of regulatory DC subsets. As DCs play a critical role in bridging the innate and adaptive immune systems, tumor immune escape mechanisms targeting this subset will have major impact on tumor immunity. Firstly, tumor-derived factors can block the differentiation of DC progenitors (e.g., IL6, vascular endothelial growth factor (VEGF), interferise with the maturation and activation of fully differentiated DCs even induce apoptosis. Furthermore, tumors can alter the differentiation program of DC precursors (via secretion of VEGF, transforming growth factor (TGF)-β, IL1β, IL13, granulocyte macrophage colony-stimulating factor (GM-CSF), prostaglandins, reactive oxygen species (ROS) and complement factors) and induce the
accumulation of immature myeloid-derived suppressor cells (MDSCs).
MDSCs release suppressive cytokines (e.g., TGFβ, IL10), arginase-1, ROS77 and indoleamine 2, 3-dioxygenase (IDO)78. IDO is an enzyme that catabolizes the degradation of L-tryptophan, an essential amino acid important for T-cell proliferation, survival and activation. MDSCs are also known to induce the development of regulatory T-cells (Tregs)79. Another way of tumor-induced de-differentiation of DCs, is the promotion of tumor-associated macrophages (TAMs or type 2 macrophages; M2), which are attracted by Th2 cytokines, glucocorticoids and growth factors in the tumor microenvironment80. TAMs, much like MDSCs, suppress a variety of immune cells, can promote Treg inhibitory functions and induce T-cell apoptosis via expression of PD-L1. Moreover, tumor-derived factors can stimulate the development of immunosuppressive regulatory DCs81 and IDO-expressing pDCs82.

An additional major suppressive cell type is the Treg, which was first described by Sakaguchi et al83. The current thinking is that there are two types of Tregs, naturally occurring and peripherally induced. They suppress a plethora of cell types by cell-cell contact (e.g., membrane-bound TGFβ, CTLA-4, and Lag3), secretion of inhibitory cytokines (e.g., IL10, TGFβ and IL35) and by ligand competition (IL2, CD80/86)84.
2. The Scope of This Thesis

Both antagonistic CTLA-4 and agonistic CD40 antibodies are in clinical use and have demonstrated promising anti-tumor effects in late-stage cancer patients. The systemic administration of such antibodies has been associated with immune-related adverse events and in the case of αCTLA-4 affecting the skin and gastrointestinal tract or with αCD40 causing cytokine-release syndrome. In this thesis, I first wanted to elucidate if systemic αCTLA-4 had an effect on our tumor model. Secondly, I aimed to investigate if local delivery of αCTLA-4 or αCD40 close to the growing tumor, where tumor antigen and anergic effector cells are present, could be an alternative to systemic dosing. The specific objectives of each paper are listed below:

2.1 Paper I
In this paper the goal was to improve the anti-tumor effects generated by TLR agonists, CpG or BCG, by targeting immune checkpoint regulators CTLA-4 and PD-1. Both signaling pathways were blocked by antibodies alone or in combination with TLR agonists to evaluate anti-tumor efficacy.

2.2 Paper II
As paper I demonstrated superior anti-tumor effects with systemic CTLA-4 blockade, we wanted herein to compare if a tumor site-directed therapy of low-dose αCTLA-4 could be an alternative to a systemic high-dose regimen, focusing on tumor growth inhibition, induction of systemic anti-tumor effects and development of tumor immunity.

2.3 Paper III
In paper III, we wanted to evaluate if low-dose αCD40 could be administered locally. Therefore, we investigated equivalent doses of local and systemic delivery, side-by-side, for efficacy, toxicity and in vivo biodistribution.
2.4 Paper IV

The aim was to evaluate if CD40 antibodies could increase their stimulatory properties and retention after local delivery. To achieve this, agonistic CD40 and biodegradable nanoparticles were mixed and assessed *in vitro* and *in vivo*. 
3. Tumor Immunotherapy

The main goal of immunotherapy is to activate or reactivate the immune system and direct it against the malignant cells, and as mentioned above, the first attempts were employed in the late 1800s by William Cooley, who injected his Coley’s toxin in cancer patients. His idea is still utilized today, through the use of Bacillus Calmette-Guérin (BCG) for treatment of superficial bladder cancer.

In the past, cancer immunotherapy has commonly been divided into active or passive, and further subdivided into specific and unspecific. Active specific immunotherapy, i.e., cancer vaccines, aims to generate a long-lasting, tumor-directed immune response in vivo and is consequently dependent on the patient’s immune system. Several different categories of cancer vaccines have been developed including protein-, or polypeptide-containing vaccines, whole cell cancer vaccines and viral vector vaccines.

Another example of active cancer immunotherapy is treatment with cytokines, which nonspecifically stimulates the immune system. The first cytokines used in patients were IFNα and IL2.

In contrast to active immunotherapy, the passive strategy delivers ready-made tumor-directed cells (i.e., adoptive cell transfer (ACT)), isolated from peripheral blood, tumor or draining LNs, or mAbs, presumably acting directly on the tumor and therefore independent on the immune system. However, lately is has become clear that both active and passive immunotherapies requires the patient’s immune system for full therapeutic efficacy. The immunomodulating mAbs used in the papers of this thesis would be classified as active as they modulate the endogenous immune response and generate tumor-directed memory. Also, tumor-targeting mAbs, like ACT, can induce indirect immune activation via the generation of tumor cell death, which can, in turn, lead to shedding of tumor antigens that can be processed by DCs and subsequently presented to tumor-directed T-cells. Further, this indirect immune activation would generate a polyclonal response to multiple tumor epitopes lasting long after the infused mAb and cells are gone.

Another exciting molecule is the bispecific T-cell engager (BiTEs), which consists of two flexibly linked antibody single chain fragments targeting epitopes on the tumor and the TCR/CD3 complex of T-cells. BiTEs have demonstrated high potency and can polyclonally activate T-cells without the need of costimulation.
3.1 Urinary Bladder Cancer

Urinary bladder cancer (UBC) is one of the first tumor types successfully treated with immunotherapy. Even though current BCG therapy is effective in a majority of patients with superficial UBC, it fails to provide responses in metastatic disease.

In 2008, UBC was listed as the ninth most common tumor type in the world with approximately 380,000 new cases and 150,000 deaths registered worldwide. There is a 3:1 ratio of men to women for developing this cancer form. Cigarette smoking accounts for the main behavioral risk factor for UBC with smokers experiencing about three-fold higher risk than non-smokers. Changed smoking habits have reduced incidence and mortality of UBC over the last 30 years in developed countries. In developing countries, Schistosomiasis infections are the major reason of squamous cell bladder carcinomas.

Most (>90%) UBCs arise from the transitional epithelial cells (or urothelium) causing transitional cell carcinoma (TCC). Tumors are staged according to invasiveness. So-called intraepithelial tumors (Ta or Tis) are localized in situ and this tumor stage is correlated with a better prognosis. When tumors invade the lamina propria (T1) or the muscle (T2-4) the outcome becomes worse with a higher risk of metastatic disease. UBC is diagnosed by cystoscopy, urography and ultrasound and the majority (70-85%) of patients present with superficial TCC (Ta, Tis or T1) at diagnosis. Localized tumors are treated with transurethral resection followed by intravesical chemotherapy, or for high-risk tumors, local BCG immunotherapy. BCG induces remission in most patients (55-75%) although up to half of responders will experience tumor recurrence within the first five years. Also, BCG therapy is associated with severe toxicity in some patients with about 20% becoming intolerant to therapy due to these side effects. For muscle-invasive UBC, radical cystectomy provides initial tumor control, but if micrometastases are observed the five-year survival rate is only 40-60%.

3.2 Monoclonal Antibodies in Tumor Immunotherapy

The importance of costimulatory and coinhibitory receptors, so-called immune checkpoint receptors, has lately gathered much attention in the field of tumor immunotherapy. As a consequence, mAbs have been developed for disrupting the tumor-induced immune tolerance in order to provoke and boost an otherwise weak anti-tumor response.

The first generation of therapeutic mAbs was of murine origin, which made them highly immunogenic with rapid induction of human anti-mouse antibody responses, thus limiting their half-life and efficacy. Later, chimeric mAbs were developed by substitution of the murine Fc domain
with that of the human through recombinant DNA technology. These chimeric mAbs resulted in improved pharmacokinetics and efficacy\(^{108-110}\). Further advances in recombinant DNA and cloning technologies generated humanized mAbs with murine hypervariable loops in the human V-segment\(^{111,112}\), and now, fully human mAbs can be developed using phage display libraries and genetically modified mice carrying human IgG loci\(^{113-116}\).

Antibodies used in cancer therapy can act in a direct and/or indirect manner. If they act directly they can activate immune cells (e.g., \(\alpha\)CD40), block growth factor receptors (\(\alpha\)VEGFR) or arrest tumor cell proliferation (\(\alpha\)HER2). Indirect effects include antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent phagocytosis (ADP) or complement-dependent cytotoxicity (CDC). Rituximab (\(\alpha\)CD20) is one such mAb, whose efficacy depends on ADCC and CDC\(^{117,118}\). Depending on which Fc\(\gamma\)R is targeted, different outcomes may develop (see section “Fc\(\gamma\) Receptors”).

Further, antibodies can be divided into direct targeting mAbs, which target molecules on the tumor itself, or the so-called immunomodulatory mAbs targeting receptors of the immune system. An advantage with the immunomodulatory mAbs is that they can elicit an immune rejection of the tumor without identifying tumor antigens, which may be down-regulated as a tumor escape mechanism.

An interesting finding has been that the evaluation criteria used for conventional chemotherapy in the clinic may underestimate response rates of immunomodulatory mAb treatment. Clinical experience with the CTLA-4 antagonist, Ipilimumab, is that responses are usually slower and an initial increase in tumor size may be observed due to inflammation. However, if responses appear they tend to be durable. Therefore, new immune-related tumor response criteria have been proposed\(^{119}\).

Figure 2 illustrates the suggested mechanism of action for the immunomodulatory mAbs used in this thesis. Briefly, in paper I and II antagonistic CTLA-4 mAbs block the intrinsic inhibitory mechanism of CTLA-4 on tumor-directed CTLs, thus maintaining CTLs in an active state. Further, one other major mechanism behind \(\alpha\)CTLA-4-mediated anti-tumor effects has recently been described as depletion of tumor-infiltrating Tregs via Fc\(\gamma\)R-expressing cells. In paper III and IV, agonistic CD40 antibodies activate APCs in a Th-independent manner and subsequently license tumor-directed CTLs to eradicate the tumor.
3.2.1 Fcγ Receptors

Recent studies with the agonistic CD40 and antagonistic CTLA-4 antibodies have highlighted the importance of crosslinking to FcγR for increased efficacy. In the murine system there are four members of FcγRs (mFcγRI, -IIB, -III and -IV) and in the human three (FcγRI, -II and -III), each with a range of allelic variants, different functions, cell distributions and affinities for IgG isotypes (Table 1). Most FcγRs can activate cellular responses by signaling through the immunoreceptor tyrosine-based activation motif (ITAM)\textsuperscript{120}. Activation of ITAM-containing receptors generally involves receptor crosslinking of a multimeric ligand. This induces full phosphorylation of ITAM tyrosines by Src kinases, which will lead to down-stream signal transduction generating immune cell activation\textsuperscript{121}. However, FcγRIIB, which is expressed widely on both hematological and non-hematological cells, contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic tail\textsuperscript{120}. The role of such receptors is to negatively regulate the activity of ITAM-containing receptors. Typically, this is done after coligation of activating and inhibitory receptors on the same cell. Receptor aggregation allows tyrosine residues in the ITIM motif to be phosphorylated by kinases
associated with the activating receptor, which will recruit phosphatases that aborts ITAM-signaling\textsuperscript{121}.

Two research groups\textsuperscript{126,127} pioneered and contributed greatly to the knowledge around the impact of IgG isotypes on αCD40 mAb action. Both groups discovered that the agonistic activity of αCD40 mAb was dependent on interaction with the inhibitory FcγRIIB. Interestingly, both mouse IgG1 and rat IgG2a, which bind moderately to FcγRIIB, presented far more agonistic activity than mouse IgG2a despite carrying the same V region.

Additionally, a recent paper investigating the mechanisms behind the anti-tumor effects generated by antagonistic αCTLA-4, revealed that upon exchange of the parental mouse IgG2b isotype to IgG2a, a dramatic improvement of efficacy was noticed. Moreover, αCTLA-4-IgG2a was more efficient, than IgG2b, in driving CD8+ T-cell expansion and depletion of Tregs in the tumor microenvironment. The authors hypothesized that the rapid reduction of Tregs was mediated via activating FcγR-expressing cells and responsible for the superior T-effector to Treg ratio generated in the tumor\textsuperscript{128}. The selective depletion of tumor-infiltrating Tregs was also

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**Table 1. FcγR expression pattern and affinities for mouse, rat and human IgG subclasses.**

<table>
<thead>
<tr>
<th>Mouse</th>
<th>FcγRI\textsubscript{ITAM}</th>
<th>FcγRII\textsubscript{ITIM}</th>
<th>FcγRIII\textsubscript{ITAM}</th>
<th>FcγRIIV\textsubscript{ITAM}</th>
<th>FcγRIIA\textsubscript{GPI}</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mouse</strong></td>
<td>IgG1</td>
<td>IgG2a</td>
<td>IgG2b</td>
<td>IgG3</td>
<td>Rat</td>
<td>Expression</td>
</tr>
<tr>
<td>FcγRI\textsubscript{ITAM}</td>
<td>NB</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>NB</td>
<td>Mono/MΦ, DC\textsuperscript{a}</td>
</tr>
<tr>
<td>FcγRIIB\textsubscript{ITIM}</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>NB</td>
<td>++</td>
<td>B, mono/MΦ, neutr, DCs, baso, MC, eos</td>
</tr>
<tr>
<td>FcγRIII\textsubscript{ITAM}</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>NB</td>
<td>+</td>
<td>NK, NKT, mono/MΦ, neutr, DC, baso, MC, eos</td>
</tr>
<tr>
<td>FcγRIIV\textsubscript{ITAM}</td>
<td>NB</td>
<td>+++</td>
<td>+++</td>
<td>NB</td>
<td>NB</td>
<td>Mono/MΦ, neutr</td>
</tr>
<tr>
<td><strong>Human</strong></td>
<td>IgG1</td>
<td>IgG2</td>
<td>IgG3</td>
<td>IgG4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FcγRI\textsubscript{ITAM}</td>
<td>+++</td>
<td>NB</td>
<td>+++</td>
<td>+++</td>
<td></td>
<td>Mono/MΦ, neutr\textsuperscript{a}, DC, MC\textsuperscript{a}</td>
</tr>
<tr>
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<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>Mono/MΦ, neutr, DC, baso, MC, eos, platelets</td>
</tr>
<tr>
<td>FcγRIIB\textsubscript{ITIM}</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>B, Mono/MΦ\textsuperscript{a}, neutr\textsuperscript{a}, DC, baso</td>
</tr>
<tr>
<td>FcγRIIC\textsubscript{ITAM}</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>NK, Mono/MΦ, neutr</td>
</tr>
<tr>
<td>FcγRIIDA\textsubscript{ITAM}</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td></td>
<td>NK, Mono/MΦ</td>
</tr>
<tr>
<td>FcγRIIB\textsubscript{GPI}</td>
<td>NB</td>
<td>+</td>
<td>+</td>
<td>NB</td>
<td></td>
<td>Neutr, baso\textsuperscript{a}</td>
</tr>
</tbody>
</table>

\textsuperscript{a}ITAM, contains an activating cytoplasmic ITAM motif; ITIM, contains an inhibitory cytoplasmic ITIM motif; GPI, contains a glycosyl-phosphatidylinositol (GPI) anchor; \textsuperscript{b}Also described as low-affinity receptors for IgE; \textsuperscript{c}Monocyte-derived DCs, but not conventional DCs\textsuperscript{122}; \textsuperscript{d}Inducible expression; \textsuperscript{e/\textsuperscript{-}}Very low expression or expressed by rare subsets; \textsuperscript{f}In Fcgr2c-ORF persons\textsuperscript{123}; Mono/MΦ, monocytes/macrophages; MC, mast cells; neut, neutrophils; baso, basophils; eos, eosinophils.

Binding is referred to as + low to no (<10\textsuperscript{6} M\textsuperscript{-1}), ++ moderate (>10\textsuperscript{6} M\textsuperscript{-1}) or +++ strong (>10\textsuperscript{7} M\textsuperscript{-1}). NB, no binding. Adapted from\textsuperscript{124,125}.
demonstrated by Simpson et al., where FcγRIV-expressing CD11b+ cells were appointed as mediators of Treg depletion most likely via ADCC or ADP. However, this group used a hamster IgG clone of αCTLA-4 in their study\textsuperscript{129}. A third paper further validating this mechanism was published by Bulliard et al., who used the parental αCTLA-4-IgG2b clone. Moreover, they observed that agonistic mAbs (IgG2a and IgG2b) targeting GITR, a member of the TNFR superfamily, also depended on activating FcγR for their in vivo efficacy\textsuperscript{130}. This is in contrast to earlier reports on other receptors of this superfamily; CD40, DR4 and DR5, where the negative FcγRIIB has been required for antibody-mediated anti-tumor activities\textsuperscript{131-133}. An explanation to this is that Tregs express CTLA-4 and GITR to much higher degree than effector T-cells, thus, these cells will therefore be the major target for depletion mediated by activating FcγRs.

The mouse αCTLA-4-IgG2b clone was used in paper I and II of this thesis. We could also see decreased tumor-infiltrating Tregs in the Panc02 tumor model (paper II) but this effect was only noticed when αCTLA-4 was combined with CpG in the MB49 tumor model of paper I. However, the differences may be related to experimental design and/or tumor model.

There are differences between mouse and human FcγR and Ig isotypes, which sometimes makes it hard to translate knowledge between these species. Most human therapeutic mAbs today carry the IgG1 isotype due to the stronger binding to a wide range of activating FcγRs, leading to potent cytotoxicity. The mouse equivalent to human IgG1 is referred to as IgG2a because of their similar functionality. However, the collected results described above indicate that the isotype of choice for immunostimulatory mAbs, such as αCD40, would expect to be the equivalent to mouse IgG1 (or rat IgG2a). Unfortunately, there is no human equivalent to these isotypes with preferential binding to FcγRIIB, assuming the effect is similar in humans. Engineering of Fc regions to enhance such binding may offer special fine-tuning of therapeutic mAbs. On the other hand, mAbs aiming for deleting its target cell, such as αCD20, would most likely benefit from the human IgG1 isotype.

3.3 CTLA-4

CTLA-4 belongs to the CD28:B7 Ig superfamily of receptors and was the first co inhibitory molecule discovered. CTLA-4 was initially believed to be involved in T-cell activation as it was isolated using an approach aiming to identify gene products important for cytolytic T-cell functions\textsuperscript{134}. However, this was counteracted by many studies supporting the negative effect of CTLA-4 signaling. It was further supported by studies of CTLA-4 knockout (KO) mice that die due fatal polyclonal CD4-dependet lymphoproliferative disease within 3-4 weeks of birth\textsuperscript{135,136}. Further, polymorphisms in the hu-
man CTLA-4 gene have been associated with different autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis\textsuperscript{137,138}. CTLA-4 was first described to be a unique T-cell marker\textsuperscript{139,140}, but expression has also been documented in a variety of non-T-cells, either normal or neoplastic including activated B-cells\textsuperscript{141}, monocytes\textsuperscript{142}, placental fibroblasts\textsuperscript{143}, muscle cells\textsuperscript{144}, leukemic cells\textsuperscript{145}, breast- and melanoma tumor cells\textsuperscript{146,147}. The functional significance of CTLA-4 in these different cell types remains to be explored, yet indication of an immune regulatory effect by monocyte-derived mDCs has been reported\textsuperscript{148}. In this thesis, the focus of CTLA-4 expression is exclusively on T-cells.

In contrast to the constitutive cell surface expression of CD28 on T-cells, CTLA-4 is absent on resting T-cells but is rapidly expressed after T-cell activation. Within 1 hour after TCR:MHC/peptide engagement, CTLA-4 mRNA can be detected and peaks at about 24-36 hours\textsuperscript{139}. At the cell surface, CTLA-4 is not readily detected until 24-48 hours post activation\textsuperscript{149}. The majority of CTLA-4 is located in intracellular compartments, such as the trans-Golgi network, endosomes and lysosomes\textsuperscript{150,151} and undergoes complex intracellular trafficking mediated by clathrin-adaptor molecules, AP-1 and AP-2. The mobilization rate of CTLA-4 from intracellular vesicles to the immunological synapse correlates with the affinity of the TCR interaction, with more efficient translocation upon stronger TCR binding\textsuperscript{152}. The accumulation of CTLA-4 in the immunological synapse is critically dependent on the interaction with its ligands, CD80 and CD86, whereas CD28 can be recruited in the absence of ligands but will not be successfully stabilized there\textsuperscript{153}.

All details on how CTLA-4 exerts its negative effect are not clear but both intrinsic and extrinsic mechanisms have been proposed. The intrinsic effects of CTLA-4 ligation involve recruitment of phosphatases (PP2A and SHP-2) to the T-cell synapse interfering with the proximal signaling of CD28 and/or TCR\textsuperscript{140,154-156}. In another model, CTLA-4 is proposed to reduce TCR- and TCR-CD28-mediated raft expression and the induction of signaling microclusters, which further downstream inhibit cell cycle progression and activation of transcription factors nuclear factor kappa B (NF-κB), NFAT and AP1\textsuperscript{157,158}. As CTLA-4 has significantly higher affinities and avidities for CD80 and CD86 compared to CD28, it can compete and reduce the positive signals generated by CD28 engagement\textsuperscript{159}. Furthermore, the TCR-induced stop signal required for stable conjugation between T-cells and APCs is inhibited resulting in reduced contact time between these cell types and, thus, decreased cytokine production and proliferation\textsuperscript{160}. Signaling can also create a B7-independent inhibition since there are non-ligand binding splice variants of CTLA-4 that inhibit T-cell activation resembling that of full-length CTLA-4\textsuperscript{161-163}. In summary, some of the intrinsic effects generated by CTLA-4 signaling are reduced IL2 transcription without affecting mRNA stabilization\textsuperscript{164-166}, production of TGFβ in Th-cells\textsuperscript{167} and blockage of
the cell cycle G0/G1 progression by inhibition of the TCR-induced production of cdk4, cdk6 and cyclin D3. The extrinsic mechanisms of CTLA-4 binding are generated by Tregs. These include reverse signaling through CD80 and CD86 on APCs leading to production of IDO. The binding of CTLA-4 can also cause transendocytosis of the B7 ligands and thereby reduce the availability for CD28, generating a higher threshold for T-cell activation and decreased APC costimulatory function. An additional soluble isoform of CTLA-4 (sCTLA-4) could potentially act in a negative manner, however, its role is not completely clear. Elevated levels of sCTLA-4 have been described in patients with autoimmune disease, but the effect on disease progression is not known.

In contrast to effector T-cells, Tregs express CTLA-4 constitutively and at high levels. Therefore, during the early studies with antagonistic αCTLA-4 there were some controversies of which cell type (effector T-cell or Treg) constituted the main target of therapy. One initial hypothesis was that the inhibitory signaling on effector T-cells was blocked by αCTLA-4. However, the high density of this molecule on Tregs and its importance for Treg suppressive capacity led to the suggestion that αCTLA-4 directly affects this T-cell compartment via depletion or by affecting their suppressive function. CTLA-4-deficient Tregs can alone induce fatal lymphoproliferative disease and the addition of CTLA-4-deficient non-Tregs augments disease, as demonstrated in CTLA-4 KO mice. Later, it was concluded in an elegant study by Peggs et al., that dual CTLA-4 blockade on both effector T-cells and Tregs was necessary for optimal anti-tumor efficacy. In contrast to the findings by Wing et al., no effect was noticed by solely blocking the Treg compartment but a partial response was evident during blockade of effector T-cells.

Nevertheless, it has been hard to find evidence supporting Treg depletion as a mechanism of action. To the contrary, both experimental models and clinical trials have confirmed that the suppressive function of Tregs is not abolished by αCTLA-4 treatment. Rather, the Tregs expand in blood and secondary lymph tissue, further supporting CTLA-4 restriction of T-cell proliferation. A turning point came from three recent studies. They demonstrated the selective depletion of tumor-infiltrating Tregs, expressing high surface levels of CTLA-4, mediated via FcγR-expressing cells (also mentioned in section “Fcγ Receptors”).

3.3.1 Anti-CTLA-4 in Preclinical Studies

Based on the knowledge of how important CTLA-4 signaling is in peripheral tolerance, it was hypothesized that antibody-mediated blockade of this molecule would reverse or reactivate tolerized T-cells and benefit tumor immunotherapy. In addition to the anti-tumor effects generated by antagonistic
CTLA-4, a fusion protein has been evaluated for treatment of autoimmune disease\(^{183}\) and in transplantation\(^{184}\). The first, and majority of studies with antagonistic CTLA-4, were pioneered by the laboratory of Professor James P. Allison. His lab initially demonstrated that syngeneic colon carcinoma and fibrosarcoma responded to CTLA-4 blockade in both prophylactic and therapeutic settings. Anti-CTLA-4 therapy was superior to CD28 agonist treatment and displayed tumor immunity\(^{185}\). This effect was later confirmed by other groups\(^{186}\) and was described to be restricted to early treatment\(^{187,188}\) and tumor immunogenicity\(^{189}\). Due to αCTLA-4 insufficiency to reduce established tumors, combinatorial therapies generating synergistic or augmented anti-tumor activities have been examined. These include different variants of vaccines\(^{190}\), antibodies against other coinhibitors (e.g., αPD-1\(^{191}\)) and/or costimulators (e.g., α41BB\(^{193}\)), Treg depletion\(^{173}\), TLR agonists (e.g., CpG\(^{191}\)), ACT\(^{193}\), cryoablation\(^{194}\), surgery\(^{195}\) and radiation\(^{196}\). The combination of αCTLA-4 and cellular vaccines of tumor cells secreting GM-CSF (Gvax)\(^{197}\) and recently FMS-like tyrosine kinase 3 ligand\(^{190}\), have been investigated with success in the B16 model. In this tumor model, the ratio of intratumoral T-effector cells to Tregs correlated with tumor rejection, an indirect measure of an αCTLA-4 response\(^{179,190}\).

A consensus drawn from preclinical studies is that the anti-tumor activity of αCTLA-4 is dependent on CTL proliferation and, in some models, Th-cells and IFN\(_\gamma\)\(^{198,199}\). Furthermore, αCTLA-4 can reverse tolerized CTLs, an ability dependent on Th-cells and IL2\(^{200}\). Collectively, these data suggest that αCTLA-4 monotherapy is efficacious in selective tumors, while combinatorial modalities have the potential to greatly augment αCTLA-4 anti-tumor efficacy.

In almost all preclinical studies of αCTLA-4, its administration has been systemic, i.e., intraperitoneal injections using a hamster-α-mouse CTLA-4 antibody clone. In addition to paper II in this thesis, there have been four other studies investigating tumor-localized αCTLA-4 therapy\(^{201-204}\). In the first paper by Tuve et al.\(^{201}\), TC-1 cells (murine lung epithelial cell line) were engineered to secrete functional αCTLA-4, which in comparison to parental cells delayed tumor growth. Recombinant αCTLA-4 was only evaluated as single intratumoral (i.t.) high-dose (200µg) injection in the parental tumor but did not demonstrate any effect. The second study by Simmons et al.\(^{202}\) also used a cellular vaccine consisting of tumor cells secreting full-length αCTLA-4 with the addition of GM-CSF (immunotherapy cells). Parental live tumor cells were injected at the dorsal side, while irradiated immunotherapy cells were inoculated in the ventral side of the animals. To evaluate these double-engineered immunotherapy cells, low-dose recombinant αCTLA-4 (two doses; 15 and 10µg) was applied together with the irradiated tumor cells only expressing GM-CSF at the vaccination site (ventral side). Better overall survival was observed after treatment with the double-engineered cellular vaccine compared to low-dose recombinant αCTLA-4.
together with Gvax. Although we used the same clone as this group, the “local administration” of recombinant \( \alpha \)CTLA-4 in this study refers to the vaccination site while in paper II it refers to the growing tumor.

In the third paper by Fransen et al.\(^ {203} \), local low-dose \( \alpha \)CTLA-4 was injected in the tumor area in a slow-release formulation (Montanide\(^ {\circ} \)). This resulted in arrested tumor growth to the same degree as a systemic high-dose, however, this route reduced antibody serum levels by thousand-fold, which decreases the risk of treatment-related toxicities.

The fourth and the last paper by Marabelle et al.\(^ {204} \), combined low-dose i.t. coinjection of \( \alpha \)CTLA-4 with \( \alpha \)OX-40 and CpG. Therapy induced depletion of tumor-infiltrating Tregs and generated systemic anti-tumor effects that eradicated disseminated disease.

3.3.2 Anti-CTLA-4 in Clinical Trials

The success of CTLA-4 blockade in preclinical experiments have encouraged the development of two fully human CTLA-4 antibodies, Ipilimumab (approved and marketed as Yervoy 2011; developed by Medarex and Bristol-Meyers Squibb (BMS), Medarex was later acquired by BMS 2009) and Tremelimumab (CP-675,206; developed by Pfizer, world-wide rights were acquired by MedImmune 2011). The majority of patients recruited to clinical trials for \( \alpha \)CTLA-4 therapy suffer from metastatic melanoma. The first Phase I study of Ipilimumab was published 2003\(^ {205} \) and subsequent studies investigating Ipilimumab alone or in combination with peptide vaccination or chemotherapy have also been published\(^ {206-208} \). In 2010, a Phase III clinical trial, enrolling more than 600 melanoma patients, demonstrated improved overall survival for the Ipilimumab treatment arm\(^ {209} \), and led to its approval as a first- and second line of treatment\(^ {210} \). This makes Ipilimumab the first immunomodulatory mAb to be licensed. Additional clinical trials of Ipilimumab are on-going in several tumor types either alone, or in combination with different chemotherapies, \( \alpha \)VEGF, B-Raf inhibition or androgen suppression\(^ {211} \).

Tremelimumab has also demonstrated durable clinical responses in early phase I and II trials\(^ {212} \) but failed to provide better overall survival than chemotherapy as first line treatment in a subsequently discontinued Phase III study\(^ {213,214} \). AstraZeneca’s MedImmune acquired worldwide rights to commercialize Tremelimumab in 2011 and is currently being explored in studies of patients with malignant mesothelioma, melanoma and advanced hepatocellular carcinoma due to chronic hepatitis C infection\(^ {211} \).

Collectively, the response rate in these studies has been low, with only 10-15% of patients experiencing objective responses\(^ {215} \). But, as mentioned above, in those limited numbers of patients where responses appear, they tend to be durable, in contrast to conventional chemotherapy. The response observed could be due to the generation of a tumor-directed memory. How-
ever, if these effects are seen in the light of the latest preclinical data, new questions arise. First, are tumor-infiltrating Tregs depleted in patients as demonstrated in mice? If so, do responding patients carry polymorphisms in their FcγR repertoires enhancing this effect? Is there a difference in the abundance of FcγR-carrying cells in the tumor upon αCTLA-4 treatment? Can preconditioning of patients increase the influx of these cells to improve Treg depletion? Some of the answers may be found in retrospective studies, but it would be very interesting to look into these questions in a prospective manner.

Although different combinations with αCTLA-4 did not generate higher response rates, a recent Phase I study combining Ipilimumab with blockade of yet another coinhibitory molecule, PD-1 (Nivolumab, BMS), produced, at the maximum tolerated dose, 53% objective responses all with tumor reduction of ≥80%[216].

As only a fraction of patients treated with CTLA-4 blockade benefits from this therapy, the search for prognostic biomarkers is constantly under investigation. Biomarkers that have correlated with anti-tumor effects of αCTLA-4 monotherapy include pre-therapy elevation of absolute lymphocyte count[217], increased surface expression of ICOS on T-cells[218,219] and up-regulation of HLA-DR/CD45RO on T-cells[220].

3.3.3 Anti-CTLA-4 Immune-Related Adverse Events

As CTLA-4 plays an important role in the maintenance of peripheral tolerance, side effects generated by blockade of this molecule have been associated with different degrees of autoimmunity, so-called immune-related adverse events (irAEs). Not surprisingly, some studies have been able to demonstrate a correlation between the presence of irAEs and treatment benefit[221]. The onset of these events is often rapid, dose-related, cumulative and schedule-dependent. The most common irAEs during αCTLA-4 therapy affect the gastrointestinal tract (e.g., enterocolitis, colitis, and diarrhea) and skin (e.g., rash, pruritus). Other less common irAEs include hepatitis, endocrinopathies (hypophysitis, hypopituitarism, adrenal insufficiency, hypothyroidism or hypogonadism) and in rare cases uveitis, pancreatitis and leukopenia. About 60% of patients treated with Ipilimumab experience irAEs and of these, almost 10-15% are of severe grade (grade III and IV). The management of irAEs has been published and involves early detection, discontinuation of mAb therapy and early symptom relief[222]. The majority of toxicities have been managed with high-dose steroids and/or supportive care. Interestingly, the anti-tumor effects generated by CTLA-4 blockade seem not to be affected by this treatment[223]. Unfortunately, preclinical toxicology studies failed to predict these events and even CTLA-4 KO mice did not suffer from the same irAEs observed in humans[135,136].
3.4 CD40

CD40 was identified in 1985 as a molecule expressed by human UBC and B-cells, and was later found to present a wider cell distribution, including cells of hematopoietic (DCs, monocytes, macrophages, NKT-cells, NK-cells) and non-hematopoietic origin (endothelial cells, epithelial cells and carcinomas). Moreover, its ligand, CD40L, is widely expressed in activated T-cells (primarily in Th-cells but also in CD8+ and γδ T-cells), monocytes, DCs, B-cells, mast cells, basophils, eosinophils, NK-cells, endothelial cells and thrombocytes. CD40 belongs to the TNFR superfamily of receptors and lack, like some other members of this family, intrinsic kinase or other signal transduction activity of its own and uses instead a series of downstream adaptor molecules for signaling. Engagement of CD40 by CD40L, typically on Th-cells, leads to trimeric clustering of CD40, which causes a conformational change and recruits family members of the TNFR associated factors (TRAFs). This multicomponent complex translocate from CD40 to the cytosol and associate with TRAF interacting kinases, in turn activating several well-known transduction pathways such as NFκB, p38/mitogen-activated protein kinase and c-Jun-NH₂-kinase. Additionally, CD40 signaling can induce Janus kinase/signal transducers and activators of transcription and phosphoinositide 3-kinase in a TRAF-independent manner. Depending on which cell type expresses CD40 and the microenvironment surrounding it, the consequence of CD40 signaling is different. For example, the CD40:CD40L interaction appears crucial for a normal humoral immune response and for DC activation. The activation of DCs is essential in tumor immunotherapy and CD40 stimulation alters DC phenotype considerably with production of anti-apoptotic factors to increase life span, secretion of cytokines (such as IL-6, IL-8, IL12, tumor necrosis factor (TNF)-α) and upregulation of accessory molecules like intracellular adhesion molecule-1, lymphocyte function-associated antigen-3, CD80 and CD86. Activation through CD40 is described as one of the most critical signals for full maturation of DCs as it enables them to crosspresent to CTLs, thereby skewing the adaptive immune system towards a Th1 response. Tumors may utilize immature or unactivated DCs since they cannot properly activate CTLs causing T-cell anergy or depletion. However, in this situation stimulatory CD40 mAbs can substitute for Th-cells and overcome T-cell tolerance, induce effective CTL responses and improve efficacy of cancer vaccines. In addition to the indirect tumor-killing capacity via DC activation, CD40 ligation of CD40+ tumors can induce apoptosis. Many tumors express CD40 under different stages of tumorigenesis including nearly 100% of B-cell malignancies.
3.4.1 Anti-CD40 in Preclinical Studies

Several studies with αCD40 have demonstrated promising results in the treatment of CD40+ and CD40− tumor models. As mentioned above, mDCs activated through CD40 display all characteristics needed for optimal crosspriming and crosspresentation to CTLs. However, studies have also observed that high levels of αCD40 can have deleterious effects causing apoptosis of CTLs and Th-cells, thus limiting successful tumor immunotherapy. As for CTLA-4 blockade, CD40 agonists have been combined in many different ways in a variety of tumor types to further improve anti-tumor efficacy. Such combinatorial treatments have involved different variants of tumor vaccines, TLR agonists (monophosphoryl lipid A) and cytokines (e.g., IL2). CD40 mAb therapy has also been investigated in a postoperative setting and delivered in a slow-release formula (Montanide) to limit mAb dissemination from the injection site.

In a majority of these studies, the hypothesis that CD40 mAb licenses DCs which subsequently activate CTLs was confirmed, however, other target and/or effector cell candidates have been proposed. These T-cell-independent mechanisms involve the action of tumoricidal macrophages, B-cells and NK-cells. CD40-activated macrophages can deplete tumor stroma generating tumor collapse, which is of major importance in e.g., stroma-rich tumors such as pancreatic cancer.

Systemic αCD40 therapy has been extensively used in preclinical models, with irAEs such as cytokine release syndrome and liver toxicity. More recently, the focus has shifted towards local administration, also employed by us in papers III and IV, where doses and consequently toxicity can be reduced but potentially with sustained systemic anti-tumor effects.

3.4.2 Anti-CD40 in Clinical Trials

Different variants to modulate the CD40 pathway have been undertaken and evaluated in Phase I clinical trials. These have been performed in patients with late-stage cancer and include recombinant CD40L trimer, adenoviral therapy and agonistic or antagonistic mAbs. Most investigations have pursued agonistic mAbs and clinical efforts have accelerated over the last years. Three mAbs have been described, the fully human CP-870,893 (Pfizer and VLST), the humanized Dacetuzumab (SGN-40, Seattle Genetics) and the chimeric Chi Lob 7/4 (University of Southampton), with most trials using CP-870,893. This clone is an IgG2 isotype and the first in-human trial was completed in 2007 where a single intravenous infusion resulted in 4 partial responses in 29 patients with a range of advanced solid tumors.

CD40 agonists have been hypothesized to synergize with chemotherapy, as they can induce tumor cell death resulting in danger signals and tumor...
antigens needed for the generation of tumor-directed CTLs. Therefore, combinatorial therapies of CP-870,893 with carboplatin and paclitaxel (advanced cancer)\textsuperscript{257} or gemcitabine (metastatic pancreatic carcinoma)\textsuperscript{250} were investigated resulting in about 20\% objective responses. Further, combinations with cisplatin and pemetrexed (advanced mesothelioma) or with the \(\alpha\)CTLA-4 mAb Tremelimumab (metastatic melanoma) have recently opened\textsuperscript{124} and will be very interesting to follow.

The second CD40 mAb, Dacetuzmumab, has been evaluated in hematological malignancies like diffuse large B-cell lymphoma (DLBCL), chronic lymphocytic leukemia, and multiple myeloma with various results. Also, this clone has been a part of a combinatorial treatment with Rituximab (\(\alpha\)CD20 mAb) and gemcitabine (DLBCL) with an encouraging overall response rate of 47\%\textsuperscript{258}. A prognostic marker, constituting a 15-gene signature, has been identified which can predict sensitivity and resistance to CD40-activating agents. The presence of this signature at baseline can, with 80\% accuracy, predict tumor eradication and progression-free survival for DLBCL patients treated with Dacetuzmumab\textsuperscript{259}.

Both CP-870,893 and Dacetuzmumab are cleared rapidly from the circulation, a property suggested to reflect a high level of target cell binding or that they do not reach saturating levels\textsuperscript{253,260}. This phenomenon was also observed in paper III, where the rat-\(\alpha\)-mouse CD40 mAb accumulated in secondary lymphoid organs and as a consequence, target APC populations upregulated their surface CD40 expression and increased in numbers.

The third CD40 mAb is the weakest agonist and has recently entered clinical testing in DLBCL patients\textsuperscript{124}. No results from this study were available at the time of printing this thesis.

3.4.3 Anti-CD40 Immune-Related Adverse Events

As CD40 is widely expressed the concerns regarding \(\alpha\)CD40 mAb drug-related toxicity include cytokine release syndromes\textsuperscript{261}, autoimmune reactions\textsuperscript{261}, thromboembolic syndromes, hyper immune activation creating activation-induced T-cell death or tolerance\textsuperscript{242,243}, and enhanced tumor angiogenesis\textsuperscript{262}. However, most of these concerns have not been observed clinically. The most common clinical irAEs are cytokine release syndrome, characterized by symptoms like transient chills, rigors and fevers, and elevation of serum TNF\(\alpha\), IL6 and liver enzymes. These irAEs have mostly been of grade II or less. Transient depletion of circulating B-cells and platelets has also been observed\textsuperscript{253,257,263}. Treatment with Dacetuzmumab has resulted in non-infectious eye disorders, notably lacking for the other agonists\textsuperscript{264}. An intriguing finding is that severe toxicity has not been detectable for \(\alpha\)CD40 mAbs in contrast to those observed with CTLA-4 blockade.
4. Results and Discussion

4.1 Paper I

In earlier publications we have demonstrated that CpG is superior to BCG in an orthotopic experimental bladder cancer model\textsuperscript{265}. In this paper, we wanted to improve the anti-tumor efficacy generated by the local TLR agonist CpG by combinatorial blockade of the negative signaling pathways CTLA-4 and PD-1. By antagonizing these coinhibitory receptors systemically using mAbs in combination with CpG we observed an augmented anti-tumor effect compared to either agent alone in a bulky tumor model. The concurrent treatment modality also increased levels of circulating tumor-directed CD107a-expressing CTLs, splenic effector Th-cells and reduced tumor-infiltrating Tregs. When CpG was instead combined with blocking PD-L1 mAbs, no additional benefit was observed. In conclusion, the simultaneous activation of APCs (by CpG) and reactivation of anergic tumor-directed T-cells (by CTLA-4 and PD-1 blockade) enhanced tumor growth inhibition possibly by eliminating tumor-infiltrating Tregs, tilting immune suppression to immune activation.

4.2 Paper II

As the CTLA-4 antagonist antibody demonstrated therapeutic efficacy in our tumor model, we wanted in this paper to determine if a tumor-site directed therapy of low-dose $\alpha$CTLA-4 could be an alternative to systemic high-dose therapy. Both administration routes were effective in the MB49 tumor model and the more aggressive Panc02 tumor model. No statistical difference in survival was observed between local low-dose and systemic high-dose $\alpha$CTLA-4 therapy, although systemic high-dose therapy generated a better response. Also, both delivery routes reduced tumor-infiltrating Tregs, but the systemically treated animals experienced accumulation of suppressor-like cells in secondary lymphoid organs, a finding absent in locally treated mice. Interestingly, low-dose $\alpha$CTLA-4, regardless of administration route, generated distant anti-tumor effects. To summarize, using local tumor-directed administration it is possible to reduce the dose of $\alpha$CTLA-4 to 1/7 of that of systemic delivery methods, and still observe anti-tumor effects. The reduced serum levels generated by local low-dose $\alpha$CTLA-4 do not promote Treg
expansion in lymphoid organs in contrast to systemic high-dose, and is enough to eradicate both primary and distant tumors. If sustained anti-tumor effects are confirmed in humans, this would pave the way for reduced toxicity and a more cost-effective treatment.

4.3 Paper III

Since local low-dose $\alpha$CTLA-4 was able to generate anti-tumor effects observed in paper II, we wanted to investigate this strategy further but changed our focus to an agonistic CD40 mAb. The rationale for using $\alpha$CD40 is that tumor-directed CTLs will not be generated unless they are properly activated by a mature and licensed APC. Thus, in paper III, locally administered agonistic CD40 antibodies were compared side-by-side with systemic administration to evaluate efficacy, toxicity and biodistribution in the experimental MB49 bladder cancer model. We concluded that local low-dose CD40 activation significantly prolonged survival compared to control animals and the anti-tumor effect was dependent on CD8+ T-cells, host CD40 expression and the presence of tumor antigen at the injection site. In vivo biodistribution studies revealed that CD40-specific mAbs were rapidly cleared from the circulation and accumulated in secondary lymphoid organs. Potential target APC populations in LNs increased in number and upregulated their surface CD40 expression upon repeated $\alpha$CD40 dosing. Systemic injection caused higher antibody uptake in liver and blood, which was also associated with elevated levels of serum haptoglobin, a murine marker of systemic inflammation. In conclusion, our results indicate that locally delivered $\alpha$CD40 mAb accumulates in the draining LN, where it could license mature, tumor antigen-presenting APCs, and subsequently activate CTLs for tumor destruction.

4.4 Paper IV

In paper IV we wanted to increase the stimulatory property and retention of locally administered $\alpha$CD40 mAbs in order to minimize toxicity. This was done by mixing them with biodegradable nanoparticles (anti-CD40-NPs). Anti-CD40-NPs enhanced the proliferative capacity of B-cells and upregulated costimulatory CD80 and CD86 receptors on DCs as well as enhanced IL12 secretion. Moreover, local treatment of MB49 tumors with anti-CD40-NPs resulted in reduced serum levels of IL6, IL10, IL12 and TNF$\alpha$ compared to treatment with soluble CD40 agonist. Taken together, anti-CD40-NPs induce synergistic immunostimulatory effects and reduce serum cytokine levels, hence limiting side effects.
The concept of local immunomodulatory therapy has slowly evolved over time. Intravesical BCG therapy has been employed since the late 1970s and is still the treatment of choice for superficial bladder cancer. Today, Adenoviral vectors, TLR agonists and mAbs are considered for tumor-site directed therapy. The benefits of local immunotherapy are many, the most important of which is the lowering of systemic concentrations. By doing so it may limit off-target side effects and consequently generate a more cost-efficient treatment. Furthermore, reactivation of resident tumor-induced anergic T-cells and activation of iDCs by local immunomodulatory therapy may induce migration of these effector cells to the target and clear disseminated disease rather than relying on the therapeutic drug itself to do so. Also, the TAAs needed for the generation of tumor-directed CTLs are located in the tumor microenvironment.

The future perspectives for the papers of this thesis can be divided into some more specific, and some more general. First, as was done for the CD40 agonist in paper III, an in vivo biodistribution study of αCTLA-4 could be performed. This information may be useful to correlate with effector mechanisms and potential side-effects to be able to optimize therapy with regard to its distribution. The recent insights of how αCTLA-4 depletes tumor-infiltrating Tregs could further be dissected by this information. Also, it would be interesting to see how and why these FcγR-carrying cells, responsible for Treg depletion, accumulate in murine tumors and if this can be translated to the human situation.

Further, as more combinatorial treatments of cancer emerge, there is a need to explore how these agents behave in vivo, e.g., if they counteract each other etc., to make sure they are optimally administered. The obvious combination for this project would be αCTLA-4 together with αCD40 and is, in fact, already under clinical investigation for selected melanoma patients. Therapeutic efficacy studies focusing on combinations of individual mAb isotypes and FcγRs may elucidate why some patients respond to therapy while others do not.

A more general aim is to adapt our local delivery strategy to a spontaneous tumor model. It is not feasible to perform different titration and combination experiments in an orthotopic model, however, there is place for such in proof-of-concept studies. We have tried intravesical instillation of mAbs, like have been done with CpG, in our orthotopic bladder cancer model but
without success. This failure is most likely due to the bigger size of the immunomodulatory mAbs compared to CpG, and will therefore be unable to penetrate the bladder wall and reach their target cells. In an attempt to come closer to the real-life situation, we have started collaboration with a group performing ultra-sound guided tumor implantation in the bladder. Accordingly, it will also be possible to administer treatment intratumorally, a model that is obviously very interesting for us.
Populärvetenskaplig sammanfattning


En tumör bildas när någon av kroppens egna celler ansamlat tillräckligt mycket skador (mutationer) i sitt DNA (arvsanlag), t.ex. på grund av strålning eller kemikalier. En del tumörer är medfödda och då finns dessa skador på DNA:t redan i det befruktade ägget.

Immunförsaret aktiveras bara fullt ut när det träffar på något som anses vara ”farligt” för oss. Det ”farliga” kan t.ex. vara en vävnadsskada, gifter från invaderande bakterier, eller att molekyler som normalt ska finnas inne i en cell läcker ut pga att våra celler dör. Om immunförsaret träffar på dessa signaler startar en immunologisk reaktion. Initialt växer tumörer ofta sakta utan att störa omgivningen och kommer därför inte uppfattas som ”farliga” av vårt immunförsvar. Det är troligen inte förrän tumören blivit för stor för sin omgivning och börjat inkräcka på närbeliggande vävnad som den verkliga aktiveringen av immunförsaret sker. Om immunförsaret inte lyckas döda alla cancerceller startar en selektionsprocess där tumören, å ena sidan, konstant försöker förändra sig (mutera) för att undvika en immunattack och immunförsaret, å andra sidan, försöker identifiera och döda dessa nya versioner av cancerceller. Om tumörens samlade mutationer leder till att den inte kan dödas av immunförsaret kan tumören expandera och växa obehindrat. Det är ofta först vid detta stadium som en tumör blir kliniskt synlig för både patienten och vårdgivaren.

Immunförsaret innehåller en mängd olika celler som utför olika funktioner vid en immunologisk reaktion. Den mest betydelsefulla celltypen vid immunterapi sägs vara T-celler. T-celler är i normala fall (dvs utan ”farliga” signaler) oaktiverade och måste stimuleras för att kunna attackera och döda tumörceller. Detta görs av en så kallad dendritisk cell (DC) som förutom att ge aktiverande signaler till T-cellen även talar om hur farligt någonting är.
genom sin egen aktiveringsgrad. Det vill säga om DCn själv är ostimulerad, vilket den är när ”farliga” signaler saknas, får inte T-cellen de aktiverande signaler den behöver och kommer att självdö. Det är just denna strategi, med flertalet nivåer av aktivering hos flera olika celltyper, som reglerar immunförsvar så att det inte ska bli överdrivet aktiverat och skada normala celler. Tyvärr kan det ibland uppstå obalans som gör att immunförsvarvet upplever ”fara” när det inte borde, eller att signalen inte försvinner. Det kan då attackera normala organ, som vid autoimmuna sjukdomar som typ 1 diabetes eller multipel skleros.


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References


A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine.