From
DEPARTMENT OF ONCOLOGY-PATHOLOGY
Karolinska Institutet, Stockholm, Sweden

TO KILL TWO BIRDS WITH ONE STONE: TARGETING MYELOID CELLS IN CANCERS

Yumeng Mao

毛郁萌

Karolinska Institutet

Stockholm 2015

About the cover:

This photo was taken by photographer/pharmacist Henry Jager, who blended milk with cream at adjusted proportions and poured the mixture into the salted water. The stunning effects were captured within a minute. In my view, this photo offers a visual interpretation of the extreme heterogeneity (the colors) and plasticity (the shapes) of myeloid cells.

Cover photo reprinted with permission from ©HenryJager (www.conartix-photo.ch).
All previously published papers were reproduced with permission from the publisher.
Published by Karolinska Institutet.
Printed by AJ E-Print AB, Stockholm, Sweden.

ISBN 978-91-7549-876-8

To Kill Two Birds with One Stone: Targeting Myeloid Cells in Cancers

THESIS FOR DOCTORAL DEGREE (Ph.D.)

Cancer Center Karolinska (CCK) Lecture Hall, R8:00, Karolinska University Hospital, Stockholm

Friday, June 12th, 2015 at 09:00.

By

Yumeng Mao

毛郁萌

Principal Supervisor:
Professor, Rolf Kiessling, M.D., Ph.D.
Karolinska Institutet
Department of Oncology-Pathology

Co-supervisors:
Docent, Andreas Lundqvist, Ph.D.
Karolinska Institutet
Department of Oncology-Pathology

Dr. Isabel Poschke, Ph.D.
German Cancer Research Center (DKFZ)
Department of Translational Cancer Research
Division of Molecular Oncology of Gastrointestinal Tumors

Opponent:
Professor, Suzanne Ostrand-Rosenberg, Ph.D.
University of Maryland
Department of Biological Sciences
Baltimore, U.S.A.

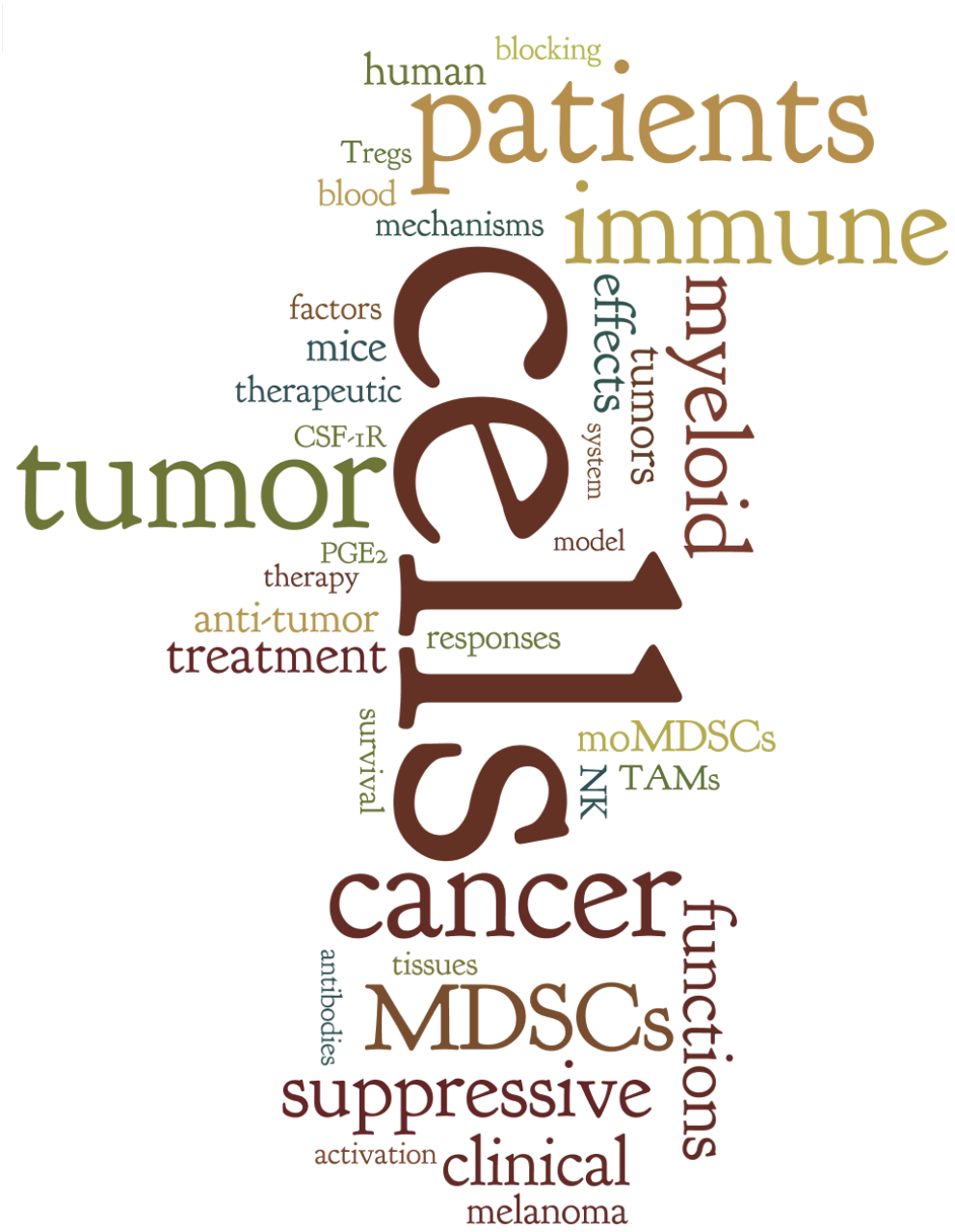
Examination Board:
Docent Susanne Gabrielsson, Ph.D.
Karolinska Institutet
Department of Medicine

Docent Angelo De Milito, Ph.D.
Karolinska Institutet
Department of Oncology-Pathology

Docent Karin Leandersson, Ph.D.
Lund University
Department of Laboratory Medicine

To my beloved grandmother, a cancer survivor since 1998

KEY WORDS



*: The graph was created using an online word cloud tool (http://www.wordle.net). It was based on the text content of this thesis, excluding acknowledgements, references and the constituent research articles.

MY PERSONAL VIEW OF THE IMMUNE SYSTEM

The few of you that might have visited my hometown, Xi'an (China), must have been impressed by the spectacular scene of the 'Terracotta Army'. However, as a local kid breathing the city's air, my favorite has always been the rectangular-shaped city walls, which has been offering security to the inner city for more than 600 years*.

Not until when I have learned more about the immune system, I started to realize how perfectly the structure of ancient Xi'an city could explain the sophisticated design of the human body. The walls, just like the skins, frequently reject life-threatening invading enemies (microbes and viruses). Within these walls, vital facilities (brain, heart, lungs etc.) and civilians (normal tissues) could function well under the protection of the highly-skilled watchmen of the city (immune system).

In general, this protecting force comprises of military troops that have large numbers of soldiers (T and B lymphocytes) as well as specialized, fast-responding fighters (NK cells) and special agents (myeloid cells). Once there is a break-in at any point of the fortification, guards will light up the beacon tower (inflammation) and the special agents will be summoned immediately. They could release explosive weapons to kill the invaders and report first-hand information to initiate military operations later on (antigen presentation).
In comparison to microbes and viruses, cancer initiation is more similar to a gangster group started within the city. In most cases, this kind of activity is quickly detected by the watchmen and terminated on the spot. However, gangster groups could use many tricks, for example fake identities or acting undercover, to avoid being recognized. When these groups have gained enough power, they could even corrupt the city's watchmen and receive assistance to spread their influences to other functioning parts (metastasis).

The special agents, myeloid cells as we mentioned earlier, normally are among the first ones to notice the gangster activities. Part of their job is to gather intelligence by infiltrating these outlawed groups and collect key information that enables effective military executions. However, due to their constant presence in the gangs, they often betray their duties and participate in illegal activities that support growth of the gangster groups.

Investigations conducted in this thesis focus on clarifying main channels that the gangster groups (tumors) employ to convert these special agents (**Study I, II** and **IV**). In detail, I aim to understand how these converted members of the immune system could slow down efficient cancer clearance (**Study I** and **II**) and block smooth information transfer to the authorities (**Study III**).

The goal of these investigations is to develop counteractive tactics that could regain the loyalty of these 'double-agents' and ultimately work from both ends to efficiently eliminate threats of the gangster groups within the city (**Study IV**).

*: The city walls in Xi'an are the most well-preserved city fortification among all Chinese cities. Its construction started in 194 B.C. and the existing part was built by the Ming Dynasty in 1370. Nowadays it is approximately 14 km long and on average 12 meters in height and 18 meters wide on the base.

ABSTRACT

Cancer progression is often accompanied by chronic inflammation and severe impairment of the immune system. In recent years, therapies eliciting tumor-specific immunity have resulted in striking tumor control and survival benefits in cancer patients. However, establishment of effective and durable immune responses is hampered by various tumor-dependent mechanisms. Besides the direct suppression mediated by tumor cells, a number of immune cell types, including regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), 'M2-biased' tumor-associated macrophages (TAMs) and regulatory dendritic cells, occur in the periphery and tumor microenvironment. These cells conduct potent inhibition of anti-tumor immunity and are associated with poor prognosis in patients. Studies included in this thesis aim to elucidate the molecular machinery that tumor cells utilize to induce suppressive functions from healthy myeloid cells (**Study I, II** and **IV**) and how the resulted suppressive myeloid cells could limit functions of T cells (**Study I**), natural killer (NK) cells (**Study II**) and differentiation of the immune-stimulating dendritic cells (DCs) (**Study III**). Finally, we tested the role of a myeloid-specific chemical inhibitor in antagonizing the induction of these suppressive myeloid cells *in vitro*. In a transgenic murine model developing highly aggressive spontaneous tumors, treatment with the inhibitor elicited robust control of established tumors and potentiated the anti-tumor effects of checkpoint blocking antibodies (**Study IV**). In summary, this thesis provides mechanistic insights for the induction of suppressive myeloid cells and demonstrates the therapeutic potential of targeting these cells for the treatment of solid tumors.

LIST OF INCLUDED STUDIES

I. **Mao Y.**, Poschke I., Wennerberg E., Pico de Coaña Y., Hansson J., Masucci G., Lundqvist A., Kiessling R.[#], Melanoma-educated $CD14^+$ cells acquire a myeloid-derived suppressor cell phenotype and are potent inhibitors of T cells via COX-2/PGE2-dependent mechanisms, *Cancer Research 73 (13): 3877-87, 2013*.

II. **Mao Y.*,** Sarhan D.*, Steven A., Seliger B., Kiessling R., Lundqvist A.[#], Inhibition of tumor-derived prostaglandin-E2 blocks the induction of myeloid-derived suppressor cells and recovers natural killer cell activity, *Clinical Cancer Research, 2014 Aug 1;20(15):4096-106.*

III. Poschke I.[#], **Mao Y.**, Adamson L., Salazar-Onfray F., Masucci G., Kiessling R., Myeloid-derived suppressor cells impair the quality of dendritic cell vaccine, *Cancer immunology, immunotherapy : CII 2012;61(6):827-38.*

IV. **Mao Y.***[#], Eissler N.*, Le Blanc K., Johnsen J.I., Kogner P., Kiessling R.[#], Targeting CSF-1R potentiates checkpoint inhibitors to control spontaneous neuroblastoma growth through modulating suppressive myeloid cells, *Manuscript*, 2015.

*: *Equal contributions; #: Corresponding authors*

SUPPORTING RESULTS

Research Articles

1. Sarhan D., Palma M., **Mao Y.**, Adamson L., Kiessling R., Mellstedt H., Österborg A. and Lundqvist A., Dendritic cell regulateion of NK-cell responses involves lymphotoxin-α, IL-12 and TGF-β, *Eur. J. Immunol., accepted, 2015*.
2. Poschke I., **Mao Y.**, Kiessling R., de Boniface J., Tumor-dependent increase of serum amino acid levels in breast cancer patients has diagnostic potential and correlates with molecular tumor subtypes, *J. Transl. Med., 11(1):290, 2013.*
3. Pico de Coaña Y., Poschke I., Gentilcore G., **Mao Y.**, Nyström M., Hansson J., Masucci G., Kiessling R., Ipilimumab treatment results in an early decrease in frequencies of granulocytic MDSCs as well as their arginase-1 production, *Cancer Immunol. Res., 1(3): 1-5, 2013.*
4. De Boniface J., **Mao Y.**, Schmidt-Mende J., Kiessling R., Poschke I., Expression patterns of the immunomodulatory enzyme Arginase 1 in blood, lymph nodes and tumor tissue of early-stage breast cancer patients, *OncoImmunology, 2012 Nov 1:8, 1305-1312.*
5. Poschke I., De Boniface J., **Mao Y.**, Kiessling R., Tumor-induced changes in the phenotype of blood-derived and tumor-associated T cells of early-stage breast cancer patients, *Int. J. Cancer, 2012 Oct 1;131(7):1611-20.*
6. De Boniface J.*, Poschke I.*, **Mao Y.**, Kiessling R., Tumor-dependent down-regulation of the ζ-chain in T and NK cells is detectable in early breast cancer and correlates with immune cell function, *Int. J. Cancer, 2012 Jul 1;131(1):129-39.*
7. Okita R., Mougiakakos D., Ando T., **Mao Y.**, Sarhan D., Wennerberg E., Lundqvist A., Mimura K., and Kiessling R., HER2/HER3 signaling regulates NK cell-mediated cytotoxicity via MHC class I chain-related molecule A/B expression in human breast cancer cells, *J. Immunol., 2012 Mar 1;188(5):2136-45*.

Reviews and Commentaries

1. **Mao Y.**, Poschke I. and Kiessling R., Tumour-induced immune suppression: role of inflammatory mediators released by myelomonocytic cells, *J. Intern. Med., 2014 Aug;276(2):154-70.*
2. Kiessling R., **Mao Y.** and Pico de Coaña Y., Myeloid suppressors decrease melanoma survival by abating tumor fighing T cells, *Clin. Cancer Res., 2014 Mar 15;20(6):1401-3.*
3. **Mao Y.**, Poschke I. and Kiessling R., Cyclooxygenase-2: Steering force of myeloid-derived suppressor cells in cancer? *OncoImmunology, 2013 2:8, e25157.*
4. Kiessling R., Okita R., Mougiakakos D., **Mao Y.**, Sarhan D., Wennerberg E., Seliger B., Lundqvist A., Mimura K., Kono K., Opposing consequences of signaling through EGF family members; escape from CTLs could be a bait for NK cells, *OncoImmunology, 2012 Oct 1;1(7):1200-01.*

Manuscript

1. **Mao Y.**, *et al.*, Interleukin-15 potentiates human natural killer cells to acquire resistance against tumor-induced immune suppression through mTOR-regulated metabolic control, 2015.

*: *Equal contributions*

CONTENTS

ABBREVIATIONS

ADCC	Antibody-dependent cellular cytotoxicity
ALL	Acute lymphoblastic leukemia
ATP	Adenosine triphosphate
CLL	Chronic lymphocytic leukemia
CCL-2	Chemokine (C-C motif) ligand 2
CCR-2	C-C chemokine receptor type 2
cGMP	Cyclic guanosine monophosphage
COX-2	Cyclooxygenase-2
Cas	*CRISPR*-associated genes
CRIPSR	Clustered regularly interspaced short palindromic repeats
CTLA-4	Cytotoxic T lymphocyte antigen-4
CXCR-2	CXC chemokine receptor type 2
DCs	Dendritic cells
FoxP3	Forehead box P3
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HIF-1α	Hypoxia induced factor-1 alpha
HMGB-1	High-mobility group box protein B-1
IDO	Indoleamine 2,3-deoxygenase
JAK	Janus Kinase
M-CSF	Macrophage colony-stimulating factor
MDSCs	Myeloid-derived suppressor cells
MHC	Major histocompatibility complex
mPGES-1	Membrane-associated PGE synthase-1
NKG2D	Natural-killer group 2, member D
NOS	Nitric oxide synthase
PBMC	Peripheral blood mononuclear cells
PD-1	Programmed cell death-1
PDE-5	Phosphodiesterase type-5
PDGF	Platelet-derived growth factor
PD-L1 or -L2	Programmed cell death ligand-1 or -2

PGE2	Prostaglandin E2
RAG-2	Recombinase-activating gene-2
RAGE	Receptor for advanced glycation endproducts
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
ScFv	Single-chain variable fragment
STAT	Signal transduction and transcription
TAA	Tumor-associated antigen
TAM	Tumor-associated macrophages
TGF-β	Transforming growth factor-beta
TLR	Toll-like receptor
TRAIL	TNF-related apoptosis-inducing ligand
Treg	Regulatory T cells
VEGF	Vascular endothelial growth factor

FOREWORD

Since the beginning of my scientific training in 2009, I have frequently heard the strong doubts about cancer immunotherapy just a few years ago. One of the common arguments that discredited the ability of the immune system in controlling established tumors was based on observations that tumors continued to progress despite being 'surrounded' by immune cells. In the clinic, boosters for the immune system, such as interleukin-2 (IL-2) or interferon-γ (IFN-γ), caused severe systemic adverse events but only showed therapeutic effects in a small number of patients. On the other hand, less toxic approaches, such as cancer vaccines, struggled to achieve satisfactory clinical responses against established solid tumors.

Recently, success stories of the uprising immunotherapies, such as 'check-point' blocking antibodies and various adoptive cell transfer strategies, have energized the research in cancer immunology once again. Massive eradication and durable tumor control have been documented in patients who have failed to respond to existing treatments. More importantly, these new approaches have 'revived' an array of classic anti-cancer drugs, to be tested at lower doses as part of the combinational approaches.

However, for anti-tumor immunity to operate optimally in a larger number of patients, we cannot avoid challenges from the extremely hostile environment in cancer patients. Some argue that this problem could be sufficiently overcome once dominant strength of the immune responses are introduced, for example by pumping in trillions of tumor-reactive T cells. This option is potentially risky due to collateral damages against healthy tissues caused by this 'unleashed' T cell army. Thus, it is reasonable to hypothesize that we may achieve a '1+1>2' situation, when immune-activating reagents are wisely combined with approaches that disarm resilient mechanisms utilized by tumor cells, such as abnormal vasculature, immune suppression, hypoxia or acidity.

The immune system is a vastly complicated network involving many distinct cell types. Therefore, we are still in great needs of in-depth knowledge on how the immune system functions in cancer patients, for example how immune cells could interact with tumor cells and regulate each other. Identification of these 'missing pieces', facilitated by refined technological advances, could help us identify new targets and develop approaches that could not only generate sufficient clinical efficacy, but also improve the quality-of-life for cancer patients.

1. SNAPSHOTS OF THE IMMUNE SYSTEM

1.1 INTRODUCTION

The textbook model divides the immune system into innate and adaptive arms. The former includes a variety of cell types that quickly respond to invading pathogens. In contrast, the latter refers to responses directed by selected fragments of pathogens and is thought to be the exclusive effectors for the establishment of immunological memory. However, as emerging evidence points towards the memory properties of certain innate immune subsets [1], it is becoming increasingly challenging to utilize the classic definitions to address current immunological questions. Thus, instead of categorizing immune cell subsets following the framework, I will try to explain the immune response as a process and introduce key elements involved in every major step.

1.2 THE FAST-RESPONDING IMMUNITY

The principle of immune protection is largely based on the 'danger signal hypothesis', which was a concept first suggested by Burnet in 1949 and refined by numerous subsequent studies [2]. In simple words, the evolutionary force has shaped the immune system to detect common features of dangerous pathogens, known as the pathogen-associated molecular patterns (PAMPs). Once healthy cells are infected, they will express the 'kill-me' signals, or damage-associated molecular patterns (DAMPs), in order to initiate immune recognitions. A group of immune cells, known as the antigen-presenting cells (APCs), bear receptors that specifically bind to PAMPs or DAMPs. Upon detection of PAMPs or DAMPs, APCs can capture the infected cells and extract antigens, which are small peptide fragments that are essential for eliciting further immune responses. Generally, this process initiates within hours after infections and the antigen-carrying APCs will migrate to lymph nodes and activate the residing T and B lymphocytes. We will have a closer look at this process in the sections below.

Besides APCs, other immune cells are also playing pivotal roles in the immediate control of invading pathogens. NK cells constitute approximately 5 to 15% of the immune cells in human peripheral blood and rapidly respond to cells lacking MHC class I surface molecules, which is often caused by viral infections [3, 4]. Previous studies have revealed that development and effector functions of NK cells are fine-tuned by a panel of inhibitory and activating molecules [5]. Recently, a hotly debated topic underlines the memory property of NK cells in an antigen-specific manner [6-9], which is traditionally considered to be exclusive for secondary immune effector cells [10]. Moreover, the complement system, which comprises a multitude of circulating or membrane-associated proteins with enzymatic activities, plays a rapid defensive role through lysis of microbes. In many cases, the fast-responding immunity is not sufficient to eradicate invading pathogens. Therefore, secondary immunity, which takes a few days to reach its maximal capacity, needs to be recruited.

1.3 THE THREE SIGNALS

1.3.1 Antigen presentation to T lymphocytes

T cell receptors (TCRs) are unique surface molecules that are essential for the activation and functions of T lymphocytes. Every TCR has a specific reactivity towards a short peptide sequence, which is presented by MHC molecules on the cell surface. Ligation between peptide-containing MHCs on APCs and TCRs can induce intracellular signal transduction cascades, which are required for the activation and expansion of T cells. There are two types of MHCs involved in the antigen recognition,

MHC class I and II. TCRs on CD8+ cytotoxic T cells (CTLs) binds to MHC class I-peptide complexes, whereas MHC class II-peptide complexes are responsible for the activation of CD4+ helper T cells.

1.3.2 Co-stimulation

For T cells to reach the full activation capacity, it is necessary to engage signal transduction mediated by co-stimulatory molecules on professional APCs. Represented by the B7 family members, for example B7.1 (CD80) and B7.2 (CD86), these molecules could bind to various receptors such as CD28 on T cells. This ligation induces vital signals for the survival and expansion of T cells. In addition, together with other adhesion molecules, binding to co-stimulatory molecules could enhance T cell activation by stabilizing immune synapses between APCs and T cells. As opposed to the co-stimulatory molecules, there are also co-inhibitory molecules that function through similar principles but negatively regulate T cells functions. This mechanism is essential to maintain immune homeostasis after infections and forms a major barrier for tumor-reactive immune responses. I will mention this pathway and its therapeutic potential for cancer treatment in later sections.

1.3.3 Cytokines

Cytokines are proteins that regulate cell functions through binding to their matching receptors. APCs can release a panel of cytokines that potentiate various functions of T cells. For example, IL-12 produced by APCs during antigen presentation could stimulate production of IFN-γ from T cells, which is a key regulator for immune defense [11]. Moreover, cytokine environment during antigen presentation could shape the functions of activated T cells, especially in the CD4+ subset.

1.4 THE SECONDARY IMMUNITY

1.4.1 T lymphocytes

As a result of the three signals, large numbers of pathogen-reactive T cells are produced through clonal expansion. These cells will then migrate to the infection sites and eliminate invading pathogens or infected host cells. CD8+ CTLs recognize cells presenting peptides by the MHC class I molecules and induce apoptosis of target cells through a variety of mechanisms, including perforin, granzymes, granulysin or membrane-bound molecules such as FasL or TRAIL. On the other hand, the classic model describes CD4+ T cells to function mainly by producing cytokines. Based on the cytokines that activate them and those released by these CD4+ T cells, they can be categorized into the Th1 or Th2 subsets. Th1 cytokines, such as IFN-γ, IL-2 and TNF-α, promote immune functions of CTLs, macrophages or NK cells. In contrast, Th2 cells produce distinct cytokines, for example TGF-β, IL-10 and IL-4, and are thought to mainly regulate humoral immune responses. The balance between Th1 and Th2 cells has been proposed to be critical in autoimmunity, allergy and cancer.

1.4.2 Humoral responses

Humoral responses are featured by the activation of B lymphocytes and production of antibodies. B cell receptors (BCRs) are membrane-bound immunoglobulins (IgG) that recognize specific antigens. Thus, different from TCRs, BCR signaling does not require the presence of MHC-peptide complexes. Instead, BCRs could directly recognize microbial surfaces. Consequently, this recognition will result in proliferation of B cells with pathogen-specific BCRs and promote their maturation into antibody-producing plasma cells. This process will lead to increased concentrations of antibodies which will bind to the pathogens and result in clearance through antibody-mediated cellular cytotoxicity (ADCC). In addition, B cells are equipped with MHC and co-stimulatory machinery and could activate and amplify antigen-specific T cells.

2. IMMUNE RESPONSES IN CONTROLLING CANCERS

2.1 HISTORICAL OVERVIEW

In 1909, Enrlich proposed that immune surveillance was engaged in the eradication of transformed cells [12] and this hypothesis was elaborated by Burnet a few decades later [13, 14]. However, several lines of experimental evidence argued that immune surveillance was not involved in limiting spontaneous or chemically induced tumors because tumor growth was comparable between immunodeficient athymic nude mice and wild-type controls [15-18]. It was later shown that the development of NK cells, γδ-T cells and some subsets of T cells were still present in athymic nude mice [19, 20]. In addition, wild-type mice indeed demonstrated substantially lower tumor incidence when the chemical carcinogen dosage was carefully titrated [21]. Similar results were obtained from RAG-2-deficient mice [22] that lack functional B and T cell populations [18, 23]. More recently, animal models created by gene-targeting technologies allowed mechanistic analysis of immunological pathways in controlling tumor development, including TCR signaling of T, NKT or γδ-T cells [24-26], synthesis of type I IFNs [27-29] and perforin [26, 30]. On the other hand, tumor progression is often accompanied by a panel of mechanisms that hamper effective clearance mediated by the immune system (section 2.2). Collectively, these observations delineated the dynamic dialogues between tumor cells and the immune system during cancer occurrence and development [31, 32].

2.2 BARRICADES FOR ANTI-TUMOR IMMUNITY

As briefly discussed in the earlier section, tumor-induced immune suppression attenuate effective anti-tumor immune responses. Even though many different cell types mediate these effects, the molecular basis of the suppression is overlapping. Below I will introduce some key aspects of these mechanisms.

2.2.1 Regulatory T cells

Regulatory T cells (Tregs) naturally occur in the thymus and are important to maintain self-tolerance in physiological conditions [33]. In malignancies and inflammation, Tregs could be induced in response to various inflammatory signals, such as IL-10, TGF-β and PGE2 [34]. Tregs belong to the CD4+ helper T cell subsets and express CD25 (IL-2Rα) on the surface and transcriptional factor FoxP3 intra-cellularly. Moreover, low expression of CD127 (IL-7Rα) was used to define Tregs in humans [35]. Numerous *in vivo* studies have demonstrated that Tregs form a substantial barrier for anti-tumor immune responses. Due to the high expression of CD25, Tregs are able to deplete IL-2 from effector T cells, therefore hamper their activation and functions [35]. In addition, Tregs are potent producers for immune-regulatory cytokines such as IL-10 or TGF-β [36, 37]. Further, Tregs could be more resistant to apoptosis in the tumor microenvironment by releasing antioxidant thioredoxin-1 [38]. These factors conduct multi-faceted effects and facilitate tumor growth and metastasis. Indeed, depleting Tregs by low-dose cytoxan potentiated the therapeutic effects of cancer vaccines in the HER2/neu transgenic mice [39].

2.2.2 Immune checkpoints

Sufficient antigen presentation requires co-stimulatory signals triggered by APCs. However, co-inhibitory molecules, also known as immune checkpoints, also exist in order to restore homeostasis after immune clearance [40]. The most well-characterized immune checkpoint to date is CTLA-4 [41, 42], which expresses at high levels on activated T cells and binds to CD80/86 with an affinity that was superior to CD28 [43], which is a T cell-activating receptor that also binds to CD80/CD86. In

addition, CTLA-4 could remove CD80/86 from APCs through trans-endocytosis [44]. Similarly, PD-1 emerges when T cells are activated [45] and can negatively regulate T cell functions and induce T cell apoptosis through ligation to PD-L1 [46, 47] or PD-L2 [48, 49]. Other co-inhibitory ligands such as B7-H3 and B7-H4 [50, 51] were also identified, but their matching receptors on T cells and detailed functions remain elusive. Expression of these immune checkpoints on tumor or immunosuppressive cells is known to be important protective mechanisms that facilitate tumor growth [52, 53] and have proven to be one of the most promising therapeutic targets for the treatment of human cancers (section 3.1).

2.2.3 Enzymatic machinery

Tumor tissues are featured by high levels of energy consumption and altered metabolic profile. Thus, production of various enzymes exhausts crucial amino acids that could support anti-tumor immunity. For example, L-arginine is extremely important for maintaining TCR signaling and T cell functions [54]. Activation of myelomonocytic cells by tumor-derived factors could lead to massive production of ARG and inducible NOS (iNOS) that results in T cell anergy by rapid depletion of L-arginine [55] and release of NO [56, 57]. However, since production of NO was shown to be one of the defending mechanisms mediated by macrophages against cancer cells [58], the role of NO on anti-tumor immunity is still not clear. A recent study showed low-dose irradiation promoted macrophage-mediated tumor rejection through the NOS pathway [59]. Even though ARG and iNOS regulate independent catalytic pathways, co-expression of these two enzymes are often observed, which leads to challenging situations for designing treatment strategies.

Another important enzyme is IDO, which catalyzes tryptophan to N-formyl-kynurenine [60]. It is an important regulatory channel for APCs to modulate T cell functions during antigen presentation through calibrating tryptophan levels [61, 62]. Tumor cells and many types of immunosuppressive cells also utilize this pathway to sabotage T cell responses [63]. Besides the direct effects, IDO activity could control other regulatory schemes in the tumor micro-environment, including COX-2/PGE2 pathway [64, 65], TGF-β or IL-10 production [66, 67]. Thus, it is becoming therapeutically appealing to target IDO activity due to the potential effects on both tumor cells and the immune system. Certainly, pharmacological inhibitors of IDO activity have demonstrated anti-tumor effects [68] and boosted chemotherapy [69] and checkpoint inhibitors [70, 71] in murine models.

2.3 IMMUNOSCORE: Creating Immunological Signatures for Cancer Classification

Observations of inflammatory immune cells in human tumor tissues date back to 1863 by pathologist Rudolf Virchow. Nowadays, it is well-documented that density of CD8+ CTL could independently predict the clinical outcome in various types of human

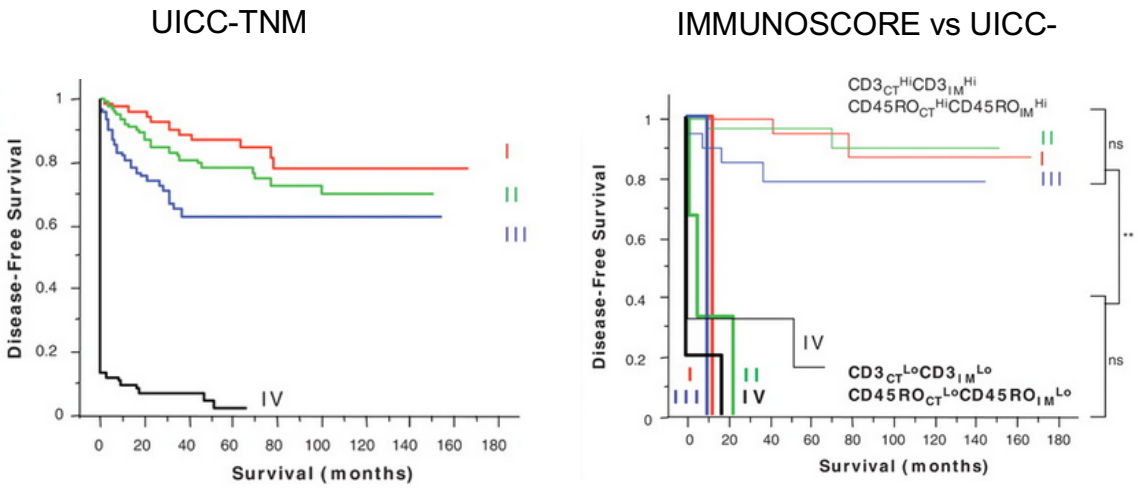


Figure 1, Cancer classification based on immune contexture. CT: Center of the tumor; IM: Invasive margin; CD3: T cell marker; CD45RO: Memory T cell marker. *Adapted from Galon et al., Science, 2006, **313**(5795): p. 1960-4. Reprinted with*

cancers [72-74]. Infiltration of NK cells has also been reported to be a positive factor in human cancers [75-77]. In contrary, the prognostic role of suppressive immune cells has been inconsistent. In some reports, 'M2-biased' macrophages [78-81] or Tregs [82-84] are associated with poor clinical outcome but correlated with better patient survival in other studies [85-87].

In a study published by Galon *et al.* [88], a large quantity of immune-related genes were screened and candidate genes were validated by tissue microarray in colorectal cancer tissues. Strikingly, it revealed that density of CD45RO+ memory T cells in the tumors provided an independent predictive factor that was complementary to the traditional histopathological classification system (**Figure 1**). In particular, late-stage tumors crowded with memory T cells may have more favorable survival than early-stage patients lacking T cell infiltration [89]. In a recent study, the intratumoral 'landscape' of 28 immune cell types was illustrated in colorectal cancer patients and different immune cells demonstrated distinct localization in the tumor [90]. Based on these findings, Immunoscore, which uses the immune contexture in human tumors as staging criteria, is proposed to be implemented in addition to the TNM classification method [91, 92]. Initiated by a few researchers focusing on colorectal cancer patients, it is to date a worldwide, multi-center investigation for various cancer types. The value of Immunoscore has provided solid evidence advocating the importance of immune surveillance during the occurrence and progression of human cancers. Particularly, these findings may have a profound future impact on the diagnosis and treatment decisions in cancer patients.

3. NEW TRENDS IN CANCER IMMUNOTHERAPY

For decades, immunotherapy struggled to prove its therapeutic efficacy in cancer patients and was never widely-accepted to be useful as a treatment option. Nowadays, therapeutic interventions eliciting tumor-reactive immunity are proven to be clinically effective even in patients with multiple metastatic lesions. In this section, I will highlight the major approaches that have shown success in clinical trials. However, it is important to point out that this is an extremely fast-evolving field that is powered by research talents across all scientific disciplines. Thus, new treatment concepts or technical advances may further improve our current view on this topic in the near future.

3.1 'CHECK-POINT' INHIBITORS

3.1.1 Unleashing T cells by CTLA-4 blockade

Immune checkpoint molecules negatively regulate immune effector cells by binding to the matching receptors. As discussed in section 2.2.2, CTLA-4 and PD-1 are two well-characterized receptors on T cells and their therapeutic potentials have been evaluated in preclinical models and clinical studies. In preclinical animal models, blocking CTLA-4 signaling effectively limited tumor growth in mice through activation of T cells [93-96]. Ipilimumab, an anti-human CTLA-4 blocking antibody, was approved by the FDA in 2011 for the treatment of metastatic melanoma and is now under investigation in patients with non-small cell lung carcinoma, small cell lung carcinoma, bladder cancer and prostate cancer. This approval was motivated by results of the landmark phase III clinical trial, which has generated durable survival advantages in metastatic melanoma patients who have failed existing therapies [97-99]. Apart from attenuating negative signals transduced, blocking antibody for CTLA-4 has demonstrated potent ability to remove Tregs in animal models by ADCC mediated by immune cells expressing FcγR [100-102]. Thus, adjusting Fc binding properties of therapeutic antibodies according to the clinical purposes may boost the *in vivo* efficacies in patients [103].

3.1.2 PD-1/PD-L as a therapeutic target

Remarkable clinical responses induced by ipilimumab have accelerated the investigation and approval of blocking antibodies against PD-1 pathway. In melanoma patients, both nivolumab (Bristol-Myers Squibb) and pembrolizumab (Merck) generated durable survival benefits [104, 105]. As a result, FDA granted permissions to these antibodies for treating human melanoma recently. Notably, clinical outcome after ipilimumab treatment did not appear to predict the efficacy of PD-1 blockade, since nivolumab enabled substantial clinical responses in patients who failed to respond to prior ipilimumab treatment [106-108]. Importantly, sequential but not concurrent administration of the two antibodies appeared to be clinically favorable because the latter resulted in severe immune-related adverse events [109]. This could be explained by the distinct regulatory role of PD-1 and CTLA-4 on the immune system [110]. Specifically, mice lacking PD-1 protein experienced tolerable autoimmune reactions [111, 112], but CTLA-4 deficiency resulted in devastating autoimmunity [113, 114]. Thus, it has been postulated that PD-1 functions through fine-tuning the threshold of T cell priming, whereas interfering CTLA-4 signaling leads to a broad activation of non-specific T cells. Even though ADCC-mediated removal of Tregs could contribute to the effects of CTLA-4 blockade, it remains to be clarified whether similar mechanisms are involved in PD-1 blocking agents.

Further therapeutic opportunities lay within PD-1 ligands PD-L1 and PD-L2, which are often expressed on tumor cells or immunosuppressive cell types. Results from several clinical trials revealed that PD-L1 blocking antibody was well-tolerated and led to promising clinical responses in patients with various solid cancers [115-117]. Existing evidence indicated that expression of PD-L1 in tumor tissues could be used as a predictive marker for the check-point blocking antibody treatment [118]. Nonetheless, it should be noted that this observation remains controversial since PD-L1 expression is not exclusive to tumor cells, but could also be expressed by fibroblasts, endothelial cells and immune cells. Expression of PD-L1 could also be controlled by external factors such as IFN-γ [119, 120], which is a cytokine produced by activated tumor-infiltrating lymphocytes (TILs) [71, 121]. Thus, expression of PD-L1 might be dynamically regulated by different treatment strategies or pathological conditions in patients.

Expression of PD-L2 was initially identified on APCs but was later demonstrated to be inducible on immune or non-immune cell types by a range of soluble factors [49, 122, 123]. It is well-documented to be a second ligand for PD-1 and transmits negative signals to T cells. Paradoxically, previous findings using PD-L2-deficient animals or blocking antibodies have implied the activating role of PD-L2 on the immune system [52, 124, 125]. In preclinical tumor models, most results available to date included PD-L2 blockade as an addition to the anti-PD-1/PD-L1 antibodies [126, 127]. Even though blocking PD-L2 indeed enhanced anti-tumor effects of other check-point blocking agents [128], PD-L2 knock-out mice conversely demonstrated more aggressive tumor progression [129]. Due to its unclear biological functions, clinical approaches towards PD-L2 are currently scarce. In a recently completed phase I study (NCT01352884), a PD-L2-IgG1 fusion protein was well tolerated and induced promising clinical responses in advanced cancers (abstract 3044, 2013 ASCO meeting). Nonetheless, it is yet to be revealed in a larger cohort of patients how this agent could potentiate anti-tumor immunity.

In order to achieve thorough blockade of the PD-1 pathway, it might be of necessity to combine anti-PD-1 and anti-PD-L1 approaches. On one hand, both PD-L1 and PD-L2 could diminish T cell activation through PD-1 signaling. On the other hand, PD-L1 was shown to inhibit proliferation and expansion of PD-1-deficient T cells [43], indicating multiple receptors could be coupled to PD-L1.

In summary, immune check-point blockers have generated encouraging clinical responses and elicited durable tumor control in patients with advanced solid tumors. However, current clinical trials are predominantly focusing on melanoma or smoking-related lung cancers, which are believed to be more immunogenic due to their high mutation rates. Thus, clinical efficacy of these agents in other cancer types remains to be explored. Taken into account that CTLA-4 and PD-1 are two of the many members in the immune check-point family, novel targets may emerge as the fundamental mechanistic landscape of these proteins is depicted.

3.1.3 Unique clinical properties of check-point inhibitors

Currently, clinical efficacy of anti-cancer treatments is mainly evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria, which measure decrease of tumor volumes after drug administration. However, immune-activating agents have demonstrated very unique response patterns in cancer patients. In certain cases, enlargement of tumor lesions or appearance of new lesions have been documented before the onset of a late clinical response after ipilimumab treatment [130, 131]. This could be explained by the distinct kinetics for establishing effective immune responses and infiltration of immune cells into tumor tissues may result in

increased tumor volumes. Therefore, it is critical to adjust the evaluation criteria for cancer immunotherapy.

Recent clinical experiences with ipilimumab revealed that check-point inhibitors may induce severe immune-related adverse events in cancer patients [132, 133]. This is a direct indicator for the potency of these agents in activating the immune system, but it also has posed challenges for clinical care of the patients. Emerging results demonstrated that blocking PD-1 or PD-L1 was associated with more tolerable toxicity. It is in line with the magnitude of autoimmunity observed in animals lacking CTLA-4 or PD-1 expressions. Consequently, in-depth knowledge of the biological functions of immune check-point pathways may be of essential for the development of novel immunotherapeutics.

3.2 ADOPTIVE CELL TRANSFER

Given that immune responses are capable of controlling tumor growth, it is reasonable to hypothesize that adoptive infusion of highly functional tumor-reactive immune cells could be effective as a therapeutic approach. Numerous investigations have been conducted and many have shown stunning anti-tumor effects. In this section, I will briefly summarize treatment strategies utilizing activated T cells or NK cells in human solid and hematological malignancies.

3.2.1 Tumor-infiltrating lymphocytes (TILs)

Solid tumor tissues are often infiltrated with T lymphocytes, which is an independent prognostic factor for clinical outcome in various types of cancer as discussed earlier. Moreover, it is generally believed that T cells in tumor tissues are recruited due to their tumor-targeting properties. Proven to be effective in human melanoma in 1988 [134, 135], TILs retrieved from surgically removed tumor tissues followed by activation with high-dose IL-2 have become an attractive treatment option. Even though not validated in the original report, it was later shown that ability of TILs to kill autologous tumor cells *in vitro* could strongly predict the response rate in patients [135, 136]. Further, transferring TILs containing both CD4+ and CD8+ T cells [137, 138], as well as lymphodepletion in patients prior to adoptive T cell transfer [138, 139] were demonstrated to be key factors for clinical efficacy. This could be due to clearance of suppressive Tregs and retention of available T cell stimuli, such as IL-2, IL-7 and IL-15 *in vivo*. These findings have introduced valuable modifications to the TILs treatment procedures. In an updated report containing 93 metastatic melanoma patients, the overall response was up to 72% and 36% of the patients treated with TILs achieved survival longer than 3 years [140]. However, this approach is only possible when sufficient amount of TILs could be generated from the same patient. To overcome this issue, alternative strategies using genetically engineered T cells were developed.

3.2.2 Creating anti-tumor T cells through genetic modifications

TCRs that recognize tumor-associated antigens (TAAs) are required for T cells to lyse tumor targets. Thus, genetic engraftment of such TCRs (TCR-T) into T cells enables their specific killing against tumor cells presenting peptides derived from TAAs [141-143]. When the TAA-specific TCR-T cells were infused, it resulted in shrinkages of tumor burdens in patients with various types of cancers [141, 144-146]. In a recently reported clinical trial, T cells equipped with TCRs specific for the tumor antigen NY-ESO-1 induced tumor regression in patients with metastatic sarcoma and melanoma [147]. Even though TAA-specific CD8+ CTLs are important for the cytolytic effects, TCR-engineered CD4+ T cells also play indispensable roles when infused simultaneously [148, 149]. This could result from their ability to produce T cell supporting cytokines.

Alternatively, T cells could be engineered to express chimeric antigen receptors (CAR-T). In these structures, the extracellular antigen specificity of a monoclonal antibody is coupled to the intracellular T cell-activating signaling domains through trans-membrane spacer molecules. Since the initial discovery, several improvements have been introduced, mainly through fine-tuning the contents of intracellular signaling domains [150, 151]. In comparison to TCR-Ts, cytolytic function of CAR-Ts does not require presence of the MHC-peptide complexes on tumor cells and T cells are sustained by multiple activating signals coupled to the CAR complexes. Therapeutic strategies using CAR-Ts targeting CD19 (CD19-CAR) have achieved remarkable success in treating refractory B cell malignancies [152-155]. In an updated report with a small patient cohort, 90% (27 out of 30) of relapsed or refractory ALL patients reached complete remission after CD19-CAR therapy and the overall survival rate was 78% at 6 months [156]. In solid tumors such as ovarian cancer [145, 157], renal cell carcinoma [158-160] and neuroblastoma [161, 162], CAR-expressing T cells were less effective in controlling tumor progression. This could be explained by the impaired persistence and survival of infused T cells caused by hostile environment both in the blood and tumor microenvironment. Currently, many ongoing clinical trials are exploring the therapeutic potential of CAR-expressing T cells as a treatment for solid and hematological malignancies [163].

3.2.3 NK cell therapy

In contrast to T cells, lysis of tumor cells mediated by NK cells is primarily based on the mismatches between killer cell Ig-like receptors (KIRs) on NK cells and MHC class I molecules on target cells. This important feature allows recipients to accommodate NK cells derived from a haploidentical family member. In addition, NK cells express FcγR on the surface and could contribute to ADCC effects triggered by tumor-binding antibodies. Moreover, death receptors on NK cells could induce apoptosis of the tumor cells through activating caspase pathways [164]. Therefore, highly-activated NK cells are suitable for treating patients with cancers. To date, most promising results with adoptive NK cell transfers were observed in patients with hematological malignancies who received allo-reactive haploidentical NK cells [165-167]. Influenced by similar resilient mechanisms as the T cells, this approach is yet to be improved in controlling tumor growth in patients with solid tumors [168-170].

3.2.4 DC-based therapy

Dendritic cells are professional APCs and are important for providing the 'three signals' during T cell priming (see 1.3). Even though a few reports acknowledged their cytotoxic functions [171, 172], DC therapy in general is thought to mediate tumor killing through enriching tumor-reactive T cells. Since most of the treatment procedures involve generating and infusing clinical grade DC products into patients, I will here categorize it as one of the cellular therapies.

In principle, DC-based therapeutics require generation of functional DCs followed by decoration with TAAs. Even though blood-derived monocytes are most frequently used, CD34+ hematopoietic progenitor cells have also been tested as precursors for maturing DCs [173, 174]. To introduce TAAs, various methods, including direct pulsing of synthetic peptides, recombinant proteins, tumor lysates or transfection-based methods have been implemented [175]. Some investigative results in small numbers of cancer patients have shown promising clinical responses [173, 174, 176-178]. As the first FDA-approved cellular therapy in 2010, Sipuleucel-T (Provenge) was one of the milestones in the history of cancer immunotherapy. This DC-based product was used to treat patients with refractory prostate cancer and could prolong overall survival for 4.1 months [179, 180]. In general, DC-based treatments are well-tolerated and

toxicity is minimal. Recently, the clinical values of naturally occurring DC subsets, such as plasmacytoid DCs have also been evaluated [181, 182]. Even though infused at low numbers, these cells were able to generate favorable immune responses in patients with melanoma.

3.2.5 Sustaining infused cells in vivo

Current protocols implemented for adoptive cell therapy require rapid activation and expansion of patient-derived T or NK cells using high-dose cytokines or agonistic antibodies. The resulted cells are extremely potent in killing tumor cells *in vitro*. Nevertheless, a marked decrease of cell numbers was observed after infusion (from one T cell in 580 PBMC one day post infusion to 1 T cell per 35.400 PBMC after 2 weeks [137]). Some argue that this reflected trafficking of the infused T cells to various organs and tumor sites. But others proposed that it was mainly due to *in vivo* apoptosis induced by cytokine withdrawal or shortened telomere length [183].

In comparison, infused at as low as 1.5×10^5 cells per kilogram body weight, CD19-CARs proliferated *in vivo* and potently controlled tumor progression in leukemia patients [152, 184]. However, when similarly designed CAR-T cells were tested in patients with solid tumors, infused cells rapidly diminished from the peripheral blood and failed to limit tumor growth efficaciously [161]. By analyzing intratumoral CAR-T cells in a preclinical animal model, recent findings suggested that inhibition of T cell signaling, rather than loss of CAR expression or antigen-carrying tumor cells could contribute to this result [185].

Thus, sustaining anti-tumor capacity of the effector cells after infusion might be the key to a successful adoptive cell therapy. This goal could be achieved through a number of approaches. Firstly, early clinical trials using anti-viral CD4+ CAR-T cells have demonstrated *in vivo* persistence of CAR-expressing T cells for almost a decade [186]. This implies that presence of antigen-specific **central memory T cells** (T_{CM}) may help prolong *in vivo* anti-tumor immunity [187]. Given the central role of IL-15 in activating memory T cells and NK cells in cancer patients [188], this cytokine holds substantial promise to extend the *in vivo* life-span of transferred T or NK cells. This point has been consolidated in experimental animal models, where tumor-specific T cells persisted *in vivo* when activated with IL-15, possibly through recruiting anti-oxidant mechanisms [189] or preventing loss of telomere [190]. Secondly, it is rational to use **combinational approaches** to sustain T cell activities. Results of a phase I clinical trial combining DC vaccines with TIL therapy in a small number of melanoma patients have been recently released [191]. The combination was safe and generated promising clinical responses and the updated protocol will include prior lymphodepletion in patients. Based on similar principles, various **protective mechanisms** could be introduced to prevent tumor-induced immune suppression. For example, co-transduction of IL-12 into CAR-T cells improved anti-tumor activity in mice by potentiating macrophage functions [192] and resisting suppression mediated by Tregs [193]. Lastly, opportunities for improving the in vivo persistence of adoptively transferred immune effector cells may underlie **novel regulatory mechanisms**. In a recent report, pre-activated antigen-specific T cells were infected with shRNA libraries that silenced greater than 1500 T cell regulatory genes. After infusion, T cells were retrieved from tumor sites and spleens and compared for the enrichment of shRNAs [194, 195]. This approach allowed the authors to identify genes that were important for T cell functions and survival, particularly in tumor microenvironment. These results highlighted that loss of *Ppp2r2d*, a gene without previously known functions in T cells, contributed substantially to survival and proliferation of transferred T cells in melanoma tumors. Therefore, manipulating these novel pathways may optimize longevity of transferred effector cells in patients with solid tumors.

4. THE 'DOUBLE AGENTS': MYELOID CELLS IN CANCERS

4.1 BACKGROUD

For decades, the dynamic interactions between chronic inflammation and cancers have been carefully dissected [196]. It has been well-documented that non-steroidal anti-inflammatory compounds, such as aspirin, prevent occurrence of various types of human cancers [197]. Local inflammation in tumor tissues is represented by increased infiltration of leukocytes, elevated levels of cytokines or chemokines and abnormal angiogenesis. Emerging evidence depicts that tumor-driven inflammation also has a systemic impact on the peripheral immune system and tumor-free organs.

In cancer patients, circulating monocytes are recruited to tumor tissues by various chemokines, such as CCL-2, and skewed into 'M2-biased' TAMs [67, 198]. In contrast to their 'M1-like' counterparts, TAMs are inefficient in producing pro-inflammatory factors such as IL-12 and TNF-α. Instead, they hamper tumor eradication through release of TGF-β, IL-10, PGE2 or activation of regulatory T cells. In addition, TAMs are an important source of VEGF and PDGF, which support abnormal vasculature in tumor tissues. Visualization tools revealed that TAMs were the chaperones for intravasation of tumor cells during cancer metastasis [199].

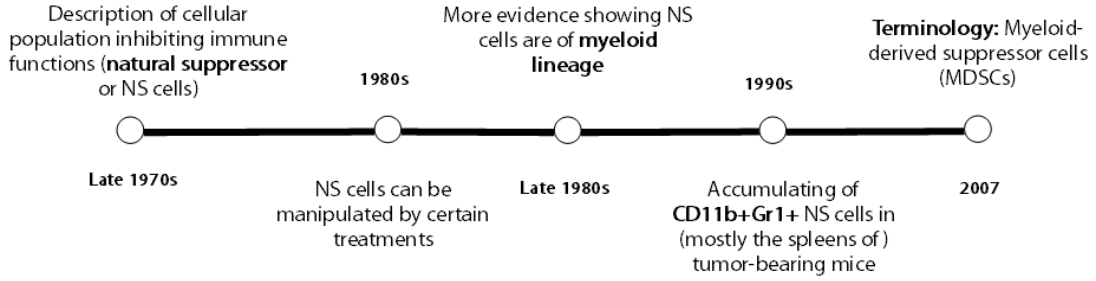


Figure 1, Brief history of myeloid-derived suppressor cells (MDSCs) in cancers.
Modified from: Talmadge and Gabrilovich, Nat. Rev. Cancer, 2013 Oct;13(10):739-52

Recently, the role of myeloid-derived suppressor cells (MDSCs) in cancers has been highlighted. Although the description of immature 'natural suppressor' cells dated back to 1970s, it was not until 2007 that the scientific nomenclature of these cells could be agreed (**Figure 1**) [200]. In tumor-bearing mice, MDSCs are defined by co-expression of CD11b and Gr1 and could be further divided into monocytic (moMDSCs) and granulocytic (grMDSCs) lineages based on the expression levels of Ly6C and Ly6G [201]. In cancer patients, phenotypic definition of MDSCs remains challenging. Several myeloid markers including CD34, CD33, CD11b, CD14, HLA-DR, CD16, CD15 or CD66b have been suggested as individual marker or in combination to detect MDSCs [202] in peripheral blood of cancer patients. Thus, the general consensus for MDSC definition emphasizes the immature phenotype and immune-suppressive functions. Due to the complex leukocyte composition in tumor tissues, functions of intratumoral MDSCs stay controversial. Previous study showed that MDSCs isolated from melanoma tumor tissues lack immune suppressive functions in comparison to the ones in the blood [203]. Conversely, later reports demonstrated that MDSCs in tumors were more potent in exerting suppression on T cells [204, 205].

In summary, TAMs and MDSCs are both heterogeneous populations that bear overlapping surface markers and employ similar immune-regulatory mechanisms in cancer patients. Since the phenotypic signatures and suppressive functions of TAMs [67, 198, 206] and MDSCs [200, 202, 207] have been extensively reviewed elsewhere,

I will instead focus on the clinical implications and targeting strategies of these cells in patients with cancers.

4.2 MYELOID CELLS AS BIOMARKERS

As mentioned in section 2.3, immune contexture in tumor tissues is a powerful prognostic indicator in cancer patients. It has been widely reported that frequencies of TAMs predicted survival of cancer patients [78-81]. In a newly proposed evaluation system (TMEM) for predicting risk for metastasis in breast cancer patients, infiltrating CD68+ cells were included as one of the essential criteria [208]. However, other studies demonstrated the positive prognostic values of TAMs [85, 209, 210]. These discrepancies could be explained by the highly tissue-dependent and versatile nature of macrophages, particularly in tumor tissues. Thus, it is becoming increasingly attractive to explore the prognostic potential of monocytic and granulocytic MDSCs in peripheral blood of cancer patients (summarized in **TABLE 1**).

Many studies have reported that levels of moMDSCs in the blood correlated well with cancer stages and predicted survival in patients with melanoma [211, 212], breast [213, 214], ovarian [215], colorectal [216], lung [217] and various types of other cancers [218, 219]. This was recently supported by the observation that increased numbers of moMDSCs reversely associated with numbers of antigen-specific CD8+ CTLs in melanoma patients [212, 220].

It is widely accepted that MDSCs form one of the main hurdles for sufficient anti-tumor immune responses. Presence of moMDSCs, but not Tregs, was observed at higher levels in colorectal cancer patients who failed to respond to an anti-cancer vaccine [221]. In addition, $CD14^{+}HLA\text{-}DR^{low/neg}$ moMDSCs were proposed to be a biomarker in patients who received a multi-epitope peptide vaccine [222]. In accordance, grMDSCs diminished from the peripheral blood in patients undergoing ipilimumab treatment [223] and frequencies of pre-treatment moMDSCs appeared at higher levels in non-responders [220, 224]. However, it should be noted that blood-derived moMDSCs and grMDSCs have distinct properties during experimental procedures *ex vivo*. While moMDSCs are present in PBMCs after gradient centrifugation, high numbers of arginase-producing grMDSCs could only be co-purified from blood of cancer patients [225]. Since cryopreservation of human PBMC could drastically change the frequencies and suppressive functions of grMDSCs [226], evaluation of this population might be limited if only frozen materials are available.

TABLE 1: Prognostic values of suppressive myeloid cells in cancer patients

Patients	Prognostic values	Other findings	Ref
Melanoma	Frequencies of moMDSCs and Tregs, but not the grMDSCs, correlated with the stage and poor prognosis in melanoma patients	Inflammatory factors, IL-1β, IFN-γ and CXCL10 in the sera correlated with the Tregs and MDSCs	[211]
	Lower moMDSCs predicted better responses to ipilimumab	No changes of moMDSCs during ipilimumab treatment	[224]
	moMDSC numbers at week 6 after ipilimumab treatment correlated with overall survival	moMDSCs were inversely associated with $CD8^{+}$ T cell frequencies	[220]

	Frequencies of moMDSCs, but not Tregs, predicted poor survival	moMDSCs reversely correlated with NY-ESO-1 and Mart-1 specific T cells	[212]
	Low TAM infiltration predicted better survival in uveal melanoma	TAMs associated with higher density of microvasicles	[227, 228]
Breast cancer	CD68/CD4/CD8 in tumor tissues predicted patient survival	Paclitaxel treatment increased M-CSF production and TAM levels	[229]

TABLE 1 (continued)

Patients	Prognostic values	Other findings	Ref
	$CD14^+Arg^+$ cells in the blood correlated with the stage of the patients	High numbers of $CD14^+Arg^+$ cells were detected in tumor tissues and draining lymph nodes	[213]
	Circulating MDSCs correlated with the stages of the disease	MDSC increased after doxorubicin and cyclophosphamide treatment	[214]
	CD68+ TAMs were used to predict metastasis (TMEM)		[208]
	TAMs were an independent prognostic factor for survival	TAMs correlated with angiogenesis and VEGF in tumors	[79]
	Higher CD163+ and CD68+ cells predicted poor survival	Localization in tumor stroma but not tumor nest was important	[230]
	Proliferative TAMs correlated with higher grade tumors	TAMs did not correlate with tumor sizes or metastasis	[81]
Ovarian Cancer	Blood MDSCs correlated with primary tumors and metastasis	MDSCs maintained the stemness of cancer cells by miRNA101	[215]
Colorectal and breast	Blood-derived MDSCs correlated with poor prognosis	$Lineage^{neg}$ cells in bone marrow were the suppressive cells	[216]
Colorectal	TAMs increased with stages and predicted poor survival	Chemokine expression, especially CCL-2 correlated with stage	[80]
	Non-responders to an anti-cancer vaccine had high blood MDSCs	Treg did not predict response	[221]
Neuroblastoma	Infiltration of inflammatory myeloid cells negatively correlated with survival	Independent from MYCN or stage	[231] **Study IV**
Non-small cell lung cancer	High moMDSCs in cancer patients and correlated with metastasis	moMDSCs suppressed T cells through ROS	[217]
Renal cell carcinoma	$CD14^+HLA\text{-}DR^{lo/neg}$ MDSCs strongly correlated with the survival of patients after cyclophosphamide and IMA901 treatment	Treg numbers before treatment also predicted responses	[222]

Various solid tumors	Common myeloid progenitors correlated with the stage of cancers	Myelopoiesis skewed towards granulocytic lineage	[218]
	Increased MDSCs and Tregs in cancer patients and MDSCs predicted poor outcome	MDSC levels correlated with higher Th2 cytokines, especially IL-13	[219]
Hodgkin's lymphoma	TAMs were associated with poor prognosis	Frequencies of TAMs predicted treatment failure rate	[78]

4.3 DRIVING FORCES FOR SUPPRESSIVE MYELOID CELLS

Tumor-driven inflammatory mediators are the most frequently cited mechanism to induce myeloid cells with suppressive functions. The majority of published studies employ well-designed *in vitro* model systems, where human or murine immune (progenitor) cells are exposed to tumor-derived factors. These findings are then validated using *in vivo* models or primary immune cells sorted from cancer patients. Even though most research articles emphasize the importance of a particular pathway, it is likely that multiple aspects are involved in regulating the diversified inflammatory network. Besides, environmental factors such as hypoxia and acidity have recently been revealed to shape the functions of myeloid cells (**TABLE 2**).

4.3.1 Established soluble factors

GM-CSF

Under physiological conditions, GM-CSF is one of the driving forces for myelopoiesis and maturation of APCs. Early studies showed the potent anti-tumor effects of GM-CSF-based vaccines in preclinical models [232]. The mechanisms of action include enhanced maturation and infiltration of 'M1-like' macrophages and dendritic cells. In humans, GM-CSF as an adjuvant therapy was shown to be clinically favorable in various types of cancer patients [233-235]. When GM-CSF was delivered to melanoma patients by an oncolytic vector (OncoVex), it decreased numbers of MDSCs as well as Tregs efficiently, and raised antigen-specific T cell responses [236]. In contrast, one clinical study using GM-CSF as an adjuvant therapy increased moMDSCs levels in melanoma patients and these cells could inhibit lymphocytes by producing TGF-β [237]. These controversial findings could be explained by the dose-dependent immune modulatory effects of GM-CSF *in vivo* [238]. In an animal model, high-dose GM-CSF, but not intermediate or low-doses, hampered efficient tumor-reactive immunity by inducing MDSCs [239]. This was supported by a recent clinical study, where low-dose GM-CSF adjuvant treatment did not increase MDSCs in pancreatic cancer patients [240]. The direct link between tumor-derived GM-CSF and induction of suppressive myeloid cells was dissected by a number of experimental models. Together with other factors including G-CSF or IL-6, recombinant GM-CSF enabled suppressive functions of human CD34+ hematopoietic stem cells [218] or primary human monocytes [216, 241, 242]. In a *Kras*-driven pancreatic tumor model, neutralization of GM-CSF abolished the induction of suppressive myeloid cells *in vivo* [243].

COX-2/PGE2

Prostaglandins (PGs) are lipids that play multifaceted roles during inflammation, autoimmunity and cancer. Cyclooxygenase-2 (COX-2) is an inducible enzyme that regulates the production of PGE2. It is often over-expressed in human cancers and indicated to be a parameter associated with poor prognosis in cancer patients [244]. Even though PGE2 initially was thought to be important in stimulating dendritic cells [245, 246], recent studies have revealed its alternative role in inducing suppressive functions in myeloid cells. In human ovarian cancer patients, the COX-2/PGE2 pathway was demonstrated to be the master regulator for suppressive functions of MDSCs via a positive feedback loop [247]. Specifically, PGE2 could activate a panel of suppressive mechanisms in human myeloid cells, including IDO, ARG1, TGF-β, PGE2 and IL-10 [247-252]. Since PGE2 is a strong activator for the JAK-STAT signaling, it is likely that this is the main pathway controlling these events [205, 253].

Interleukin-1

IL-1 is another widely reported factor in driving the induction of suppressive myeloid cells in cancers. In a recent study, IL-1/IL-1R interaction was significantly enhanced on monocytes isolated from patients with kidney malignancies and drove molecular

mechanisms to support angiogenesis and cancer invasion [254]. In preclinical models, tumor-derived IL-1β could augment mRNA expression of iNOS and ROS in myeloid cells, and could therefore be a potential target for cancer immunotherapy [254-257].

TABLE 2: Induction mechanisms of suppressive myeloid cells in cancers

Methods	Tumor types	Essential factors	Suppressive mechanisms	Ref
Patients	Malignant melanoma	GM-CSF (adjuvant therapy)	TGF-β	[237]
		STAT-3 (AG490)	ROS, ARG1	[253]
	Ovarian	PGE2	PGE2, IDO, IL-10, ARG1 (mRNAs)	[247]
	Renal cell carcinoma	IL-1/IL-1R	Angiogenesis and invasiveness	[254]
	Head and neck	STAT-3 (Sttatic or siRNA)	ARG1	[205]
***In vitro* tumor co-culture**	Human early-passage melanoma tumor cells	COX-2/PGE-2 (Celecoxib)	PGE2, ROS	[250]
	Various human solid tumor cell lines	GM-CSF, IL-1β, IL-6, VEGF, TNFα	ARG1, ROS	[258]
	Pancreatic human tumor cell lines	COX-2/PGE2	ARG1 (expression)	[259]
	Human glioblastoma tumor cell lines	Not tested	PD-L1 (higher expression)	[260]
***In vitro* cytokine mixture**	Seven human tumor types	GM-CSF, G-CSF and IL-6 (used on $CD34^+$ progenitor cells)	Not tested	[218]
	Human ovarian cancer	PGE2	PGE2, IDO, IL-10, ARG1 (mRNAs)	[251]
	Human solid tumors	GM-CSF+G-CSF	ARG1 (higher expression)	[216]
	Various human cancers	PGE2	TGF-β, ROS	[248]
		GM-CSF+G-CSF	IDO, Tregs	[241]
		GM-CSF+IL-6 (Possible other factors)	iNOS, TGF-β, ARG1, VEGF (mRNAs)	[242]
***In vivo* murine models**	Breast cancer (4T1)	COX-2/PGE2 (shRNA, inhibitor)	Not tested	[248, 261]
		IL-1β	iNOS, ROS (higher expression)	[255-257]
		S100A8/A9	Arginase (high expression)	[262]

	Various tumors	HMGB-1	IL-10	[263]
	Colon and lymphoma (CT26 and EL-4)	S100A9 (silencing and transgenic)	ROS (expression)	[264]
	Lymphoma (EL-4)	Hypoxia, HIF-1α	Arginase (expression)	[265]
		SIRT-1/HIF-1α, Glycolysis	Arginase, IL-10, TGF-β (expression)	[266]
	Neuroblastoma (NXS2)	ATP-P2X7R	ROS, ARG1, TGF-β1 (expression)	[267]
	Melanoma (B16F10)	GM-CSF	ARG1, iNOS	[268]
	Melanoma (MT-RET-1)	VEGF, iNOS	ROS, STAT-3 (higher expression)	[269]
	Melanoma (B16F10), Lung cancer (LLC)	Hypoxia, HIF-1α	PD-L1	[270]
		Lactic acid, HIF-1α	ARG1 (mRNA)	[271]
	B cell lymphoma (A20) Melanoma (B78H1)	GM-CSF (vaccine)	iNOS	[239]
	Pancreatic cancer (*KRAS*-driven model)	GM-CSF (antibody or silencing)	ARG1, iNOS	[243]
	Lymphoma (EL-4)	Retinoblastoma-1 (*Rb-1*) (in moMDSCs)	Not tested	[272]

4.3.2 Emerging inflammatory factors

Other mediators involved in inflammation and autoimmunity also take part in cancer-induced modulation of the immune system. S100 proteins are conserved among vertebrates and possess calcium-binding properties [273]. Among all 21 members, S100A8 and S100A9 are by far the most investigated in suppressive myeloid cells [274]. These two proteins can form heterodimers (S100A8/9) and skew myelopoiesis towards a suppressive phenotype by binding to TLR4 and RAGE [262, 264]. Accordingly, higher levels of soluble S100A8/9 were observed in blood of cancer patients and correlated with MDSC frequencies [275, 276]. Quinoline-3-carboxamide is a clinically validated compound for the treatment of autoimmunity and inflammatory disorders. A recent study identified that it directly interacted with S100A9, engaging both Zn++ and Ca++ [277]. Provided that S100A9 was strongly associated with poor clinical outcome in prostate cancer patients [278], tasquinimod has been tested in randomized clinical trials [279, 280] and is currently under phase III clinical investigation as a treatment of metastatic prostate cancers (NCT01234311).

Another pro-inflammatory protein that interacts with TLR4 and RAGE is HMGB-1 [281]. It was originally recognized for its regulatory functions on DNA conformation but later shown to be released by macrophages during sepsis [282, 283]. In tumor-bearing mice, HMGB-1 could potentiate the production of IL-10 via NFκB pathway in MDSCs and directly inhibit functions of T cells [263]. In addition, post-surgical removal of colon cancer tumor masses in mice resulted in elevated production of HMGB-1, which

facilitated the recruitment of MDSCs [284]. This could partially contribute to cancer recurrence after surgery in patients with solid tumors. In addition, human tumor cells can produce HMGB-1 in an autocrine fashion to sustain their survival and proliferation [285], potentially through modulating mitochondrial activities [286]. Conversely, radiotherapy or irradiation were reported to induce immunogenic cell death by triggering release of HMGB-1 and antigen presentation mediated by TLR4 on DCs [287-289]. These findings imply the dual role of HMGB-1 in regulating the interplay between tumor cells and the immune system. During cancer progression, HMGB-1 is released as a mechanism to prevent immune surveillance. When tumor masses are assaulted by anti-cancer agents, massive production of HMGB-1 in combination with other pro-inflammatory factors and tumor-associated antigens promote activation of APCs and T cell priming. However, retention of HMGB-1 after the anti-cancer treatments recruits immune cells with tumor-promoting properties and contributes to a favorable environment for cancer recurrence.

4.3.3 Hypoxic and metabolic control

Cancer cells are highly proliferative and can avoid programmed cell death. Consequently, the abnormal metabolic and acidic landscape in the tumor tissues creates a hostile environment for anti-tumor immune effectors. In normal tissues, HIF-1α is maintained at low levels in myeloid cells, but could be elevated in MDSCs or TAMs by tumor-induced hypoxia [265, 270, 290] or lactic acid [271, 291]. This activation subsequently results in enhanced immune expression of suppressive factors such as arginase and immune check-point PD-L1 on myeloid cells. Moreover, fast-growing tumor cells feature high concentrations of ATP in the adjacent tissues and the P2X7 receptor on myeloid cells could capture the available ATP and promote synthesis of arginase, ROS and TGF-β [267]. In line with these findings, differentiation of MDSCs was shown to be powered by mTOR/HIF-1α-regulated glycolytic activity [266]. Even though the metabolic alterations in cancer cells have been extensively discussed [292], it is only recently that mTOR-mediated metabolic control was shown

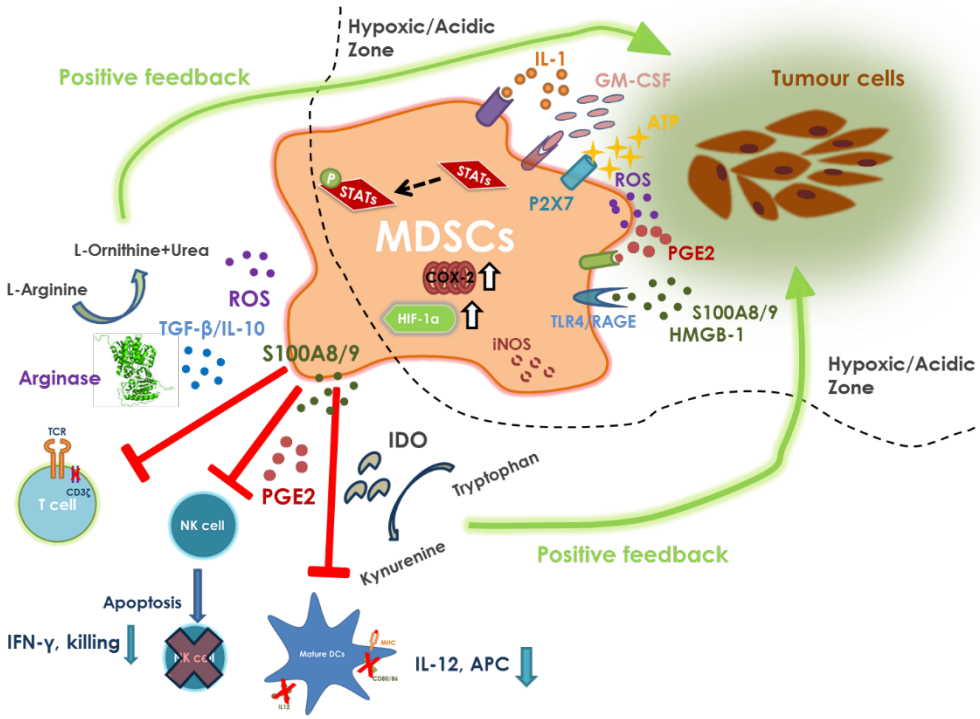


Figure 2, Functional overview of interactions between MDSCs and tumors.
Updated from: Mao, Poschke and Kiessling, J. Intern. Med., 2014 Aug;276(2):154-70.

to regulate the development of immune cells [293, 294]. Thus, it is reasonable to hypothesize that tumor-induced metabolic rearrangements may have a great impact on the immune composition in cancer patients. In-depth understanding of these mechanisms could reveal novel targets for therapeutic interventions in cancer treatment.

4.3.4 The 'Jemaa el-Fnaa'

The activation and differentiation of suppressive myeloid cells are guarded by inter-connected mechanistic networks (**Figure 2**). Tumor-induced metabolic and oxidative changes promote myeloid cells to acquire suppressive functions through a HIF-1α-mediated machinery. Meanwhile, a variety of tumor-derived factors, such as GM-CSF, PGE2, IL-1 and IL-6, are potent inducers for JAK-STAT signaling pathways, which in turn activate production of suppressive factors from myeloid cells. Through STAT signaling, TNFα, TGF-β, VEGF [295], PGE2 [296, 297] or the hypoxic environment [298] also enhance the production of pro-inflammatory HMGB-1 or S100 protein family members. On the other hand, suppressive myeloid cells release soluble factors such as microRNAs, ROS or various inflammatory mediators and deplete essential metabolites from the adjacent areas. This collectively reinforces the abnormal metabolic and oxidative milieu, which facilitates tumor invasion and migration. Even though less discussed in this thesis, interactions among immune cells, or between immune cells and endothelial cells or fibroblasts, amplify the cellular complexity in the solid tumor microenvironment. Thus, instead of illustrating this network as many independent one-to-one dialogues, it resembles my experience in the seemingly 'chaotic' night market 'Jemaa el-Fnaa' in Marrakesh (Morocco). Thousands of vendors and customers are entering and exiting the market and simultaneously interacting with each other. Even though people bargain for different goods, the negotiation methods and tactics are largely overlapping.

4.4 TARGETING SUPPRESSIVE MYELOID CELLS

Suppressive myeloid cells in cancers are extremely heterogeneous and characterized by the production of inhibitory factors but lacking immune-stimulatory potential. Because they often share surface antigens with immune-stimulatory myeloid cells, it remains challenging to specifically target these cells for the treatment of human cancers. In general, existing results support that blockade of tumor-induced inflammation is an efficient way to decrease the numbers and functions of suppressive myeloid cells. Standard therapies, such as surgery or radiation, also have an impact on the suppressive myeloid cells. Even though certain chemotherapeutics have been reported to ameliorate the expansion of suppressive myeloid cells, other cytostatic agents have demonstrated a promoting role. While the detailed mechanisms remain to be revealed, recent findings suggest that targeted therapy or immunotherapy also reduce suppressive myeloid cells in cancer patients. In this section, I have summarized the recently reported *in vivo* modulatory effects of anti-cancer treatments on suppressive myeloid cells, especially in cancer patients (**TABLE 3**).

4.4.1 Anti-cancer treatments and suppressive myeloid cells

Standard surgical procedure or cytostatic drugs have recently demonstrated their role on suppressive myeloid cells in cancer patients. Removal of tumor burdens by surgery decreased the number of arginase-producing $CD14^+$ cells in the blood of breast cancer patients [213]. Chemotherapeutics, such as gemcitabine, capecitabine [240], or trabectedin [299], reduced circulating MDSCs in cancer patients, possibly by inducing selective cell death through the caspase-8 pathway [299]. In contrary, treatments

using doxorubicin, cyclophosphamide [214] or paclitaxel [229] have resulted in elevated numbers of MDSCs in cancer patients. These observations were in agreement with observations in tumor-bearing mice [229, 299-302]. Paradoxically, paclitaxel [303-305] and doxorubicin [306] could reduce accumulation of MDSCs in preclinical models. This could be explained by the distinct dosing and concurrent treatment agents used in humans or mice. Although not yet shown in humans, 5-fluorouracil specifically deleted MDSCs and recovered anti-tumor T cell responses in mice [307]. Furthermore, low-dose irradiation enabled re-activation of TAMs to an anti-tumor phenotype that was featured by high production of iNOS and potently controlled tumor growth in mice [59].

Novel anti-cancer agents, such as targeted therapy or immunotherapy, have also shown modulatory roles on suppressive myeloid cells in patients with cancers. Vemurafinib, an inhibitor targeting a *BRAF* mutation in cancer cells, could decrease moMDSCs and grMDSCs in peripheral blood of melanoma patients [308]. However, it remains to be elucidated whether this effect was directly on myeloid cells or resulted from rapid tumor shrinkage. The checkpoint inhibitor ipilumumab also induced a marked decrease of grMDSCs in the blood of melanoma patients within weeks [223]. Even though moMDSCs frequencies were unchanged during the antibody therapy, pre-treatment levels of moMDSCs were associated with treatment outcome [224]. Additionally, adoptive transfer of highly activated lymphocytes reduced MDSCs in the peripheral blood of cancer patients, but it did not seem to predict treatment outcome in these patients [309].

TABLE 3: Modulation of suppressive myeloid cells in preclinical and clinical studies

Subjects	Tumor types	Treatments	Key effects	Ref
HUMAN	Breast cancer	Surgery	Decreased levels of $CD14^+Arg^+$ cells in the blood	[213]
		Doxorubicin, cyclophosphamide	Higher levels of circulating MDSCs	[214]
		Paclitaxel prior to surgery	Increased levels of $CD11b^+CD14^+$ cells in the blood	[229]
	Pancreatic cancer	Gemcitabine, Capecitabine, vaccine and GM-CSF	Reduction of MDSCs in the blood	[240]
	Diffuse-type giant cell tumor	Anti-CSF-1R mAb	Reduction of $CD68^+CD163^+$ cells and tumor regression	[310]
	Various solid tumors	VEGF-Trap	No effects on MDSCs but enhanced DC maturation	[311]
	Metastatic prostate cancers	Anti-CCL-2 mAb (Calumab, Phase II)	No responses, probably due to insufficient neutralization	[312]
	Soft tissue sarcoma	Trabectedin	Selective depletion of blood monocytes through caspase-8 pathway	[299]

	Renal cell carcinoma	Adoptive T and NK therapy	Reduction of MDSCs in blood	[309]
		Sunitinib	Reduction of MDSCs but no correlation to tumor burden	[313]
			Reduced immature myeloid cells but better CD1c+ DCs	[314]
		All-trans-retinoic acid (ATRA)	Reduction of MDSCs and maturation to DCs	[315, 316]
	Small cell lung cancer	All-trans-retinoic acid (ATRA)	Decreased MDSCs and improved antigen-specific response to p53	[317]
	Head and neck	25-hydroxyvitamin D3	Decreased $CD34^+$ cells in the blood and increased HLA-DR	[318]
		Tadalafil (PDE-5 inhibitor)	Significant reduction of MDSCs in the tumor and blood at intermediate doses	[319]
	Advanced stage melanoma	Vemurafinib (*BRAF* inhibitor)	Decrease of both moMDSCs and gr MDSCs in the blood	[308]

TABLE 3 (continued)

Subjects	Tumor types	Treatments	Key effects	Ref
HUMAN	Advanced stage melanoma	Ipilimumab (anti-CTLA-4 mAb)	Reduced numbers of Arginase-producing grMDSCs, but no effects on moMDSCs	[223]
		Denileukin Diftitox (ONTAK)	Induction of $STAT\text{-}3^{hi}$ toleragenic DCs and Tregs	[320]
		GM-CSF vaccine (OncoVex)	Decreased MDSC and Tregs in vaccinated patients	[236]
		GM-CSF adjuvant	Expansion of $CD14^+HLA\text{-}DR^{lo/neg}$ moMDSCs	[237]
		DC vaccine	Induction of IDO in the vaccine production and higher IDO^+FoxP3^+ cells after infusion	[321]
MOUSE	Glioblastoma (inducible) Cervical (transplantable)	CSF-1R inhibitor (BLZ945)	Repolarization of M2-like macrophages to M1-like; delayed tumor growth	[301, 322]
	Neuroblastoma (MYCN-driven)	CSF-1R inhibitor (BLZ945)	Reduction of MDSCs and M2-like macrophages, significant control of established tumors	**Study IV**
	Breast cancer (MMTV-PyMT)	Paclitaxel (PTX) +CSF-1R inhibitor	PTX induced TAM infiltration and CSF-1R blocking increased the anti-tumor effects	[229, 301]

		(PLX3397 or BLZ945)		
		Anti-CCL2 mAb	Reduced macrophage infiltration to metastases in lungs	[323]
	Breast cancer (4T1, EMT6)	Doxorubicin (DOX) +T cell transfer	Depletion of MDSCs and decrease of IDO production	[306]
	Prostate cancer (RM-1, -3)	Radiotherapy +CSF-1R inhibitor (PLX3397)	Blocking CSF-1R improved anti-tumor immunity mediated by irradiation by depleting TAMs and moMDSCs	[324]
	Pancreatic cancer (orthotopic)	Checkpoint antibody +CSF-1R inhibitor (PLX3397)	CSF-1R blockade eliminated TAMs and moMDSCs and potentiated better response of anti-PD1/CTLA4 mAb	[325]
	Melanoma (B16, SM-1)	T cell transfer +CSF-1R inhibitor (PLX3397)	CSF-1R inhibition improved efficacy of antigen-specific adoptive T cell transfer	[326]
	Lewis lung carcinoma (3LL)	Anti-VEGFR-2 mAb (DC101) +CSF-1R inhibitor (GW2580)	Decreased MDSCs in tumors; best tumor control when combined	[327]
		Sunitinib	Reduction of MDSCs and improve mIL12+anti-4-1BB activation	[328]

TABLE 3 (continued)

Subjects	Tumor types	Treatments	Key effects	Ref
MOUSE	Lymphoma (EL-4)	Pepti-body (against S100A9)	Peptide-Fc fusion protein depleted MDSCs by binding to membrane S100A9	[329]
		5-Fluorouracil	Selective depletion of MDSCs and improved T cell response	[307]
	B cell lymphoma (A20HA)	Various chemotherapies	Cyclophosphamide, doxorubicin and melphalan induced MDSCs; Gemicitabine reduced MDSCs	[302]
	Melanoma (B16, LLC)	Dopamine	Depletion of CD115+Gr1+ moMDSCs and improved anti-tumor immunity	[330]
	Prostate (CR Myc-Cap) Melanoma (B16-h5T4)	Tasquinimod (S100A9 inhibitor)	Decrease of MDSCs and M2-like macrophages; increase of T cell infiltration	[331]

	Melanoma (MT-RET-1)	L-NIL (iNOS inhibitor)	Reduction of MDSCs and loss of suppressive functions	[269]
	Melanoma (*Ret* transgenic) or colon cancer (CT-26)	Sildenafil (PDE-5 inhibitor)	Reduction of MDSCs and inflammatory factors	[332, 333]
		Paclitaxel	Reducing MDSCs by promoting maturation	[303-305]
		Cyclophsphamide	Induction of MDSCs	[300]
	Pancreatic cancer (RT-5)	Low-dose irradiation	Promotion of $iNOS^+$ M1-like macrophages and increased T cell response	[59]
	Human RCC (Xenograft A498)	IL-1R antagonist	Abrogated tumor promoting TAMs and delayed tumor growth	[254]
	Fibroma (MN/MCA1)	Trabectedin	Depletion of MDSCs and TAMs; delayed tumor growth	[299]
	Neuroblastoma (MYCN transgenic) Glioma (inducible) Mesothelioma	COX-2 inhibitor (Aspirin, Celecoxib, SC58236)	Decreased TAMs and MDSCs, delayed tumor growth	[261, 334-336]
	Fibrosarcoma, Lymphoma (EL-4)	Gr-1 antibody	Complete tumor protection by eliminating MDSCs	[329, 337]
	Melanoma (B16F10)	Depletion of $CCR2^+$ MDSCs (antibody)	Blocked monocyte trafficking to tumors and improved $CD8^+$ T cell therapy	[268]
	Sarcoma (RMS)	Depletion of $CXCR2^+$ MDSCs (antibody)	Enhanced T cell activation and effects of anti-PD-1 mAb	[338]
	Colon cancer ($APC^{min/+}$)	CXCR2 pepducin	Blocked the formation of spontaneous tumors	[339]

4.4.2 Alleviating inflammation

Given that chronic inflammation is tightly intertwined with cancer progression, agents with anti-inflammatory properties have been extensively explored in patients with cancer. The preventive effects of COX-2 inhibitors on cancer incidence have been proven in large numbers of patients [197]. Besides the direct limiting effects on tumor cells, modulation of suppressive myeloid cells could contribute to this protection. In experimental settings, genetic ablation of tumor-derived COX-2 [248] or pharmacological inhibition of COX-1/2 [261, 334-336] efficiently limited the accumulation of suppressive myeloid cells in tumor-bearing animals and translated into slower tumor growth *in vivo*. However, it should be noted that COX-2 inhibitors often are associated with cardiovascular toxicity [340]. A recent study using genomic analysis of COX-2 deficient mice revealed that more than 1000 genes were differentially expressed in the kidney, which caused dysregulation of nitric oxide synthase [341]. Therefore, inhibitors targeting alternative checkpoints of the COX-2 pathway, for example the enzyme mPGES-1 [342], should be explored.

Other inflammatory pathways could also be drugable targets for blocking suppressive myeloid cells. A chemical inhibitor, tasquinimod, specifically binds to S100A9 and was able to enhance the anti-tumor T cell response in animal models by removing MDSCs and TAMs [331]. It is currently being validated in a phase III clinical trial in metastatic prostate cancer patients (NCT01234311). Recently, a novel 'pepti-body' mediated potent deletion of MDSCs *in vivo* through ligation to membrane-bound S100A9 [329]. In addition, antagonizing IL-1R signaling could block TAM functions and attenuate human tumor invasiveness in a xenograft model [254].

A few pharmacological compounds that were originally designed to resolve physiological inconveniences have demonstrated anti-tumor capacity by re-shaping tumor-induced inflammatory landscape. PDE-5 inhibitors, such as tadalafil or sildenafil, which antagonize cyclic GMP degradation and induce release of NO, efficiently controlled tumor growth by blunting induction of suppressive myeloid cells in head and neck cancer patients [319] and pre-clinical models [332, 333]. This is in line with another study, where low-dose irradiation potentiated the functions of iNOS-producing myeloid cells [59]. On the other hand, an inhibitor blocking iNOS activity was shown to be effective in controlling tumor progression by attenuating suppressive myeloid cells [269]. These findings may reflect the dual role of NO production during progression and treatment of solid tumors.

4.4.3 Restraining induction signals

As described in section 4.3, the precise induction pathway for suppressive myeloid cells is still unclear. In mice, depletion methods using antibodies targeting Gr-1 are commonly used [329, 337]. However, Gr-1 is not expressed in humans and myeloid cells quickly recover once the antibody treatment is discontinued. Thus, restraining induction signals of suppressive myeloid cells is clearly more beneficial as a therapeutic option.

Among all the key pathways, antagonizing M-CSF receptor (CSF-1R) has to date demonstrated the most profound therapeutic potential. RG7155, an antibody developed by Roche showed consistent effects to eliminate TAMs in pre-clinical murine models, non-human primates and cancer patients [310]. Data from a phase I clinical trial (NCT01494688) in patients with pigmented villonodular synovitis (PVNS) disclosed during the ASCO annual meeting in 2014 (abstract 10504) confirmed the safety of the treatment and 9 out of 10 patients showed progression-free survival for up to 17 months. In addition, chemical inhibitors against the tyrosine kinase associated with CSF-1R signaling, such as BLZ945 (Novartis) or PLX3397 (Roche) also demonstrated encouraging results in a number of studies, as monotherapy [301, 322] or in combination with radiotherapy [324], chemotherapy [229, 301], checkpoint inhibitors [325], adoptive T cell transfer [326] or anti-angiogenic antibody [327]. However, in a phase II clinical trial, PLX3397 did not show benefits for the progression-free survival in patients with recurrent glioblastoma (abstract 2023, 2014 ASCO annual meeting). Starting from January in 2015, the first clinical trial combining the anti-PD-1 mAb (Bristol-Mayer Squibb) and anti-CSF-1R mAb (Five Prime) was initiated in 6 different types of human solid tumors.

It is worth pointing out that the *in vivo* mechanisms of action of CSF-1R blockers are yet to be clarified. In a few pre-clinical tumor models, CSF-1R inhibition as a monotherapy only resulted in moderate tumor control, despite efficient *in vivo* depletion of TAMs [301, 324, 326, 327]. In contrast, other studies [301, 322] including **study IV** in this thesis showed potent therapeutic effects of CSF-1R blockade, potentially through re-programming myeloid cells in the tumors. Of note, the CSF-1R blocking antibody depleted TAMs but elevated numbers of MDSCs in the tumors [310].

Besides the distinct inflammatory nature of each murine tumor model, the *in vivo* stability, permeability or kinetics of the compound in various organs may greatly influence the treatment outcome. In all of the studies, notably, CSF-1R inhibition enabled superior synergistic effects in the respective combinatorial settings. This confirms that suppressive myeloid cells form one of the major resistance mechanisms towards anti-cancer therapies and could be utilized as a therapeutic target.

Based on the similar principle, sunitinib inhibits multiple receptor tyrosine kinases including CSF-1R, CD117, flt3, and could also block the induction of suppressive myeloid cells. In patients with renal cell carcinoma, sunitinib efficiently decreased the numbers of immature MDSCs [313, 314] and enhanced the maturation of CD1c+ DCs [314]. In addition, sunitinib could potentially elicit similar effects in lung cancer patients, as indicated in an *in vivo* murine model [328].

4.4.4 Blocking mobility

Leukocyte trafficking is guided by a variety of chemokines and often skewed by tumor-derived factors. In malignant conditions, suppressive myeloid cells are recruited in response to the inflammatory milieu in the tumor microenvironment. Chemokine (C-C motif) ligand 2 (CCL-2), released by tumors is key to the infiltration of inflammatory

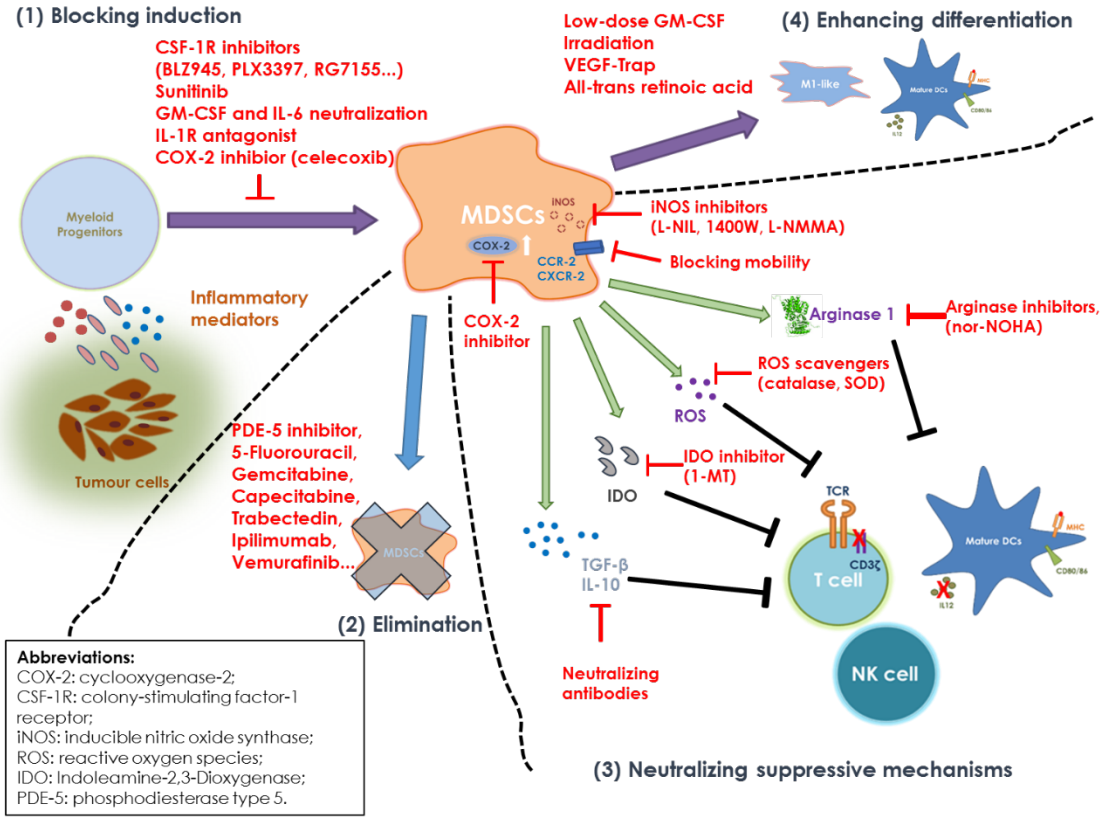


Figure 3, Targeting strategies for suppressive myeloid cells in cancers.

myeloid cells [323]. A therapeutic antibody (Calumab) against CCL-2 has been tested in a phase II clinical trial in metastatic prostate cancer patients. The response rate was poor, which could be due to the insufficient neutralization of CCL-2 in patients [312]. To overcome this problem, CCR-2, the receptor for CCL-2 has been evaluated as an alternative target. Indeed, blocking CCR-2 with a therapeutic antibody depleted MDSCs from tumor-bearing mice and synergized with adoptive CD8+ T cell transfer [268].

Another important migratory molecule is CXCR-2, which is essential for recruiting myeloid cells during inflammation-driven tumorigenesis [343]. In mice, limiting CXCR-2 functions on circulating myeloid cells greatly prevented their infiltration into tumor tissues [339] and boosted the anti-tumor effects of anti-PD-1 blockade [338].

4.4.5 Reprogramming activation

Myeloid cells are extremely plastic and their functions are substantially influenced by the surrounding factors. Monocytes isolated from blood could be primed *in vitro* to immune-stimulatory DCs for cancer treatment or acquire tolerogenic properties for combating autoimmune diseases. Thus, an appealing approach for cancer treatment is to promote the re-activation of suppressive myeloid cells *in vivo*. To some extent, this could be achieved by using GM-CSF, which enabled the maturation of MDSCs to DCs [236]. However, it should be carefully calibrated since high-dose GM-CSF may support the expansion of MDSCs *in vivo* [237]. Another agent that has potent reprogramming function of suppressive myeloid cells is all-trans-retinoic acid (ATRA), which is structurally similar to vitamin A and is used to treat various malignancies [344]. It could induce a DC-like phenotype and trigger IL-12 production from monocytes *in vitro* [345] and enhance *in vivo* efficacy of cancer vaccines [346]. When tested in patients with renal cell carcinoma or lung cancers, MDSCs were diminished from the blood, potentially due to maturation towards functional DCs [315-317] mediated by the intra-cellular accumulation of glutathione [347]. Moreover, a VEGF blocker (VEGF-trap) [348] has also promoted the maturation of DCs in cancer patients, but it did not decrease the numbers of MDSCs [311].

4.4.6 To Kill two birds with one stone

Suppressive myeloid cells possess multi-faceted functions in sustaining cancer occurrence, progression and metastasis and are one of the major barriers for successful therapeutic interventions in cancer immunotherapy. To date, tremendous efforts have been invested to design and validate pharmacological compounds that could efficiently target these mechanisms. In brief, four main strategies, including **1)** blocking the induction, **2)** eliminating the presence, **3)** disarming the suppressive machinery and **4)** facilitating the maturation, have been proposed (**Figure 3**). From my point of view, it is risky to unselectively neutralize immune modulatory factors, since many of them, such as TGF-β, ROS, PGE2 or iNOS, also play pivotal physiological roles in humans. Although elimination of suppressive myeloid cells has been observed in patients receiving certain anti-cancer agents, the mechanistic details are yet to be clarified. Therefore, it might be more plausible to combine approaches that limit tumor-driven induction of suppressive myeloid cells, with stimulatory signals that potentiate their functional maturation. Together, this may not only remove immunosuppressive barricades, but also create an environment that is favorable for anti-tumor immunity, both in the periphery and in the tumor microenvironment.

5. IMMUNOTHERAPY: WHERE ARE WE HEADING?

5.1 INTRODUCTION

Combating cancers with the patient's own immune system is being gradually acknowledged as an attractive therapeutic option. However, a range of hurdles need to be overcome for achieving curative effects in patients with advanced solid tumors. Throughout this thesis, I have highlighted the challenges and opportunities posed by myeloid cells with immunosuppressive properties. However, it is important to keep in mind that this is only one of the many mechanisms that tumor cells employ to prevent effective immune clearance. We also cannot ignore that different cancer types may have distinct inflammatory profiles, leading to diversified immune landscapes. Here, I will handpick some emerging aspects that hold promises towards improved efficacy of cancer immunotherapy.

5.2 COMBINATION THERAPY: Going Beyond the Immunity

5.2.1 Restoration of immune functions

The unprecedented success of cancer immunotherapy has raised excitements in redesigning current cancer treatment regimens. One general notion is to pursue a '1+1>2' scenario, where the existing therapeutics that trigger anti-tumor immunity could be used simultaneously to boost the effects of immunotherapy.

Chemotherapeutics and radiation therapy are known to modulate immune cell functions including the myeloid compartment (section 4.4.1). In addition, these treatments are thought to increase the permeability of solid tumor tissues and directly regulate molecular properties of cancer cells, making them more sensitive to immune cell mediated lysis [349]. Indeed, synergistic effects could be achieved when chemotherapy or radiation was combined with checkpoint blocking antibodies [350-353]. Because high-dose cytostatic drugs or radiotherapy could limit immune cell functions by inducing cell death, it may be more reasonable to implement the 'metronomic dosing' [354] of the agents in such combinations. This notion was supported by observations in preclinical models, where fractionated radiotherapy, but not a single high dose, boosted the anti-tumor effects of anti-CTLA-4 treatment [355]. These synergistic effects of fractionated radiotherapy with checkpoint blockade, particularly for the PD-1/PD-L1 axis could be explained by the modulation of PD-L1 expression on tumor cells [350].

Besides suppressive myeloid cells, many players in the cancer-induced immunosuppressive network could be targeted in the combinatorial approaches [356]. For example, immunosuppressive regulatory T cells (Tregs) may cause the failure of efficient immune surveillance against cancer cells. Depletion of Tregs has long been investigated as a potential treatment strategy for cancers [357-359]. The anti-tumor effects of anti-CTLA-4 mAb in mice were partially due to removal of Tregs by ADCC. Moreover, inhibitors for key suppressive mechanisms, such as IDO, have also generated additive effects as part of combinational approaches in preclinical models [70, 71, 360].

5.2.2 Correction of vasculature

Initially, anti-angiogenic therapy was believed to function through exhausting nutrient supplies for cancer cells by destroying their blood supplies. However, later evidence indicated that normalization of vascular architecture in the solid tumor microenvironment might be more beneficial [361], possibly due to the repolarization of M2-like macrophages and enhanced destruction through infiltrating immune cells [362, 363]. VEGF was previously shown to have a direct role on T cell development

and induced exhaustion of CD8+ T cells in tumors [364-366]. In accordance, blocking VEGF potentiated anti-tumor effects of DC vaccines [367] and adoptive T cell therapy [368] in murine models and supported significantly longer patient survival when combined with chemotherapy [369]. In melanoma patients, anti-angiogenic antibody could enhance immune cell infiltration after ipilimumab treatment [370]. Similar effects could be expected when VEGF blockade is combined with inhibition of the PD-1/PD-L1 pathway. In a recent report, tumor-derived VEGF collaborated with IL-10 and PGE2 to induce death ligand expression on endothelial cells, which resulted in apoptosis of infiltrating CD8+ T cells [371]. Consequently, combining COX-2 inhibitor with anti-VEGF antibody abolished these mechanisms and restored anti-tumor immunity.

5.2.3 Multi-tasking therapeutics

Antibody engineering technology allows production of artificial proteins that merge two antigen specificities. Typically, one part of the 'bi-specific' antibody recognizes tumor-associated surface proteins, while the other part could trigger T cell activation, for example through CD3 signaling. In addition, some products contain the Fc domain, which engages ADCC mediated by NK cells or macrophages. Therefore, bi-specific antibodies are extremely potent in directing tumor-specific killing through multiple cytotoxic machineries [372]. The first bi-specific antibody approved for clinical usage was catumaxomab, which recognized EpCAM and simultaneously triggered T cell activation through CD3 signaling [373]. Based on similar concepts, a collection of bi-specific antibodies were designed and investigated for cancer treatments [372].

However, severe adverse events induced by bi-specific antibodies hamper the clinical application in a wider range of cancer patients. This is partially due to the anti-CD3 fragment, which elicits excessive T cell activation *in vivo*. Decreased dosing could alleviate toxicity but also jeopardize the anti-tumor efficacy. In a preclinical model, this issue could be compensated by combining low-dose anti-GD2 bi-specific antibody with DC vaccines [374]. Alternatively, such toxicity might be attenuated if the anti-CD3 domain is replaced by fragments that liberate tumor-reactive T cells from inhibitory pathways, such as PD-1 or PD-L1 signaling. This approach may facilitate an antigen-directed activation of T cells in the proximity to tumor cells, which could be further supported by effector cells engaged through ADCC. For cancer types that lack common antigens, multiple immune checkpoint blocking fragments, or fragments potentiating reprogramming of suppressive myeloid cells, such as anti-CSF-1R, could be collaborated.

Another novel concept that is currently under clinical development is the production of high-affinity, antigen-specific monoclonal TCRs. These proteins recognize defined epitopes of tumor-associated antigens presented by MHC class I molecules. The linked anti-CD3 ScFv could attract and activate T cells to conduct specific lysis of the tumor cells [375, 376]. In comparison to monoclonal antibodies, this approach could target antigens that are derived intra-cellularly. It also circumvents the laborious preparation procedure of TCR-transduced T cells required for adoptive T cell therapy.

Besides engineered biological products, some naturally existing proteins could also play multi-faceted roles in directing immune responses. CD80 provides co-stimulation through CD28 during T cell priming, which is often interrupted by immune checkpoint molecules in the tumor microenvironment. Thus, the soluble form of CD80 protein is likely to restore T cell functions by blocking immune checkpoint interactions, as well as offering additional co-stimulatory signals. Indeed, a CD80-Fc fusion protein improved functions of human and murine T cells, even more pronounced than blocking antibodies against the PD-1/PD-L1 axis [377, 378]. This indicates that previously unidentified receptors are involved in CD80 ligation. However, CD80 has been

reported to be expressed at high levels on the surface of MDSCs in cancer patients [253] and tumor-bearing mice [379], and has been proposed to be one of the suppressive mechanisms against T cells. Of note, the CD80-Fc fusion protein activated, rather than inhibited human and murine T cell functions *in vitro*. This suggests that membrane-bound CD80 may have distinct biological functions to the soluble proteins. Even though the *in vivo* efficacy remains to be seen, the CD80-Fc fusion protein may amplify anti-tumor capacity via elimination of Tregs or PD-L1+ cells by ADCC.

5.2.4 Risks analysis

Novel concepts of combination therapy are accompanied with previously unrecorded concerns and clinical complications. For example, concurrently administrating blocking antibodies for CTLA-4 and PD-1 amplified the autoimmune toxicity associated with either antibody alone [109]. In another case, devastating liver toxicity was reported when inhibition of *BRAF* oncogene mutation was combined with ipilimumab [380]. These cases restate the necessity of conducting risk assessments while new combinatorial approaches are being clinically investigated, even if the individual treatments have been approved separately by the regulatory agencies.

5.3 TECHNOLOGICAL ADVANCES

Currently, the enthusiasm towards cancer immunotherapy is immense. However, we still cannot underestimate challenges from immune suppression and the potential risk in using immune-stimulatory agents that elicit immune responses unselectively. Thus, we need to be able to improve exclusively tumor-specific immunity and accurately manipulate immunosuppressive mechanisms. To reach this goal, we need technical advances that enable comprehensive analysis of the human immune system and precise modulation of immune cell subsets.

5.3.1 Biomaterials and immunotherapy

Advances in biomedical material research hold great promises in improving clinical efficacy and safety of cancer immunotherapeutics. Encapsulation of biological products, for example cytokines or antibodies, into engineered nanoscale vehicles, could optimize their *in vivo* stability and pharmacokinetics. This is particularly attractive for agents that have severe systemic adverse effects. In a proof-of-principle study, a nanoporous material supported gradual release of the anti-CTLA-4 mAb *in vivo* and the anti-tumor effects were improved [381]. It is also possible to equip nanoparticles with multiple immune stimulatory properties, creating controllable doses of personalized therapeutic ‘cocktails’. For example, nanoparticles conjugated with co-stimulatory anti-CD137 mAb and IL-2 induced profound anti-tumor effects in tumor-bearing mice [382]. When decorated with ‘anchors’ recognizing surface molecules on tumor cells, it is possible for the systemically injected nanoparticles to locally deliver agents that otherwise induce systemic adverse events. Similarly, nanoparticles could be used to maintain *in vivo* activity of adoptively transferred anti-tumor effector cells by specific delivery of immune activating factors [383, 384]. Moreover, it is possible to specifically and more efficiently target or reprogram suppressive myeloid cells using these approaches.

Some naturally occurring nanoscale vesicles could also be used as novel therapeutic approaches. Exosomes are released as ‘messengers’ from biologically functional cells and encapsulate contents that could conduct versatile properties on the immune system. Tumor-derived exosomes have been shown to induce suppressive myeloid cells by delivering factors such as PGE2, TGF-β [385] or membrane-bound Hsp72 [386]. On the other hand, exosomes shed from DCs carry co-stimulatory molecules

and are able to stimulate antigen-specific immune responses. Therefore, it has economically attractive to utilize these exosome as the DC-surrogates in treating cancer patients [387]. Several studies have proven that exosomes derived from DCs were immune stimulatory and potentiated *in vivo* protective effects in tumor-bearing mice [388, 389], through activation of T and B cells [390, 391].

Currently, the majority of cancer vaccines inject peptides, proteins or DNA plasmids that contain potential T cell epitopes directly into patients. In most cases, this approach elicits protective immune responses against the given antigen(s), but has modest therapeutic effects against established tumors [392]. Thus, encapsulating tumor-associated antigens into biomedical materials may be advantageous for cancer vaccine approaches by prolonging *in vivo* exposure, specific delivery to APCs or enabling co-delivery of adjuvants [393]. A number of studies focused on delivering antigens and adjuvants to residing DCs in lymph nodes [394, 395]. An emerging perspective is to program dendritic cells *in situ* by implanting nano-scaffolds containing tumor-associated antigens [396]. A recent update from the same group utilized nano-scaffolds with self-assembling properties after implantation. This allowed formation of a 3D mesoporous structure, where immune cells from the host animal could be primed against tumor-associated antigens [397]. This resulted in controlled and durable release of immune activating contents and recruited substantial tumor-rejecting humoral and cellular immune mechanisms.

5.3.2 Mega-analysis of immune responses

The immunological response to cancer occurrence or therapeutic interventions is a fine-tuned network of numerous parallel events. Contents in the extracellular matrix, cell surface proteins or intracellular signaling pathways collaboratively govern the success of treatment strategies. Therefore, a comprehensive overview of these components and the subsequent signaling cascades has substantial prognostic and therapeutic implications in guiding the development of cancer immunotherapy.

Development of multi-color flow cytometry was a milestone achievement and this method is currently widely used for analyzing immunological profile in cancer patients. Using fluorochrome-conjugated antibodies, a sophisticatedly designed flow cytometry platform allows detection of 10 to 15 proteins simultaneously. When appropriate lineage markers are included, the results reflect cellular properties of a defined immune cell subset at a given time. However, immune cell populations are extremely heterogeneous and analysis of large numbers of functional pathways are also required to accurately dissect major disease- or treatment-related cellular alternations. Therefore, technological advances empowering massive data-recording and processing are in great demand.

Cytometry by Time-of-Flight (CyToF) is a powerful cell detection method with significantly improved protein detection capacity. Instead of fluorophores, antibodies are labeled with element isotopes and recorded by subsequent mass spectrometry [398, 399]. This approach potentiates measurement of up to (theoretically) 100 parameters at the same time and circumvents the compensation step, a procedure that is required for correcting spectral overlaps among different fluorochromes. In one of the first studies using this technology, 34 parameters were characterized by CyToF, in order to depict the hematopoietic hierarchy and response to pharmacological inhibitors [400]. A later study analyzed the virus-specific CD8+ T cells and identified previously less appreciated complexity within the population [401]. Application of CyToF technology has also been extended for imaging tumor tissues. Recently, 32 parameters were measured simultaneously in breast cancer tumor tissues and the extremely heterogeneous sub-populations in the tumor microenvironment could be

delineated [402]. Although these platforms are not currently applicable to fulfill routine clinical demands, they hold great potential to reveal vital information towards in-depth understanding of the anti-tumor immunity.

Given the heterogeneity of myeloid compartment, CyToF platform may offer a powerful tool to scrutinize the regulatory network during the development and activation of suppressive myeloid cells. In addition, anti-tumor T cell responses after immunotherapy could be better illustrated not only in the peripheral blood, but also in solid tumor tissues. This may allow us to uncover novel therapeutic targets and prognostic markers that facilitate our understanding on tumor-induced immune suppression and guide the development of novel treatment strategies.

5.3.3 Precise genome editing

The CRISPR-Cas9 system is a natural defensive mechanism utilized by bacteria and archaea, in order to prevent incorporation of foreign DNAs into their own genomes [403]. Guided by a short RNA sequence, the Cas9 endonuclease could use molecular scissors to cut on a precise point and disable the functions of invading DNAs. With appropriate engineering, the CRISPR-Cas9 system could be used as a tool to modify genome on the desired locations accurately [404]. It has shown promising clinical implications, particularly for correcting genetic flaws in human stem cells [405, 406]. For the treatment of cancers, some studies encourage direct injection of CRISPR-Cas9 *in vivo*, which targets and corrects cancer-driven mutations. However, this approach should be carefully evaluated since the injected agents could be neutralized by the host's immune system, thus may have low penetration into the tumor tissues.

Seattle-based corporation Dendreon, known to develop the first FDA-approved DC vaccine approach, claimed bankruptcy at the end of 2014. The high treatment cost and the modest clinical benefits might be the main hurdles for Provenge, their prostate cancer vaccine, to be commercially appealing for a large number of patients. It definitely does not discredit the clinical efficacy of DC-based therapies. Rather it reflected the challenges of implementing cell-based therapies in the real-life scenario.

Alternatively, RNA-guided genome editing may be utilized to improve immune cell functions against human cancers. After acquisition of the GMP facilities from Dendreon, pharmaceutical giant Novartis is leading the way to evaluate CD19-CAR T cells for the treatment of hematological malignances in a phase II clinical trial. It is now becoming clear that the CRISPR-Cas9 technology will be incorporated into this treatment. Even though the detailed applications are not yet disclosed, a few potential modifications could be speculated. Firstly, the current treatment strategy of CD19-CARs requires isolation and transduction of autologous T cells for each individual patient. It is a labor-intensive procedure that requires tremendous amounts of dedication and expertise. Therefore, if the HLA class I molecules and the intrinsic TCRs could be silenced from the CAR-transduced T cells, it will be possible to prepare universal CD19-CAR T cell products that are not destroyed by the host's immune system or perturb graft-versus-host reactions. This could be a key step to implement the treatment in a more standardized and cost-effective manner. Secondly, certain molecules hampering *in vivo* functions of the adoptively transferred T cells, for example PD-1 or CTLA-4, could be removed using the CRISPR-Cas9 system. This step restricts the functional enhancement to tumor-reactive T cells, avoiding unselective activation of T cells often induced by checkpoint blocking antibodies. Furthermore, upon establishment, the genome-editing tools could modify genes that are crucial for the *in vivo* durability of the adoptively transferred T cells, such as *Ppp2r2d*. This might be less critical for the success of CD19-CAR T cells but could have substantial implications for adoptive cell therapies against solid tumors.

5.4 INTERDISCIPLINARY FRAMEWORK FOR CANCER IMMUNOTHERAPY

In the modern day cancer research, the rigid boundaries among research disciplines are diminishing. Although studies of cancer genetics are still the mainstay for many cancer types, associations between genomic instability and inflammation have been elucidated. Powerful next generation sequencing platforms are now employed to pinpoint mutations that may contain neo-epitopes that guide potent T cell responses. Moreover, development of high through-put analytical approaches, such as CyToF, requires specialists in bioinformatics for reliable data interpretation and validation. Rapid advances in biotechnology and molecular biology have broadened the genetic editing arsenal with superior accuracy and specificity. Nano-technology inventions promise greater future potency and safety for today's medicine. Although these are just very few examples, it is evident to me that tumor immunologists can no longer dissect complicated research questions and develop effective anti-cancer therapies without key contributions from other research disciplines. The interdisciplinary framework that marries a wide range of expertise and know-how today, is the foundation for an improved patient survival tomorrow.

6. SUMMARY OF THE MAJOR FINDINGS

The central aim of this thesis is to elucidate how tumor-derived factors could induce suppressive functions from healthy myeloid cells. The detailed interactions between patient-derived MDSCs and T cells, NK cells and DCs were analyzed in **study I**, **II** and **III**, respectively. **Study IV** extended this evaluation to the myelopoiesis process of human hematopoietic stem cells and suppressive myeloid cells were explored as a therapeutic target in mice developing aggressive spontaneous tumors.

6.1 **Study I:** TUMOR-DRIVEN INDUCTION OF MDSC IS MEDIATED BY COX-2/PGE2

Motivation

Human melanoma is believed to be one of the cancer types that is responsive to immunotherapy due to its high mutation rates associated with UV-induced damages. We and many other groups have noted the accumulation of suppressive MDSCs in the peripheral blood of melanoma patients and these cells may hamper effective anti-tumor immunity. However, the mechanisms of their induction remain unclear. Thus, we sought to analyze the precise pathways that regulate the induction of MDSCs.

Experimental Design

To dissect the detailed mechanisms, I established a co-culture model, where primary human monocytes from healthy donors were exposed to early-passage melanoma tumor cell lines expanded from fresh patient biopsies. Next, the tumor-educated monocytes were retrieved from cultures and their phenotype and T cell inhibitory functions were evaluated. A variety of pharmacological inhibitors were added during the co-culture, to analyze the key regulators driving tumor-induced alterations.

To assure that our *in vitro* findings were clinically relevant, we purified monocytes from blood of patients with advanced melanoma. These cells were subsequently added to autologous T cells and the suppressive mechanisms were compared to those obtained from *in vitro* induced MDSC-like cells.

Main Findings

After *in vitro* co-culture with early-passage melanoma tumor cells, healthy monocytes acquired a phenotype resembling $CD14^{+}HLA\text{-}DR^{low/-}$ moMDSCs in melanoma patients. In addition, MDSC-like cells and patient-derived monocytes potently inhibited

T cell effector functions. Mechanistically, COX-2/PGE2 pathway was involved both during the induction of MDSC-like cells and inhibition of T cells because a selective COX-2 inhibitor (Celecoxib) or a PGE2 neutralizing antibody could restore T cell functions. Finally, adding synthetic PGE2 to monocytes increased their ability to inhibit T cells.

Outlook of the Study

One major limitation of the co-culture model was that it did not reflect the impact of tumor-derived factors on myelopoiesis, which may directly contribute to the immobilization of progenitor cells and production of immature myeloid cells in the peripheral organs. In addition, we utilized a panel of inhibitors, in order to dissect the main channels that led to the induction of MDSCs. However, selection of these inhibitors were based on previously reported pathways. Thus, a comprehensive comparison between control monocytes and MDSC-like cells by genomic or proteomic screening may identify novel pathways that are involved in this process.

6.2 **Study II:** MDSCS SUPPRESS NK CELLS THROUGH TGF-β

Motivation

NK cells play an important role in modulating tumor growth and metastasis and could be used as a therapeutic approach to treat human cancers. Although interactions between MDSCs and T cells have been frequently reported, the effect of MDSCs on NK cells is controversial. Therefore, we aimed to analyze the precise interplay between these two immune cell types in patients with melanoma.

Experimental Design

We utilized PGE2-treated human monocytes and autologous NK cells as an *in vitro* model to study their interactions. To validate the mechanisms, we performed experiments using moMDSCs and NK cells isolated from blood of advanced stage melanoma patients. Further, we inoculated control or COX-2-silenced murine tumors in mice and compared the frequencies of MDSCs by flow cytometry and *in vivo* cytolytic capacity of NK cells by live imaging in tumor-bearing mice.

Main Findings

We demonstrated that PGE2 could trigger the production of soluble TGF-β from healthy monocytes through the PGE2 receptors EP2 and EP4. This was the major NK suppressive mechanism utilized by PGE2-treated monocytes and moMDSCs from melanoma patients. *In vivo*, COX-2 silencing resulted in a marked decrease of MDSCs in the spleens and a concurrent increase of cytotoxicity mediated by NK cells in the tumor-bearing mice.

Relevance of the Study

Currently, the impact of cancer-driven MDSCs on NK cells in tumor-bearing mice is controversial. MDSCs were reported to suppress NK cells [407] through membrane-bound TGF-β [408]. However, another report indicated the stimulatory role of MDSCs on NK cells in a NKG2D-dependent manner [409]. In melanoma patients, interplay between MDSCs and NK cells, particularly the molecular mechanisms, are much less investigated.

Our results demonstrate how tumor-derived PGE2 could drive the induction of MDSCs that suppress NK cells in patients with advanced melanoma. It revealed a previously unknown connection between PGE2 and the release of TGF-β from human primary monocytes. COX-2 inhibitors may have off-target effects or a direct impact on the suppressive functions of MDSCs. Thus, our approach using tumor cells with genetic ablation of COX-2 *in vivo* allowed specific analysis of the impact of tumor-derived COX-2/PGE2 on the induction of MDSCs and inhibition on NK cells. In summary, this study expanded our current understanding on the multi-faceted role of tumor-derived PGE2, which recruited MDSCs to suppress NK cells in cancer patients. Thus, targeting COX-2/PGE2 pathway could synergize with the anti-tumor effects of adoptive NK cell therapy.

6.3 **Study III:** MDSCS IMPAIR THE MATURATION OF DENDRITIC CELLS

Motivation

Currently, DC-based therapy often relies on maturation of monocytes purified from blood of cancer patients. Inevitably, moMDSCs, with known ability to suppress various types of immune cells, will be present during the process. Thus, it is important to evaluate the role of moMDSCs and how their presence could influence quality of the resulting DC vaccines.

Experimental Design

The clinical materials were obtained from stage IV melanoma patients who were enrolled in a phase I clinical study (MAT01) [191], where DC vaccines were combined with adoptive T cell transfer. Patients underwent leukapheresis and monocytes and lymphocytes were enriched in different cell fractions by elutriation. Monocytic MDSCs were isolated based on the low surface expression of HLA-DR and HLA-DR+ cells from the same donor were used as controls. Next, moMDSCs were mixed with HLA-DR+ cells at escalating ratios, to evaluate their influence on the quality of DC maturation.

Main Findings

We found that the presence of moMDSCs did not alter the yield of DCs but had a negative impact on DC quality. In detail, high frequency of moMDSCs at the beginning of DC maturation procedure blocked the up-regulation of activation markers and co-stimulatory signals on DCs and impaired the ability of DCs to take up antigens, to migrate and stimulate T cell functions.

Relevance of the Study

This study is part of an ongoing clinical trial, where patients received cellular therapy combining DC vaccines and adoptive T cell therapy. Our data provided critical information regarding the impact of moMDSCs on the overall quality of DC-based vaccines. Even though moMDSCs could be matured upon stimulation, their performance was sub-optimal in comparison to the HLA-DR+ cells. In addition, their presence had a 'by-stander' effect, which negatively affected the maturation of HLA-DR+ cells. Based on these results, there is an ongoing research project in our group, where optimized protocols for DC maturation are being tested.

6.4 **Study IV:** CSF-1R INHIBITION AS A POTENT APPROACH TO BOOST ANTI-TUMOR IMMUNITY

Motivation

Neuroblastoma is one of the most common solid cancer type among infants. Despite the aggressive therapies, high-risk patients still suffer from poor survival and there is a great need for novel treatments. Inflammation-driven expansion of suppressive myeloid cells play pivotal roles in neuroblastoma-mediated immune suppression and these cells could independently predict worse clinical outcome in neuroblastoma patients [231]. Thus, it might be a suitable cancer type for therapeutic interventions by targeting these cells.

As mentioned in earlier sections, it is challenging to target suppressive myeloid cells specifically due to their heterogeneous nature. CSF-1R signaling is important for the survival and development of myeloid cells and we sought to inhibit this pathway to limit the induction of suppressive myeloid cells.

Experimental Design

To evaluate the immune system in neuroblastoma patients, in particular the myeloid compartment, we analyzed publically available expression datasets by searching the R2 database (see 6.5.3). In order to elucidate the impact of neuroblastoma-derived factors on the differentiation of myeloid cells, we established 3 *in vitro* models (**Figure 4**), where human CD34+ hematopoietic stem cells, primary monocytes and murine bone marrow cells were cultured in the presence of neuroblastoma tumor-conditioned medium (TCM). In addition, inhibitors of CSF-1R signaling were added into these cultures and the subsequent phenotypic and functional changes of myeloid cells were evaluated. To test the therapeutic potential of CSF-1R inhibition *in vivo*, we employed a mouse model, in which human oncogene *MYCN* drives the development of highly

aggressive spontaneous neuroblastoma from adrenal medullas. Mice bearing palpable abdominal tumors were treated with a selective CSF-1R inhibitor, BLZ945, or in combination with checkpoint blocking antibodies against the PD-1/PD-L1 axis. After 10 days, tumor weights and the immunological profiles were compared among groups.

Main Findings

In comparison to benign neurofibroma tissues, we found that neuroblastoma tissues expressed higher levels of M-CSF and CSF-1R. Indeed, both M-CSF and CSF-1R+ myeloid cells predicted poor survival in patients without prior treatments. These findings were validated using *in vitro* culture models, where CSF-1R signaling was proven to modulate myelopoiesis of human CD34+ hematopoietic stem cells and murine bone marrow cells and enabled suppressive functions from primary human monocytes. When mice were treated with CSF-1R inhibitor in the therapeutic setting, the progression of highly aggressive tumors was efficiently controlled. This was mainly due to the specific effects on suppressive myeloid cells *in vivo*. To our surprise, checkpoint blocking antibodies against PD-1/PD-L1 had no impact on the suppressive myeloid cells and failed to control tumor growth in the TH-*MYCN* mice. Combining CSF-1R inhibition with checkpoint inhibitors enabled striking anti-tumor effects that were superior to either treatment alone.

Relevance of the Study

It is well-appreciated that tumor-derived factors facilitate the engraftment of hematopoietic stem cells in the periphery, leading to splenic production of immature myeloid cells in mice [410]. However, these effects were less understood in humans. In this study, we comprehensively elucidated the impact of CSF-1R signaling on early differentiation and local activation of suppressive myeloid cells in humans and mice. These models could be potentially applicable to dissect myeloid cell functions in various types of solid tumors.

According to published findings using the TH-*MYCN* murine model of high-risk human neuroblastoma, it is extremely difficult to achieve meaningful therapeutic effects due to the known short treatment window and rapid disease progression once the tumors are established and palpable [411]. Although targeting suppressive myeloid cells have long been considered as an attractive option to improve cancer immunotherapy in other cancer types, our study is the first documentation of the *in vivo* synergistic potency between a small molecule CSF-1R inhibitor and checkpoint blocking antibodies in controlling aggressive progression of spontaneous tumors. In fact, the relative success in novel treatment of these animals resulted in prolonged survival but none of the animals was cured or even showed shrinkage of tumors contrasting our current results [412, 413]. Therefore, the study has a unique strength in that the combination therapy resulted in outstanding curative effects in 75% of the treated mice and prevented the aggressive progression of large tumors among the remaining ones.

Given that the therapeutic efficacy of PD-1 blockade and the prognostic values of suppressive myeloid cells are being tested in various human cancers, this study may have a broad implication for the therapy of human cancers.

6.5 TECHNICAL DETAILS

6.5.1 In vitro models to study suppressive myeloid cells in humans and mice

To investigate the induction of suppressive myeloid cells, three *in vitro* models were established, where human primary monocytes, cord blood-derived CD34+ hematopoietic progenitor cells or murine bone marrow cells were cultured in the presence of tumor-derived factors (**Figure 4**).

The model co-culturing primary human monocytes with tumor cell lines reflected how differentiated myeloid cells could gain suppressive functions under the influence of tumor-derived factors. To maximize the physiological relevance, freshly isolated melanoma tumor cells were expanded from lymph node metastases of advanced stage melanoma patients and utilized at early passages (<7 passages).

The monocyte-tumor co-cultures have limited power to reflect the influence of tumor-derived factors on myelopoiesis, which may result in accumulation of immature suppressive myeloid cells in the periphery. To overcome this issue, I established an *in vitro* model, where CD34+ hematopoietic stem cells purified from cord blood were matured in the presence of tumor-conditioned medium. This allows us to more accurately dissect the impact of tumor-derived factors on the early differentiation of the myeloid compartment.

Based on several previously published reports [243, 414], we utilized an additional *in vitro* model, where murine bone marrow cells were cultured in the presence of tumor-conditioned medium. This essential model offered a time effective method that allowed us to select myeloid-modulating compounds before testing in the *in vivo* models.

Collectively, these three independent approaches enabled us to gain in-depth understanding of the induction and functions of suppressive myeloid cells in cancer patients and tumor-bearing animals. More importantly, we were able to select compounds in a cost- and time-efficient manner that may have a greater *in vivo*

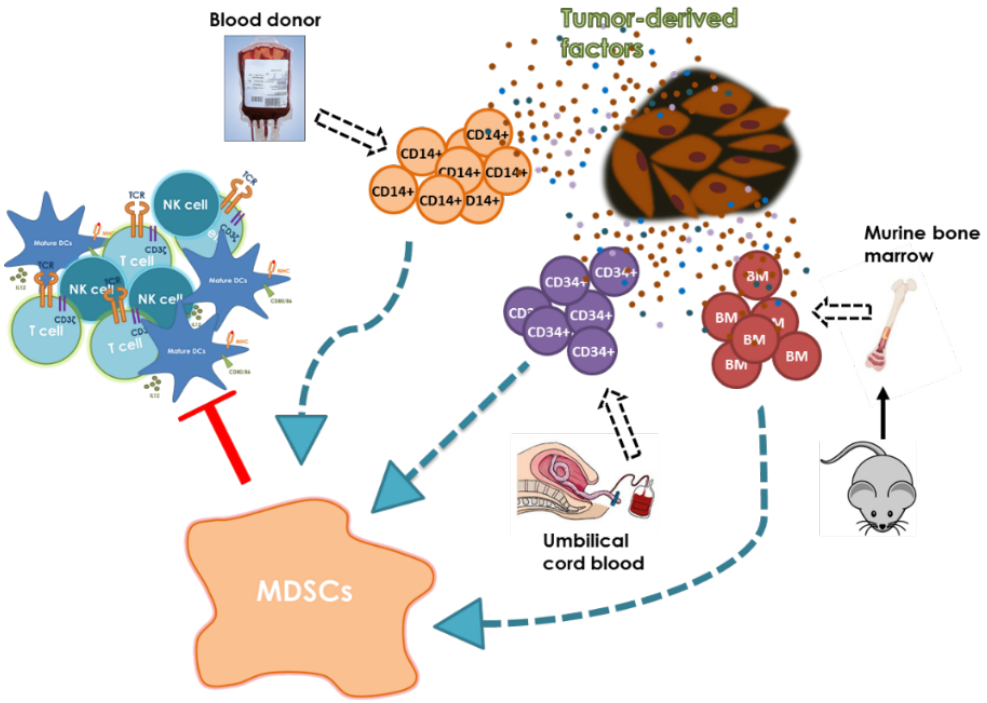


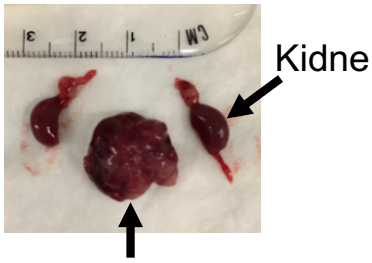
Figure 4, *In vitro* models to study tumor-induced differentiation of suppressive myeloid cells.

impact.

6.5.2 The TH-MYCN neuroblastoma murine model

The transgenic murine model that spontaneously develops neuroblastoma was established by Weiss *et al.* in 1997 [415]. In this model, human proto-oncogene *MYCN* is controlled by tyrosine hydroxylase promotor (TH-*MYCN*), which is active during early development of migrating cells in the neural crest [416]. Thus, TH-*MYCN* transgenic mice express high levels of human MYCN protein in the adrenal gland and paraspinal ganglia, which results in induction of spontaneous neuroblastoma tumors that resemble the aggressive growth pattern of high-risk neuroblastoma patients. Following the tumor incidence and treatment window suggested by a previous study [417], mice received 3 abdominal palpations per week and spontaneously arising tumors were harvested 10 days after detection. As shown in **Figure 5**, large spontaneous tumors arise from the adrenal gland in these mice and situate between kidneys in the abdominal cavity. The non-treated control mice on average developed tumors weighing 1.5 grams, approximately 7.5 % of the body mass (20 grams).

Figure 5, Representative



6.5.3 The R2 database

The 'R2: microarray analysis and visualization platform (http://r2.amc.nl)' is a public database containing enormous amounts of valuable expression datasets extracted from various types of healthy tissues, tumor tissues or cell lines. The purpose of this database is to create a user-friendly environment for biologists to test relevant hypotheses using existing datasets. In certain patient datasets, additional pathological information or clinical outcome of the patient cohorts are also available. Thus, this platform provides an extremely efficient tool for researchers who lack immediate access to clinical samples. In **study IV** of this thesis, we have retrieved key clinical information supporting the relevance of targeting CSF-1R+ myeloid cells in human neuroblastoma datasets. However, given that genomic information cannot completely reflect dynamic changes of the biological network, we have extensively validated the results by protein detection methods or assays enabling functional evaluations at the cellular level or in the *in vivo* environment.

7. ACKNOWLEDGEMENTS

I consider myself extremely fortunate since so many people have offered generous support or expressed genuine interests towards my research. Of course the pages below are far from sufficient to express all my appreciations.

The supervisors

Rolf, it really has been a privilege to spend these years in your lab and I truly enjoyed every single day of it. I am sincerely grateful for the opportunity and freedom to pursue research ideas that excite me. Your support and encouragements have always been prompt whenever necessary. Not only has my doctoral training equipped me with the critical scientific knowledge, but also it broadened my horizon immensely as an independent researcher.

Andreas, I have always enjoyed the possibility to knock on your door and discuss burning thoughts or share exciting progresses. You kept your 'cool' and was open-minded even towards some of my 'craziest' suggestions. Your mentoring style has made the work fun and encouraged my creativity.

Isabel, you introduced me to some of the most important techniques I know today and your presence catalyzed my smooth integration into the projects and the group. You have nicely demonstrated the key features of a successful doctoral training and I continuously benefit from your sharp comments and constructive criticisms.

Karl-Erik and **Ingegerd Hellström**, your mentoring in Seattle initiated my scientific adventure in the field of tumor immunology. Even though we no longer work together, I have never stopped receiving guidance and encouragements from you. I am one of the many who have been inspired by your life-long passion and dedication towards research.

Key contributors to study II and IV

Dhifaf, from the very first day, you impressed me with your tremendous motivation on research. But what really inspired me, is your maturity and persistence under difficult circumstances. These qualities have made our collaboration efficient and highly enjoyable.

Nina, you are one of the most dedicated and well-trained scientists and I truly appreciate your high level of research integrity and scientific credentials. Your endless efforts in our collaboration have contributed some of the most exciting results in this thesis and pushed the project to a completely different level.

The tumor immunology society on the CCK floor 01

My personal development has been stimulated by the unique scientific 'micro-environment' on the first floor. Every previous and present member of the Kiessling/Lundqvist group has in her or his own way influenced my work. It is indeed a luxury for me to not only maintain positive work relations, but also establish personal connections with many of my colleagues.

Maarten, it has really been a pleasure to have you as one of my fellow PhD students from the beginning. I am always amazed by your enthusiasm on research and the amounts of effort you are willing to invest on helping others. I look forward to your success after summer! **Erik** (broshan!), your positive attitude and great sense of humor have in many occasions rescued me from devastating situations. **Yago**, we travelled most often to international conferences and you are the expert to upgrade days from interesting to AWESOME. **Tom**, you impressed me during your internship and I hope your talents in research will come across your bright medical career in the future. **Tanja**, thanks for sharing with me your expertise on T cells and also the fascinating stories from the north. **Veronika**, thanks for being well-organized and energetic as the next dedicated FACS guru. **Kristina**, I am sure you will produce super NK cells in no time. **Helena**, I always enjoyed the time when we could exchange thoughts over a cup of coffee. **Jeroen**, I appreciate that you have broadened my view with your knowledge and **Yuya**, I am sure you will help a lot of patients in Japan. **Maria** and **Ulrika**, your essential daily contributions are critical for the function of the entire group. Finally, thanks to the good time with the recent group members **Bettina**, **Kevin**, and **Iva**.

Previous postdoctoral fellows **Dimi, Riki, Alvaro, Behavesh, Simona, Chiara and Takashi,** you all have substantially influenced my research, especially in the beginning. My work was also supported by the excellent previous group members, **Maxi-Lu, Jochen, Özcan, Nicole, Christina, Madhura, Giusy, Sara, Renee, Mamud, Franziska** and **Aline.**

Collaborators and friends

Special thanks to the neuroblastoma experts, **Per, John Inge, Lena-Maria, Linda, Jelena, Anna, Malin, Cecilia** and **Lotta**, for the support and interest on our collaboration. **Ola, Laia, Vincent, Shuo** and **Carl**, thank you for your patience in introducing me to the mysterious RNA world and being so great hosts at the SciLife pub. **Xiaonan** and **Stig**, it was fantastic to get acquainted with the powerful seahorse platform. Inclusion of the precious patient materials was made possible by the efficient clinical partnership with **Johan, Giuseppe, Maria Wolodarski, Roger, Suzanne** and **Jana**.

Majken, **Tatjana, Jeanette**, **Emma** and **Charlotte**, myeloid cells are the way to go! Thanks to the extremely friendly floor members **Tina, Torbjörn, Anders, Du Juan, Cecilia, Nathalie, Linnea, Andreas, Lars** and congratulations to **Nikolas** for the recent promotion as a father! In addition, the Thursday floor Fika has always been enjoyable with **Elle, Elizabeth, Lars Adamson, Amir, Mohammed, Barbro, Ingrid, Ann, Salam, Kia, Eva-Lena** and **Fariba.**

My time at CCK has been made very interactive by the pub crew, **Sara, Sophia, Nathalie, Ran,** and the OPUS editorial members, **Emma** (the boss), **Matheus** and **Iryna**. **Mahdi**, thanks for coaching me on how to get into shape in the gym. I appreciate the friendship established in Stockholm with **张烨，刘超，烁哥，沁子姐，毕然，王宇，Alireza, Pinar, Mindy, Patricia, Mikael, Gianluca, Xinsong, Caroline, Lidi, Mei and Qiang**.

Also for the advice and help received from **Lars Holmgren, Angelo, Dan,** and **Erika**. And thanks for the tricks and tips on fixing the FACS machine from **Juan**.

Lastly, to my external mentor **Karin Aasa**, I highly value your career advice that help me to develop my professional qualifications.

External collaborators

Stanley, it was my huge pleasure to host you twice here in Stockholm and it intrigued me every time we discussed the work. **Barbara** and **Andre**, your excellent contributions in study II are always highly appreciated. **Mike**, I appreciate your insightful comments and a great sense of humor.

I also thank **Novartis** for providing the BLZ945 compound for the study IV and the scientific discussions with Novartis researchers **Jim** and **Marion**.

致我的家人 (To my family)

爸爸和妈妈，如果没有你们对我从小到大孜孜不倦的督促和教导，很难想象我会在学业上有今天的成绩。自从我出国以来，你们竭尽所能的在精神上鼓励我，在经济上支持我，所以我才可能放手追逐自己的兴趣和梦想。我特别感激的是，你们总在尝试理解我的想法和接纳我的决定，这对我来说是非常宝贵的支持。另外，我很幸运现在每次回国还能有九十岁高龄的**姥姥和姥爷**的关爱和陪伴，你们是全家人的纽带和精神支柱。**大舅，舅妈，霁姐和 John**，我很感激当年在西雅图时你们对我的照顾和支持。**二舅，小舅，雷，雯姐和两位舅妈**，我从小就在你们的关爱中长大。即便是现在，你们也在尽可能的对我的生活和工作提供帮助。这些不求回报的关怀是我前进的动力。因为篇幅有限，我没办法对亲朋好友们一一致谢，但是你们的帮助和鼓励我都会铭记在心！

The Eissler Family

Nina, you are a person with such a gentle heart and you sincerely care about people around you. It calms me down every time I look into your beautiful eyes. I can't describe how lucky I have been to have your support during my PhD. **Maria**, you welcomed me every time with open arms and delicious wines. Your courage and dedication always inspired me. **Katrin** and **Christoph**, and your adorable daughters **Hannah** and **Mathilda**, thank you for the great

hospitality and you make my every visit memorable. I am sure you will enjoy the summer here! **Tilo** and **Ruth,** and your beautiful children **Inga** and **Jan,** it has always been a blast to meet you and share the joy in life.

Funding agencies

The six travel grants from **Radiumhemmets Forskningsfonder** allowed me to develop by sharing my research around the globe. In addition, part of the study IV was supported by a grant from the **Robert Lundberg's Memorial Foundation**.

The studies included in this thesis are generously supported by grants received from the Swedish Cancer Society, the Swedish Medical Research Council, the Cancer Society of Stockholm, the ALF-project grant from Stockholm City Council, the Knut and Alice Wallenberg's Foundation, the Karolinska-sponsored Center for Immune Modulatory Therapies for Autoimmunity and Cancer (IMTAC), the Swedish Childhood Cancer Foundation, and the Neuroblastoma and CNS Tumor Network of Sweden (NBCNS).

8. CITED ARTICLES

1. Bell, E., *Memory responses: Fitter, faster, better innate immune cells.* Nat Rev Immunol, 2014. **14**(8): p. 517.
2. Matzinger, P., *The danger model: a renewed sense of self.* Science, 2002. **296**(5566): p. 301-5.
3. Lodoen, M.B. and L.L. Lanier, *Viral modulation of NK cell immunity.* Nat Rev Microbiol, 2005. **3**(1): p. 59-69.
4. Karre, K., et al., *Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy.* Nature, 1986. **319**(6055): p. 675-8.
5. Freud, A.G. and M.A. Caligiuri, *Human natural killer cell development.* Immunol Rev, 2006. **214**: p. 56-72.
6. Peng, H., et al., *Liver-resident NK cells confer adaptive immunity in skin-contact inflammation.* J Clin Invest, 2013. **123**(4): p. 1444-56.
7. O'Leary, J.G., et al., *T cell- and B cell-independent adaptive immunity mediated by natural killer cells.* Nat Immunol, 2006. **7**(5): p. 507-16.
8. Paust, S., et al., *Critical role for the chemokine receptor CXCR6 in NK cell-mediated antigen-specific memory of haptens and viruses.* Nat Immunol, 2010. **11**(12): p. 1127-35.
9. Romee, R., et al., *Cytokine activation induces human memory-like NK cells.* Blood, 2012. **120**(24): p. 4751-60.
10. Paust, S. and U.H. von Andrian, *Natural killer cell memory.* Nat Immunol, 2011. **12**(6): p. 500-8.
11. Trinchieri, G., *Interleukin-12: a cytokine produced by antigen-presenting cells with immunoregulatory functions in the generation of T-helper cells type 1 and cytotoxic lymphocytes.* Blood, 1994. **84**(12): p. 4008-27.
12. Enrlich, P., *Ueber den jetzigen stand der karzinomforschung.* Ned. Tijdschr. Geneeskd., 1909. **5**: p. 273-290.
13. Burnet, M., *Cancer: a biological approach. III. Viruses associated with neoplastic conditions. IV. Practical applications.* Br Med J, 1957. **1**(5023): p. 841-7.
14. Burnet, M., *Cancer; a biological approach. I. The processes of control.* Br Med J, 1957. **1**(5022): p. 779-86.
15. Rygaard, J. and C.O. Povlsen, *The mouse mutant nude does not develop spontaneous tumours. An argument against immunological surveillance.* Acta Pathol Microbiol Scand B Microbiol Immunol, 1974. **82**(1): p. 99-106.
16. Stutman, O., *Tumor development after 3-methylcholanthrene in immunologically deficient athymic-nude mice.* Science, 1974. **183**(4124): p. 534-6.
17. Klein, G., *Immune surveillance--a powerful mechanism with a limited range.* Natl Cancer Inst Monogr, 1976. **44**: p. 109-13.
18. Qin, Z. and T. Blankenstein, *A cancer immunosurveillance controversy.* Nat Immunol, 2004. **5**(1): p. 3-4; author reply 4-5.
19. Ikehara, S., et al., *Functional T cells in athymic nude mice.* Proc Natl Acad Sci U S A, 1984. **81**(3): p. 886-8.
20. Maleckar, J.R. and L.A. Sherman, *The composition of the T cell receptor repertoire in nude mice.* J Immunol, 1987. **138**(11): p. 3873-6.
21. Engel, A.M., et al., *Methylcholanthrene-induced sarcomas in nude mice have short induction times and relatively low levels of surface MHC class I expression.* APMIS, 1996. **104**(9): p. 629-39.
22. Shankaran, V., et al., *IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity.* Nature, 2001. **410**(6832): p. 1107-11.
23. Shinkai, Y., et al., *RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement.* Cell, 1992. **68**(5): p. 855-67.
24. Smyth, M.J., et al., *Differential tumor surveillance by natural killer (NK) and NKT cells.* J Exp Med, 2000. **191**(4): p. 661-8.
25. Girardi, M., et al., *Regulation of cutaneous malignancy by gammadelta T cells.* Science, 2001. **294**(5542): p. 605-9.

26. Street, S.E., E. Cretney, and M.J. Smyth, *Perforin and interferon-gamma activities independently control tumor initiation, growth, and metastasis.* Blood, 2001. **97**(1): p. 192-7.
27. Street, S.E., et al., *Suppression of lymphoma and epithelial malignancies effected by interferon gamma.* J Exp Med, 2002. **196**(1): p. 129-34.
28. Kaplan, D.H., et al., *Demonstration of an interferon gamma-dependent tumor surveillance system in immunocompetent mice.* Proc Natl Acad Sci U S A, 1998. **95**(13): p. 7556-61.
29. Dunn, G.P., et al., *A critical function for type I interferons in cancer immunoediting.* Nat Immunol, 2005. **6**(7): p. 722-9.
30. van den Broek, M.E., et al., *Decreased tumor surveillance in perforin-deficient mice.* J Exp Med, 1996. **184**(5): p. 1781-90.
31. Dunn, G.P., et al., *Cancer immunoediting: from immunosurveillance to tumor escape.* Nat Immunol, 2002. **3**(11): p. 991-8.
32. Dunn, G.P., L.J. Old, and R.D. Schreiber, *The immunobiology of cancer immunosurveillance and immunoediting.* Immunity, 2004. **21**(2): p. 137-48.
33. Sakaguchi, S., et al., *Immunologic tolerance maintained by CD25+ CD4+ regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance.* Immunol Rev, 2001. **182**: p. 18-32.
34. Yamaguchi, T. and S. Sakaguchi, *Regulatory T cells in immune surveillance and treatment of cancer.* Semin Cancer Biol, 2006. **16**(2): p. 115-23.
35. Vignali, D.A., L.W. Collison, and C.J. Workman, *How regulatory T cells work.* Nat Rev Immunol, 2008. **8**(7): p. 523-32.
36. Sakaguchi, S., *Naturally arising CD4+ regulatory t cells for immunologic self-tolerance and negative control of immune responses.* Annu Rev Immunol, 2004. **22**: p. 531-62.
37. Piccirillo, C.A., et al., *CD4(+)CD25(+) regulatory T cells can mediate suppressor function in the absence of transforming growth factor beta1 production and responsiveness.* J Exp Med, 2002. **196**(2): p. 237-46.
38. Mougiakakos, D., et al., *Increased thioredoxin-1 production in human naturally occurring regulatory T cells confers enhanced tolerance to oxidative stress.* Blood, 2011. **117**(3): p. 857-61.
39. Ercolini, A.M., et al., *Recruitment of latent pools of high-avidity CD8(+) T cells to the antitumor immune response.* J Exp Med, 2005. **201**(10): p. 1591-602.
40. Chen, L., *Co-inhibitory molecules of the B7-CD28 family in the control of T-cell immunity.* Nat Rev Immunol, 2004. **4**(5): p. 336-47.
41. Alegre, M.L., K.A. Frauwirth, and C.B. Thompson, *T-cell regulation by CD28 and CTLA-4.* Nat Rev Immunol, 2001. **1**(3): p. 220-8.
42. Walker, L.S. and D.M. Sansom, *The emerging role of CTLA4 as a cell-extrinsic regulator of T cell responses.* Nat Rev Immunol, 2011. **11**(12): p. 852-63.
43. Butte, M.J., et al., *Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses.* Immunity, 2007. **27**(1): p. 111-22.
44. Qureshi, O.S., et al., *Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4.* Science, 2011. **332**(6029): p. 600-3.
45. Ishida, Y., et al., *Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death.* EMBO J, 1992. **11**(11): p. 3887-95.
46. Dong, H., et al., *Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion.* Nat Med, 2002. **8**(8): p. 793-800.
47. Freeman, G.J., et al., *Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation.* J Exp Med, 2000. **192**(7): p. 1027-34.
48. Tseng, S.Y., et al., *B7-DC, a new dendritic cell molecule with potent costimulatory properties for T cells.* J Exp Med, 2001. **193**(7): p. 839-46.
49. Latchman, Y., et al., *PD-L2 is a second ligand for PD-1 and inhibits T cell activation.* Nat Immunol, 2001. **2**(3): p. 261-8.
50. Sica, G.L., et al., *B7-H4, a molecule of the B7 family, negatively regulates T cell immunity.* Immunity, 2003. **18**(6): p. 849-61.

51. Zou, W. and L. Chen, *Inhibitory B7-family molecules in the tumour microenvironment.* Nat Rev Immunol, 2008. **8**(6): p. 467-77.
52. Liu, X., et al., *B7DC/PDL2 promotes tumor immunity by a PD-1-independent mechanism.* J Exp Med, 2003. **197**(12): p. 1721-30.
53. Driessens, G., J. Kline, and T.F. Gajewski, *Costimulatory and coinhibitory receptors in anti-tumor immunity.* Immunol Rev, 2009. **229**(1): p. 126-44.
54. Rodriguez, P.C., et al., *L-arginine consumption by macrophages modulates the expression of CD3 zeta chain in T lymphocytes.* J Immunol, 2003. **171**(3): p. 1232-9.
55. Bronte, V. and P. Zanovello, *Regulation of immune responses by L-arginine metabolism.* Nat Rev Immunol, 2005. **5**(8): p. 641-54.
56. MacMicking, J., Q.W. Xie, and C. Nathan, *Nitric oxide and macrophage function.* Annu Rev Immunol, 1997. **15**: p. 323-50.
57. Weinberg, J.B., *Nitric oxide production and nitric oxide synthase type 2 expression by human mononuclear phagocytes: a review.* Mol Med, 1998. **4**(9): p. 557-91.
58. Bogdan, C., *Nitric oxide and the immune response.* Nat Immunol, 2001. **2**(10): p. 907-16.
59. Klug, F., et al., *Low-dose irradiation programs macrophage differentiation to an iNOS(+)/M1 phenotype that orchestrates effective T cell immunotherapy.* Cancer Cell, 2013. **24**(5): p. 589-602.
60. Shimizu, T., et al., *Indoleamine 2,3-dioxygenase. Purification and some properties.* J Biol Chem, 1978. **253**(13): p. 4700-6.
61. Mellor, A.L. and D.H. Munn, *IDO expression by dendritic cells: tolerance and tryptophan catabolism.* Nat Rev Immunol, 2004. **4**(10): p. 762-74.
62. Terness, P., J.J. Chuang, and G. Opelz, *The immunoregulatory role of IDO-producing human dendritic cells revisited.* Trends Immunol, 2006. **27**(2): p. 68-73.
63. Prendergast, G.C., *Immune escape as a fundamental trait of cancer: focus on IDO.* Oncogene, 2008. **27**(28): p. 3889-900.
64. Braun, D., R.S. Longman, and M.L. Albert, *A two-step induction of indoleamine 2,3 dioxygenase (IDO) activity during dendritic-cell maturation.* Blood, 2005. **106**(7): p. 2375-81.
65. Marshall, B., D.B. Keskin, and A.L. Mellor, *Regulation of prostaglandin synthesis and cell adhesion by a tryptophan catabolizing enzyme.* BMC Biochem, 2001. **2**: p. 5.
66. Belladonna, M.L., et al., *Cutting edge: Autocrine TGF-beta sustains default tolerogenesis by IDO-competent dendritic cells.* J Immunol, 2008. **181**(8): p. 5194-8.
67. Mantovani, A., et al., *Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes.* Trends Immunol, 2002. **23**(11): p. 549-55.
68. Hou, D.Y., et al., *Inhibition of indoleamine 2,3-dioxygenase in dendritic cells by stereoisomers of 1-methyl-tryptophan correlates with antitumor responses.* Cancer Res, 2007. **67**(2): p. 792-801.
69. Muller, A.J., et al., *Inhibition of indoleamine 2,3-dioxygenase, an immunoregulatory target of the cancer suppression gene Bin1, potentiates cancer chemotherapy.* Nat Med, 2005. **11**(3): p. 312-9.
70. Holmgaard, R.B., et al., *Indoleamine 2,3-dioxygenase is a critical resistance mechanism in antitumor T cell immunotherapy targeting CTLA-4.* J Exp Med, 2013. **210**(7): p. 1389-402.
71. Spranger, S., et al., *Up-regulation of PD-L1, IDO, and T(regs) in the melanoma tumor microenvironment is driven by CD8(+) T cells.* Sci Transl Med, 2013. **5**(200): p. 200ra116.
72. Naito, Y., et al., *CD8+ T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer.* Cancer Res, 1998. **58**(16): p. 3491-4.
73. Sato, E., et al., *Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer.* Proc Natl Acad Sci U S A, 2005. **102**(51): p. 18538-43.
74. Haanen, J.B., et al., *Melanoma-specific tumor-infiltrating lymphocytes but not circulating melanoma-specific T cells may predict survival in resected advanced-stage melanoma patients.* Cancer Immunol Immunother, 2006. **55**(4): p. 451-8.

75. Ishigami, S., et al., *Prognostic value of intratumoral natural killer cells in gastric carcinoma.* Cancer, 2000. **88**(3): p. 577-83.
76. Kondo, E., et al., *Preoperative natural killer cell activity as a prognostic factor for distant metastasis following surgery for colon cancer.* Dig Surg, 2003. **20**(5): p. 445-51.
77. Villegas, F.R., et al., *Prognostic significance of tumor infiltrating natural killer cells subset CD57 in patients with squamous cell lung cancer.* Lung Cancer, 2002. **35**(1): p. 23-8.
78. Steidl, C., et al., *Tumor-associated macrophages and survival in classic Hodgkin's lymphoma.* N Engl J Med, 2010. **362**(10): p. 875-85.
79. Leek, R.D., et al., *Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma.* Cancer Res, 1996. **56**(20): p. 4625-9.
80. Bailey, C., et al., *Chemokine expression is associated with the accumulation of tumour associated macrophages (TAMs) and progression in human colorectal cancer.* Clin Exp Metastasis, 2007. **24**(2): p. 121-30.
81. Campbell, M.J., et al., *Proliferating macrophages associated with high grade, hormone receptor negative breast cancer and poor clinical outcome.* Breast Cancer Res Treat, 2011. **128**(3): p. 703-11.
82. Katz, S.C., et al., *Regulatory T cell infiltration predicts outcome following resection of colorectal cancer liver metastases.* Ann Surg Oncol, 2013. **20**(3): p. 946-55.
83. Nakagawa, K., et al., *Low infiltration of peritumoral regulatory T cells predicts worse outcome following resection of colorectal liver metastases.* Ann Surg Oncol, 2015. **22**(1): p. 180-6.
84. Adotevi, O., et al., *A decrease of regulatory T cells correlates with overall survival after sunitinib-based antiangiogenic therapy in metastatic renal cancer patients.* J Immunother, 2010. **33**(9): p. 991-8.
85. Forssell, J., et al., *High macrophage infiltration along the tumor front correlates with improved survival in colon cancer.* Clin Cancer Res, 2007. **13**(5): p. 1472-9.
86. Correale, P., et al., *Regulatory (FoxP3+) T-cell tumor infiltration is a favorable prognostic factor in advanced colon cancer patients undergoing chemo or chemoimmunotherapy.* J Immunother, 2010. **33**(4): p. 435-41.
87. Frey, D.M., et al., *High frequency of tumor-infiltrating FOXP3(+) regulatory T cells predicts improved survival in mismatch repair-proficient colorectal cancer patients.* Int J Cancer, 2010. **126**(11): p. 2635-43.
88. Galon, J., et al., *Type, density, and location of immune cells within human colorectal tumors predict clinical outcome.* Science, 2006. **313**(5795): p. 1960-4.
89. Pages, F., et al., *In situ cytotoxic and memory T cells predict outcome in patients with early-stage colorectal cancer.* J Clin Oncol, 2009. **27**(35): p. 5944-51.
90. Bindea, G., et al., *Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer.* Immunity, 2013. **39**(4): p. 782-95.
91. Galon, J., et al., *Towards the introduction of the 'Immunoscore' in the classification of malignant tumours.* J Pathol, 2014. **232**(2): p. 199-209.
92. Galon, J., et al., *Cancer classification using the Immunoscore: a worldwide task force.* J Transl Med, 2012. **10**: p. 205.
93. Leach, D.R., M.F. Krummel, and J.P. Allison, *Enhancement of antitumor immunity by CTLA-4 blockade.* Science, 1996. **271**(5256): p. 1734-6.
94. Walunas, T.L., et al., *CTLA-4 can function as a negative regulator of T cell activation.* Immunity, 1994. **1**(5): p. 405-13.
95. van Elsas, A., A.A. Hurwitz, and J.P. Allison, *Combination immunotherapy of B16 melanoma using anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing vaccines induces rejection of subcutaneous and metastatic tumors accompanied by autoimmune depigmentation.* J Exp Med, 1999. **190**(3): p. 355-66.
96. Hurwitz, A.A., et al., *Combination immunotherapy of primary prostate cancer in a transgenic mouse model using CTLA-4 blockade.* Cancer Res, 2000. **60**(9): p. 2444-8.

97. Hodi, F.S., et al., *Improved survival with ipilimumab in patients with metastatic melanoma.* N Engl J Med, 2010. **363**(8): p. 711-23.
98. Wolchok, J.D., et al., *Ipilimumab monotherapy in patients with pretreated advanced melanoma: a randomised, double-blind, multicentre, phase 2, dose-ranging study.* Lancet Oncol, 2010. **11**(2): p. 155-64.
99. Phan, G.Q., et al., *Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma.* Proc Natl Acad Sci U S A, 2003. **100**(14): p. 8372-7.
100. Laurent, S., et al., *The engagement of CTLA-4 on primary melanoma cell lines induces antibody-dependent cellular cytotoxicity and TNF-alpha production.* J Transl Med, 2013. **11**: p. 108.
101. Simpson, T.R., et al., *Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma.* J Exp Med, 2013. **210**(9): p. 1695-710.
102. Wang, C., et al., *In vitro characterization of the anti-PD-1 antibody nivolumab, BMS-936558, and in vivo toxicology in non-human primates.* Cancer Immunol Res, 2014. **2**(9): p. 846-56.
103. Furness, A.J., et al., *Impact of tumour microenvironment and Fc receptors on the activity of immunomodulatory antibodies.* Trends Immunol, 2014. **35**(7): p. 290-8.
104. Topalian, S.L., et al., *Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab.* J Clin Oncol, 2014. **32**(10): p. 1020-30.
105. Hamid, O., et al., *Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma.* N Engl J Med, 2013. **369**(2): p. 134-44.
106. Robert, C., et al., *Nivolumab in previously untreated melanoma without BRAF mutation.* N Engl J Med, 2015. **372**(4): p. 320-30.
107. Weber, J.S., et al., *Safety, efficacy, and biomarkers of nivolumab with vaccine in ipilimumab-refractory or -naive melanoma.* J Clin Oncol, 2013. **31**(34): p. 4311-8.
108. Robert, C., et al., *Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial.* Lancet, 2014. **384**(9948): p. 1109-17.
109. Wolchok, J.D., et al., *Nivolumab plus ipilimumab in advanced melanoma.* N Engl J Med, 2013. **369**(2): p. 122-33.
110. Okazaki, T., et al., *A rheostat for immune responses: the unique properties of PD-1 and their advantages for clinical application.* Nat Immunol, 2013. **14**(12): p. 1212-8.
111. Nishimura, H., et al., *Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor.* Immunity, 1999. **11**(2): p. 141-51.
112. Nishimura, H., et al., *Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice.* Science, 2001. **291**(5502): p. 319-22.
113. Tivol, E.A., et al., *Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4.* Immunity, 1995. **3**(5): p. 541-7.
114. Waterhouse, P., et al., *Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4.* Science, 1995. **270**(5238): p. 985-8.
115. Brahmer, J.R., et al., *Safety and activity of anti-PD-L1 antibody in patients with advanced cancer.* N Engl J Med, 2012. **366**(26): p. 2455-65.
116. Brahmer, J.R., et al., *Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates.* J Clin Oncol, 2010. **28**(19): p. 3167-75.
117. Powles, T., et al., *MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer.* Nature, 2014. **515**(7528): p. 558-62.
118. Herbst, R.S., et al., *Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients.* Nature, 2014. **515**(7528): p. 563-7.
119. Loke, P. and J.P. Allison, *PD-L1 and PD-L2 are differentially regulated by Th1 and Th2 cells.* Proc Natl Acad Sci U S A, 2003. **100**(9): p. 5336-41.

120. Chen, J., et al., *Interferon-gamma-induced PD-L1 surface expression on human oral squamous carcinoma via PKD2 signal pathway.* Immunobiology, 2012. **217**(4): p. 385-93.
121. Wimberly, H., et al., *PD-L1 expression correlates with tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy in breast cancer.* Cancer Immunol Res, 2014.
122. Kinter, A.L., et al., *The common gamma-chain cytokines IL-2, IL-7, IL-15, and IL-21 induce the expression of programmed death-1 and its ligands.* J Immunol, 2008. **181**(10): p. 6738-46.
123. Lesterhuis, W.J., et al., *Platinum-based drugs disrupt STAT6-mediated suppression of immune responses against cancer in humans and mice.* J Clin Invest, 2011. **121**(8): p. 3100-8.
124. Zhang, Y., et al., *Regulation of T cell activation and tolerance by PDL2.* Proc Natl Acad Sci U S A, 2006. **103**(31): p. 11695-700.
125. Salama, A.D., et al., *Critical role of the programmed death-1 (PD-1) pathway in regulation of experimental autoimmune encephalomyelitis.* J Exp Med, 2003. **198**(1): p. 71-8.
126. Parekh, V.V., et al., *PD-1/PD-L blockade prevents anergy induction and enhances the anti-tumor activities of glycolipid-activated invariant NKT cells.* J Immunol, 2009. **182**(5): p. 2816-26.
127. He, Y.F., et al., *Blocking programmed death-1 ligand-PD-1 interactions by local gene therapy results in enhancement of antitumor effect of secondary lymphoid tissue chemokine.* J Immunol, 2004. **173**(8): p. 4919-28.
128. Okudaira, K., et al., *Blockade of B7-H1 or B7-DC induces an anti-tumor effect in a mouse pancreatic cancer model.* Int J Oncol, 2009. **35**(4): p. 741-9.
129. Shin, T., et al., *In vivo costimulatory role of B7-DC in tuning T helper cell 1 and cytotoxic T lymphocyte responses.* J Exp Med, 2005. **201**(10): p. 1531-41.
130. Wolchok, J.D., et al., *Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria.* Clin Cancer Res, 2009. **15**(23): p. 7412-20.
131. Callahan, M.K., J.D. Wolchok, and J.P. Allison, *Anti-CTLA-4 antibody therapy: immune monitoring during clinical development of a novel immunotherapy.* Semin Oncol, 2010. **37**(5): p. 473-84.
132. Beck, K.E., et al., *Enterocolitis in patients with cancer after antibody blockade of cytotoxic T-lymphocyte-associated antigen 4.* J Clin Oncol, 2006. **24**(15): p. 2283-9.
133. Di Giacomo, A.M., M. Biagioli, and M. Maio, *The emerging toxicity profiles of anti-CTLA-4 antibodies across clinical indications.* Semin Oncol, 2010. **37**(5): p. 499-507.
134. Rosenberg, S.A., et al., *Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. A preliminary report.* N Engl J Med, 1988. **319**(25): p. 1676-80.
135. Rosenberg, S.A., et al., *Treatment of patients with metastatic melanoma with autologous tumor-infiltrating lymphocytes and interleukin 2.* J Natl Cancer Inst, 1994. **86**(15): p. 1159-66.
136. Schwartzentruber, D.J., et al., *In vitro predictors of therapeutic response in melanoma patients receiving tumor-infiltrating lymphocytes and interleukin-2.* J Clin Oncol, 1994. **12**(7): p. 1475-83.
137. Yee, C., et al., *Adoptive T cell therapy using antigen-specific CD8+ T cell clones for the treatment of patients with metastatic melanoma: in vivo persistence, migration, and antitumor effect of transferred T cells.* Proc Natl Acad Sci U S A, 2002. **99**(25): p. 16168-73.
138. Dudley, M.E., et al., *Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma.* J Clin Oncol, 2005. **23**(10): p. 2346-57.
139. Dudley, M.E., et al., *Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes.* Science, 2002. **298**(5594): p. 850-4.

140. Rosenberg, S.A., et al., *Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy.* Clin Cancer Res, 2011. **17**(13): p. 4550-7.
141. Morgan, R.A., et al., *Cancer regression in patients after transfer of genetically engineered lymphocytes.* Science, 2006. **314**(5796): p. 126-9.
142. Zhao, Y., et al., *Primary human lymphocytes transduced with NY-ESO-1 antigen-specific TCR genes recognize and kill diverse human tumor cell lines.* J Immunol, 2005. **174**(7): p. 4415-23.
143. Morgan, R.A., et al., *High efficiency TCR gene transfer into primary human lymphocytes affords avid recognition of melanoma tumor antigen glycoprotein 100 and does not alter the recognition of autologous melanoma antigens.* J Immunol, 2003. **171**(6): p. 3287-95.
144. Johnson, L.A., et al., *Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen.* Blood, 2009. **114**(3): p. 535-46.
145. Kershaw, M.H., et al., *A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer.* Clin Cancer Res, 2006. **12**(20 Pt 1): p. 6106-15.
146. Parkhurst, M.R., et al., *T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis.* Mol Ther, 2011. **19**(3): p. 620-6.
147. Robbins, P.F., et al., *Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1.* J Clin Oncol, 2011. **29**(7): p. 917-24.
148. Kuball, J., et al., *Cooperation of human tumor-reactive CD4+ and CD8+ T cells after redirection of their specificity by a high-affinity p53A2.1-specific TCR.* Immunity, 2005. **22**(1): p. 117-29.
149. Frankel, T.L., et al., *Both CD4 and CD8 T cells mediate equally effective in vivo tumor treatment when engineered with a highly avid TCR targeting tyrosinase.* J Immunol, 2010. **184**(11): p. 5988-98.
150. Urba, W.J. and D.L. Longo, *Redirecting T cells.* N Engl J Med, 2011. **365**(8): p. 754-7.
151. Sadelain, M., R. Brentjens, and I. Riviere, *The promise and potential pitfalls of chimeric antigen receptors.* Curr Opin Immunol, 2009. **21**(2): p. 215-23.
152. Porter, D.L., et al., *Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia.* N Engl J Med, 2011. **365**(8): p. 725-33.
153. Kochenderfer, J.N. and S.A. Rosenberg, *Treating B-cell cancer with T cells expressing anti-CD19 chimeric antigen receptors.* Nat Rev Clin Oncol, 2013. **10**(5): p. 267-76.
154. Kebriaei, P., et al., *Infusing CD19-directed T cells to augment disease control in patients undergoing autologous hematopoietic stem-cell transplantation for advanced B-lymphoid malignancies.* Hum Gene Ther, 2012. **23**(5): p. 444-50.
155. Kochenderfer, J.N., et al., *B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells.* Blood, 2012. **119**(12): p. 2709-20.
156. Maude, S.L., et al., *Chimeric antigen receptor T cells for sustained remissions in leukemia.* N Engl J Med, 2014. **371**(16): p. 1507-17.
157. Kandalaft, L.E., D.J. Powell, Jr., and G. Coukos, *A phase I clinical trial of adoptive transfer of folate receptor-alpha redirected autologous T cells for recurrent ovarian cancer.* J Transl Med, 2012. **10**: p. 157.
158. Lamers, C.H., et al., *Treatment of metastatic renal cell carcinoma with CAIX CAR-engineered T cells: clinical evaluation and management of on-target toxicity.* Mol Ther, 2013. **21**(4): p. 904-12.
159. Lamers, C.H., et al., *Process validation and clinical evaluation of a protocol to generate gene-modified T lymphocytes for imunogene therapy for metastatic renal cell carcinoma: GMP-controlled transduction and expansion of patient's T lymphocytes using a carboxy anhydrase IX-specific scFv transgene.* Cytotherapy, 2006. **8**(6): p. 542-53.

160. Lamers, C.H., et al., *Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: first clinical experience.* J Clin Oncol, 2006. **24**(13): p. e20-2.
161. Pule, M.A., et al., *Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma.* Nat Med, 2008. **14**(11): p. 1264-70.
162. Louis, C.U., et al., *Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma.* Blood, 2011. **118**(23): p. 6050-6.
163. Aranda, F., et al., *Trial Watch: Adoptive cell transfer for anticancer immunotherapy.* Oncoimmunology, 2014. **3**: p. e28344.
164. Vivier, E., et al., *Functions of natural killer cells.* Nat Immunol, 2008. **9**(5): p. 503-10.
165. Ruggeri, L., et al., *Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants.* Science, 2002. **295**(5562): p. 2097-100.
166. Miller, J.S., et al., *Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer.* Blood, 2005. **105**(8): p. 3051-7.
167. Ruggeri, L., et al., *Natural killer cell alloreactivity for leukemia therapy.* J Immunother, 2005. **28**(3): p. 175-82.
168. Iliopoulou, E.G., et al., *A phase I trial of adoptive transfer of allogeneic natural killer cells in patients with advanced non-small cell lung cancer.* Cancer Immunol Immunother, 2010. **59**(12): p. 1781-9.
169. Geller, M.A., et al., *A phase II study of allogeneic natural killer cell therapy to treat patients with recurrent ovarian and breast cancer.* Cytotherapy, 2011. **13**(1): p. 98-107.
170. Parkhurst, M.R., et al., *Adoptive transfer of autologous natural killer cells leads to high levels of circulating natural killer cells but does not mediate tumor regression.* Clin Cancer Res, 2011. **17**(19): p. 6287-97.
171. Lakomy, D., et al., *Cytotoxic dendritic cells generated from cancer patients.* J Immunol, 2011. **187**(5): p. 2775-82.
172. Manna, P.P. and T. Mohanakumar, *Human dendritic cell mediated cytotoxicity against breast carcinoma cells in vitro.* J Leukoc Biol, 2002. **72**(2): p. 312-20.
173. Banchereau, J., et al., *Immune and clinical responses in patients with metastatic melanoma to CD34(+) progenitor-derived dendritic cell vaccine.* Cancer Res, 2001. **61**(17): p. 6451-8.
174. Mackensen, A., et al., *Phase I study in melanoma patients of a vaccine with peptide-pulsed dendritic cells generated in vitro from CD34(+) hematopoietic progenitor cells.* Int J Cancer, 2000. **86**(3): p. 385-92.
175. Palucka, K. and J. Banchereau, *Dendritic-cell-based therapeutic cancer vaccines.* Immunity, 2013. **39**(1): p. 38-48.
176. Nestle, F.O., et al., *Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells.* Nat Med, 1998. **4**(3): p. 328-32.
177. Su, Z., et al., *Immunological and clinical responses in metastatic renal cancer patients vaccinated with tumor RNA-transfected dendritic cells.* Cancer Res, 2003. **63**(9): p. 2127-33.
178. Geiger, J.D., et al., *Vaccination of pediatric solid tumor patients with tumor lysate-pulsed dendritic cells can expand specific T cells and mediate tumor regression.* Cancer Res, 2001. **61**(23): p. 8513-9.
179. Kantoff, P.W., et al., *Sipuleucel-T immunotherapy for castration-resistant prostate cancer.* N Engl J Med, 2010. **363**(5): p. 411-22.
180. Small, E.J., et al., *Placebo-controlled phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone refractory prostate cancer.* J Clin Oncol, 2006. **24**(19): p. 3089-94.
181. Tel, J., et al., *Natural human plasmacytoid dendritic cells induce antigen-specific T-cell responses in melanoma patients.* Cancer Res, 2013. **73**(3): p. 1063-75.
182. Tel, J., et al., *Human plasmacytoid dendritic cells are equipped with antigen-presenting and tumoricidal capacities.* Blood, 2012. **120**(19): p. 3936-44.

183. Zhou, J., et al., *Telomere length of transferred lymphocytes correlates with in vivo persistence and tumor regression in melanoma patients receiving cell transfer therapy.* J Immunol, 2005. **175**(10): p. 7046-52.
184. Kalos, M., et al., *T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia.* Sci Transl Med, 2011. **3**(95): p. 95ra73.
185. Moon, E.K., et al., *Multifactorial T-cell hypofunction that is reversible can limit the efficacy of chimeric antigen receptor-transduced human T cells in solid tumors.* Clin Cancer Res, 2014. **20**(16): p. 4262-73.
186. Scholler, J., et al., *Decade-long safety and function of retroviral-modified chimeric antigen receptor T cells.* Sci Transl Med, 2012. **4**(132): p. 132ra53.
187. Klebanoff, C.A., et al., *Central memory self/tumor-reactive CD8+ T cells confer superior antitumor immunity compared with effector memory T cells.* Proc Natl Acad Sci U S A, 2005. **102**(27): p. 9571-6.
188. Conlon, K.C., et al., *Redistribution, Hyperproliferation, Activation of Natural Killer Cells and CD8 T Cells, and Cytokine Production During First-in-Human Clinical Trial of Recombinant Human Interleukin-15 in Patients With Cancer.* J Clin Oncol, 2015. **33**(1): p. 74-82.
189. Kesarwani, P., et al., *Promoting thiol expression increases the durability of antitumor T-cell functions.* Cancer Res, 2014. **74**(21): p. 6036-47.
190. Li, Y., et al., *IL-15 activates telomerase and minimizes telomere loss and may preserve the replicative life span of memory CD8+ T cells in vitro.* J Immunol, 2005. **174**(7): p. 4019-24.
191. Poschke, I., et al., *A phase I clinical trial combining dendritic cell vaccination with adoptive T cell transfer in patients with stage IV melanoma.* Cancer Immunol Immunother, 2014. **63**(10): p. 1061-71.
192. Chmielewski, M., et al., *IL-12 release by engineered T cells expressing chimeric antigen receptors can effectively Muster an antigen-independent macrophage response on tumor cells that have shut down tumor antigen expression.* Cancer Res, 2011. **71**(17): p. 5697-706.
193. Pegram, H.J., et al., *Tumor-targeted T cells modified to secrete IL-12 eradicate systemic tumors without need for prior conditioning.* Blood, 2012. **119**(18): p. 4133-41.
194. Zhou, P. and K.W. Wucherpfennig, *Discovering cancer immunotherapy targets in vivo.* Oncoimmunology, 2014. **3**: p. e28500.
195. Zhou, P., et al., *In vivo discovery of immunotherapy targets in the tumour microenvironment.* Nature, 2014. **506**(7486): p. 52-7.
196. Balkwill, F. and A. Mantovani, *Inflammation and cancer: back to Virchow?* Lancet, 2001. **357**(9255): p. 539-45.
197. Algra, A.M. and P.M. Rothwell, *Effects of regular aspirin on long-term cancer incidence and metastasis: a systematic comparison of evidence from observational studies versus randomised trials.* Lancet Oncol, 2012. **13**(5): p. 518-27.
198. Balkwill, F., K.A. Charles, and A. Mantovani, *Smoldering and polarized inflammation in the initiation and promotion of malignant disease.* Cancer Cell, 2005. **7**(3): p. 211-7.
199. Wyckoff, J.B., et al., *Direct visualization of macrophage-assisted tumor cell intravasation in mammary tumors.* Cancer Res, 2007. **67**(6): p. 2649-56.
200. Talmadge, J.E. and D.I. Gabrilovich, *History of myeloid-derived suppressor cells.* Nat Rev Cancer, 2013. **13**(10): p. 739-52.
201. Youn, J.I., et al., *Subsets of myeloid-derived suppressor cells in tumor-bearing mice.* J Immunol, 2008. **181**(8): p. 5791-802.
202. Poschke, I. and R. Kiessling, *On the armament and appearances of human myeloid-derived suppressor cells.* Clin Immunol, 2012. **144**(3): p. 250-68.
203. Gros, A., et al., *Myeloid cells obtained from the blood but not from the tumor can suppress T-cell proliferation in patients with melanoma.* Clin Cancer Res, 2012. **18**(19): p. 5212-23.

204. Gorgun, G.T., et al., *Tumor-promoting immune-suppressive myeloid-derived suppressor cells in the multiple myeloma microenvironment in humans.* Blood, 2013. **121**(15): p. 2975-87.
205. Vasquez-Dunddel, D., et al., *STAT3 regulates arginase-I in myeloid-derived suppressor cells from cancer patients.* J Clin Invest, 2013. **123**(4): p. 1580-9.
206. Qian, B.Z. and J.W. Pollard, *Macrophage diversity enhances tumor progression and metastasis.* Cell, 2010. **141**(1): p. 39-51.
207. Ostrand-Rosenberg, S. and P. Sinha, *Myeloid-derived suppressor cells: linking inflammation and cancer.* J Immunol, 2009. **182**(8): p. 4499-506.
208. Rohan, T.E., et al., *Tumor microenvironment of metastasis and risk of distant metastasis of breast cancer.* J Natl Cancer Inst, 2014. **106**(8).
209. Ohno, S., et al., *The degree of macrophage infiltration into the cancer cell nest is a significant predictor of survival in gastric cancer patients.* Anticancer Res, 2003. **23**(6D): p. 5015-22.
210. Zhou, Q., et al., *The density of macrophages in the invasive front is inversely correlated to liver metastasis in colon cancer.* J Transl Med, 2010. **8**: p. 13.
211. Jiang, H., et al., *Elevated chronic inflammatory factors and myeloid-derived suppressor cells indicate poor prognosis in advanced melanoma patients.* Int J Cancer, 2014.
212. Weide, B., et al., *Myeloid-derived suppressor cells predict survival of patients with advanced melanoma: comparison with regulatory T cells and NY-ESO-1- or melan-A-specific T cells.* Clin Cancer Res, 2014. **20**(6): p. 1601-9.
213. de Boniface, J., et al., *Expression patterns of the immunomodulatory enzyme arginase 1 in blood, lymph nodes and tumor tissue of early-stage breast cancer patients.* Oncoimmunology, 2012. **1**(8): p. 1305-1312.
214. Diaz-Montero, C.M., et al., *Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy.* Cancer Immunol Immunother, 2009. **58**(1): p. 49-59.
215. Cui, T.X., et al., *Myeloid-derived suppressor cells enhance stemness of cancer cells by inducing microRNA101 and suppressing the corepressor CtBP2.* Immunity, 2013. **39**(3): p. 611-21.
216. Solito, S., et al., *A human promyelocytic-like population is responsible for the immune suppression mediated by myeloid-derived suppressor cells.* Blood, 2011. **118**(8): p. 2254-65.
217. Huang, A., et al., *Increased CD14(+)HLA-DR (-/low) myeloid-derived suppressor cells correlate with extrathoracic metastasis and poor response to chemotherapy in non-small cell lung cancer patients.* Cancer Immunol Immunother, 2013. **62**(9): p. 1439-51.
218. Wu, W.C., et al., *Circulating hematopoietic stem and progenitor cells are myeloid-biased in cancer patients.* Proc Natl Acad Sci U S A, 2014. **111**(11): p. 4221-6.
219. Gabitass, R.F., et al., *Elevated myeloid-derived suppressor cells in pancreatic, esophageal and gastric cancer are an independent prognostic factor and are associated with significant elevation of the Th2 cytokine interleukin-13.* Cancer Immunol Immunother, 2011. **60**(10): p. 1419-30.
220. Kitano, S., et al., *Computational algorithm-driven evaluation of monocytic myeloid-derived suppressor cell frequency for prediction of clinical outcomes.* Cancer Immunol Res, 2014. **2**(8): p. 812-21.
221. Kimura, T., et al., *MUC1 vaccine for individuals with advanced adenoma of the colon: a cancer immunoprevention feasibility study.* Cancer Prev Res (Phila), 2013. **6**(1): p. 18-26.
222. Walter, S., et al., *Multipeptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival.* Nat Med, 2012. **18**(8): p. 1254-61.
223. Pico de Coana, Y., et al., *Ipilimumab treatment results in an early decrease in the frequency of circulating granulocytic myeloid-derived suppressor cells as well as their Arginase1 production.* Cancer Immunol Res, 2013. **1**(3): p. 158-62.

224. Meyer, C., et al., *Frequencies of circulating MDSC correlate with clinical outcome of melanoma patients treated with ipilimumab.* Cancer Immunol Immunother, 2014. **63**(3): p. 247-57.
225. Schmielau, J. and O.J. Finn, *Activated granulocytes and granulocyte-derived hydrogen peroxide are the underlying mechanism of suppression of t-cell function in advanced cancer patients.* Cancer Res, 2001. **61**(12): p. 4756-60.
226. Kotsakis, A., et al., *Myeloid-derived suppressor cell measurements in fresh and cryopreserved blood samples.* J Immunol Methods, 2012. **381**(1-2): p. 14-22.
227. Bronkhorst, I.H., et al., *Detection of M2-macrophages in uveal melanoma and relation with survival.* Invest Ophthalmol Vis Sci, 2011. **52**(2): p. 643-50.
228. Makitie, T., et al., *Tumor-infiltrating macrophages (CD68(+) cells) and prognosis in malignant uveal melanoma.* Invest Ophthalmol Vis Sci, 2001. **42**(7): p. 1414-21.
229. DeNardo, D.G., et al., *Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy.* Cancer Discov, 2011. **1**(1): p. 54-67.
230. Medrek, C., et al., *The presence of tumor associated macrophages in tumor stroma as a prognostic marker for breast cancer patients.* BMC Cancer, 2012. **12**: p. 306.
231. Asgharzadeh, S., et al., *Clinical significance of tumor-associated inflammatory cells in metastatic neuroblastoma.* J Clin Oncol, 2012. **30**(28): p. 3525-32.
232. Dranoff, G., *GM-CSF-based cancer vaccines.* Immunol Rev, 2002. **188**: p. 147-54.
233. Spitler, L.E., et al., *Adjuvant therapy of stage III and IV malignant melanoma using granulocyte-macrophage colony-stimulating factor.* J Clin Oncol, 2000. **18**(8): p. 1614-21.
234. Peoples, G.E., et al., *Combined clinical trial results of a HER2/neu (E75) vaccine for the prevention of recurrence in high-risk breast cancer patients: U.S. Military Cancer Institute Clinical Trials Group Study I-01 and I-02.* Clin Cancer Res, 2008. **14**(3): p. 797-803.
235. Kanerva, A., et al., *Antiviral and antitumor T-cell immunity in patients treated with GM-CSF-coding oncolytic adenovirus.* Clin Cancer Res, 2013. **19**(10): p. 2734-44.
236. Kaufman, H.L., et al., *Local and distant immunity induced by intralesional vaccination with an oncolytic herpes virus encoding GM-CSF in patients with stage IIIc and IV melanoma.* Ann Surg Oncol, 2010. **17**(3): p. 718-30.
237. Filipazzi, P., et al., *Identification of a new subset of myeloid suppressor cells in peripheral blood of melanoma patients with modulation by a granulocyte-macrophage colony-stimulation factor-based antitumor vaccine.* J Clin Oncol, 2007. **25**(18): p. 2546-53.
238. Parmiani, G., et al., *Opposite immune functions of GM-CSF administered as vaccine adjuvant in cancer patients.* Ann Oncol, 2007. **18**(2): p. 226-32.
239. Serafini, P., et al., *High-dose granulocyte-macrophage colony-stimulating factor-producing vaccines impair the immune response through the recruitment of myeloid suppressor cells.* Cancer Res, 2004. **64**(17): p. 6337-43.
240. Annels, N.E., et al., *The effects of gemcitabine and capecitabine combination chemotherapy and of low-dose adjuvant GM-CSF on the levels of myeloid-derived suppressor cells in patients with advanced pancreatic cancer.* Cancer Immunol Immunother, 2014. **63**(2): p. 175-83.
241. Zoso, A., et al., *Human fibrocytic myeloid-derived suppressor cells express IDO and promote tolerance via Treg-cell expansion.* Eur J Immunol, 2014.
242. Lechner, M.G., D.J. Liebertz, and A.L. Epstein, *Characterization of cytokine-induced myeloid-derived suppressor cells from normal human peripheral blood mononuclear cells.* J Immunol, 2010. **185**(4): p. 2273-84.
243. Bayne, L.J., et al., *Tumor-derived granulocyte-macrophage colony-stimulating factor regulates myeloid inflammation and T cell immunity in pancreatic cancer.* Cancer Cell, 2012. **21**(6): p. 822-35.
244. Harris, S.G., et al., *Prostaglandins as modulators of immunity.* Trends Immunol, 2002. **23**(3): p. 144-50.

245. Legler, D.F., et al., *Prostaglandin E2 is generally required for human dendritic cell migration and exerts its effect via EP2 and EP4 receptors.* J Immunol, 2006. **176**(2): p. 966-73.
246. Krause, P., et al., *Prostaglandin E2 is a key factor for monocyte-derived dendritic cell maturation: enhanced T cell stimulatory capacity despite IDO.* J Leukoc Biol, 2007. **82**(5): p. 1106-14.
247. Obermajer, N., et al., *PGE(2)-induced CXCL12 production and CXCR4 expression controls the accumulation of human MDSCs in ovarian cancer environment.* Cancer Res, 2011. **71**(24): p. 7463-70.
248. Mao, Y., et al., *Inhibition of tumor-derived prostaglandin-e2 blocks the induction of myeloid-derived suppressor cells and recovers natural killer cell activity.* Clin Cancer Res, 2014. **20**(15): p. 4096-106.
249. Lanzinger, M., et al., *Ambivalent effects of dendritic cells displaying prostaglandin E2-induced indoleamine 2,3-dioxygenase.* Eur J Immunol, 2012. **42**(5): p. 1117-28.
250. Mao, Y., et al., *Melanoma-educated CD14+ cells acquire a myeloid-derived suppressor cell phenotype through COX-2-dependent mechanisms.* Cancer Res, 2013. **73**(13): p. 3877-87.
251. Obermajer, N., et al., *Positive feedback between PGE2 and COX2 redirects the differentiation of human dendritic cells toward stable myeloid-derived suppressor cells.* Blood, 2011. **118**(20): p. 5498-505.
252. Cheon, H., et al., *Prostaglandin E2 augments IL-10 signaling and function.* J Immunol, 2006. **177**(2): p. 1092-100.
253. Poschke, I., et al., *Immature immunosuppressive CD14+HLA-DR-/low cells in melanoma patients are Stat3hi and overexpress CD80, CD83, and DC-sign.* Cancer Res, 2010. **70**(11): p. 4335-45.
254. Chittezhath, M., et al., *Molecular profiling reveals a tumor-promoting phenotype of monocytes and macrophages in human cancer progression.* Immunity, 2014. **41**(5): p. 815-29.
255. Elkabets, M., et al., *IL-1beta regulates a novel myeloid-derived suppressor cell subset that impairs NK cell development and function.* Eur J Immunol, 2010. **40**(12): p. 3347-57.
256. Bunt, S.K., et al., *Reduced inflammation in the tumor microenvironment delays the accumulation of myeloid-derived suppressor cells and limits tumor progression.* Cancer Res, 2007. **67**(20): p. 10019-26.
257. Bunt, S.K., et al., *Inflammation induces myeloid-derived suppressor cells that facilitate tumor progression.* J Immunol, 2006. **176**(1): p. 284-90.
258. Lechner, M.G., et al., *Functional characterization of human Cd33+ and Cd11b+ myeloid-derived suppressor cell subsets induced from peripheral blood mononuclear cells co-cultured with a diverse set of human tumor cell lines.* J Transl Med, 2011. **9**: p. 90.
259. Thakur, A., et al., *Microenvironment generated during EGFR targeted killing of pancreatic tumor cells by ATC inhibits myeloid-derived suppressor cells through COX2 and PGE2 dependent pathway.* J Transl Med, 2013. **11**: p. 35.
260. Rodrigues, J.C., et al., *Normal human monocytes exposed to glioma cells acquire myeloid-derived suppressor cell-like properties.* Neuro Oncol, 2010. **12**(4): p. 351-65.
261. Sinha, P., et al., *Prostaglandin E2 promotes tumor progression by inducing myeloid-derived suppressor cells.* Cancer Res, 2007. **67**(9): p. 4507-13.
262. Sinha, P., et al., *Proinflammatory S100 proteins regulate the accumulation of myeloid-derived suppressor cells.* J Immunol, 2008. **181**(7): p. 4666-75.
263. Parker, K.H., et al., *HMGB1 enhances immune suppression by facilitating the differentiation and suppressive activity of myeloid-derived suppressor cells.* Cancer Res, 2014. **74**(20): p. 5723-33.
264. Cheng, P., et al., *Inhibition of dendritic cell differentiation and accumulation of myeloid-derived suppressor cells in cancer is regulated by S100A9 protein.* J Exp Med, 2008. **205**(10): p. 2235-49.

265. Corzo, C.A., et al., *HIF-1alpha regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment.* J Exp Med, 2010. **207**(11): p. 2439-53.
266. Liu, G., et al., *SIRT1 limits the function and fate of myeloid-derived suppressor cells in tumors by orchestrating HIF-1alpha-dependent glycolysis.* Cancer Res, 2014. **74**(3): p. 727-37.
267. Bianchi, G., et al., *ATP/P2X7 axis modulates myeloid-derived suppressor cell functions in neuroblastoma microenvironment.* Cell Death Dis, 2014. **5**: p. e1135.
268. Lesokhin, A.M., et al., *Monocytic CCR2(+) myeloid-derived suppressor cells promote immune escape by limiting activated CD8 T-cell infiltration into the tumor microenvironment.* Cancer Res, 2012. **72**(4): p. 876-86.
269. Jayaraman, P., et al., *Tumor-expressed inducible nitric oxide synthase controls induction of functional myeloid-derived suppressor cells through modulation of vascular endothelial growth factor release.* J Immunol, 2012. **188**(11): p. 5365-76.
270. Noman, M.Z., et al., *PD-L1 is a novel direct target of HIF-1alpha, and its blockade under hypoxia enhanced MDSC-mediated T cell activation.* J Exp Med, 2014. **211**(5): p. 781-90.
271. Colegio, O.R., et al., *Functional polarization of tumour-associated macrophages by tumour-derived lactic acid.* Nature, 2014. **513**(7519): p. 559-63.
272. Youn, J.I., et al., *Epigenetic silencing of retinoblastoma gene regulates pathologic differentiation of myeloid cells in cancer.* Nat Immunol, 2013. **14**(3): p. 211-20.
273. Marenholz, I., C.W. Heizmann, and G. Fritz, *S100 proteins in mouse and man: from evolution to function and pathology (including an update of the nomenclature).* Biochem Biophys Res Commun, 2004. **322**(4): p. 1111-22.
274. Bresnick, A.R., D.J. Weber, and D.B. Zimmer, *S100 proteins in cancer.* Nat Rev Cancer, 2015. **15**(2): p. 96-109.
275. Wang, L., et al., *Increased myeloid-derived suppressor cells in gastric cancer correlate with cancer stage and plasma S100A8/A9 proinflammatory proteins.* J Immunol, 2013. **190**(2): p. 794-804.
276. Zhao, F., et al., *S100A9 a new marker for monocytic human myeloid-derived suppressor cells.* Immunology, 2012. **136**(2): p. 176-83.
277. Bjork, P., et al., *Identification of human S100A9 as a novel target for treatment of autoimmune disease via binding to quinoline-3-carboxamides.* PLoS Biol, 2009. **7**(4): p. e97.
278. Hermani, A., et al., *Calcium-binding proteins S100A8 and S100A9 as novel diagnostic markers in human prostate cancer.* Clin Cancer Res, 2005. **11**(14): p. 5146-52.
279. Pili, R., et al., *Phase II randomized, double-blind, placebo-controlled study of tasquinimod in men with minimally symptomatic metastatic castrate-resistant prostate cancer.* J Clin Oncol, 2011. **29**(30): p. 4022-8.
280. Bratt, O., et al., *Open-label, clinical phase I studies of tasquinimod in patients with castration-resistant prostate cancer.* Br J Cancer, 2009. **101**(8): p. 1233-40.
281. Sims, G.P., et al., *HMGB1 and RAGE in inflammation and cancer.* Annu Rev Immunol, 2010. **28**: p. 367-88.
282. Wang, H., et al., *HMG-1 as a late mediator of endotoxin lethality in mice.* Science, 1999. **285**(5425): p. 248-51.
283. Yanai, H., et al., *Conditional ablation of HMGB1 in mice reveals its protective function against endotoxemia and bacterial infection.* Proc Natl Acad Sci U S A, 2013. **110**(51): p. 20699-704.
284. Li, W., et al., *HMGB1 recruits myeloid derived suppressor cells to promote peritoneal dissemination of colon cancer after resection.* Biochem Biophys Res Commun, 2013. **436**(2): p. 156-61.
285. Jube, S., et al., *Cancer cell secretion of the DAMP protein HMGB1 supports progression in malignant mesothelioma.* Cancer Res, 2012. **72**(13): p. 3290-301.
286. Kang, R., et al., *The HMGB1/RAGE inflammatory pathway promotes pancreatic tumor growth by regulating mitochondrial bioenergetics.* Oncogene, 2014. **33**(5): p. 567-77.

287. Apetoh, L., et al., *Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy.* Nat Med, 2007. **13**(9): p. 1050-9.
288. He, Y., et al., *Tissue damage-associated "danger signals" influence T-cell responses that promote the progression of preneoplasia to cancer.* Cancer Res, 2013. **73**(2): p. 629-39.
289. Suzuki, Y., et al., *Immunogenic tumor cell death induced by chemoradiotherapy in patients with esophageal squamous cell carcinoma.* Cancer Res, 2012. **72**(16): p. 3967-76.
290. Doedens, A.L., et al., *Macrophage expression of hypoxia-inducible factor-1 alpha suppresses T-cell function and promotes tumor progression.* Cancer Res, 2010. **70**(19): p. 7465-75.
291. Ohashi, T., et al., *Dichloroacetate improves immune dysfunction caused by tumor-secreted lactic acid and increases antitumor immunoreactivity.* Int J Cancer, 2013. **133**(5): p. 1107-18.
292. Cairns, R.A., I.S. Harris, and T.W. Mak, *Regulation of cancer cell metabolism.* Nat Rev Cancer, 2011. **11**(2): p. 85-95.
293. Araki, K., et al., *mTOR regulates memory CD8 T-cell differentiation.* Nature, 2009. **460**(7251): p. 108-12.
294. Marcais, A., et al., *The metabolic checkpoint kinase mTOR is essential for IL-15 signaling during the development and activation of NK cells.* Nat Immunol, 2014. **15**(8): p. 749-57.
295. Hiratsuka, S., et al., *Tumour-mediated upregulation of chemoattractants and recruitment of myeloid cells predetermines lung metastasis.* Nat Cell Biol, 2006. **8**(12): p. 1369-75.
296. Chandramouli, A., et al., *The induction of S100p expression by the Prostaglandin E(2) (PGE(2))/EP4 receptor signaling pathway in colon cancer cells.* Cancer Biol Ther, 2010. **10**(10): p. 1056-66.
297. Miao, L., et al., *Prostaglandin E2 stimulates S100A8 expression by activating protein kinase A and CCAAT/enhancer-binding-protein-beta in prostate cancer cells.* Int J Biochem Cell Biol, 2012. **44**(11): p. 1919-28.
298. Grebhardt, S., et al., *Hypoxia and HIF-1 increase S100A8 and S100A9 expression in prostate cancer.* Int J Cancer, 2012. **131**(12): p. 2785-94.
299. Germano, G., et al., *Role of macrophage targeting in the antitumor activity of trabectedin.* Cancer Cell, 2013. **23**(2): p. 249-62.
300. Sevko, A., et al., *Cyclophosphamide promotes chronic inflammation-dependent immunosuppression and prevents antitumor response in melanoma.* J Invest Dermatol, 2013. **133**(6): p. 1610-9.
301. Strachan, D.C., et al., *CSF1R inhibition delays cervical and mammary tumor growth in murine models by attenuating the turnover of tumor-associated macrophages and enhancing infiltration by CD8 T cells.* Oncoimmunology, 2013. **2**(12): p. e26968.
302. Ding, Z.C., et al., *Immunosuppressive myeloid cells induced by chemotherapy attenuate antitumor CD4+ T-cell responses through the PD-1-PD-L1 axis.* Cancer Res, 2014. **74**(13): p. 3441-53.
303. Michels, T., et al., *Paclitaxel promotes differentiation of myeloid-derived suppressor cells into dendritic cells in vitro in a TLR4-independent manner.* J Immunotoxicol, 2012. **9**(3): p. 292-300.
304. Sevko, A., et al., *Application of paclitaxel in low non-cytotoxic doses supports vaccination with melanoma antigens in normal mice.* J Immunotoxicol, 2012. **9**(3): p. 275-81.
305. Sevko, A., et al., *Antitumor effect of paclitaxel is mediated by inhibition of myeloid-derived suppressor cells and chronic inflammation in the spontaneous melanoma model.* J Immunol, 2013. **190**(5): p. 2464-71.
306. Alizadeh, D., et al., *Doxorubicin eliminates myeloid-derived suppressor cells and enhances the efficacy of adoptive T-cell transfer in breast cancer.* Cancer Res, 2014. **74**(1): p. 104-18.

307. Vincent, J., et al., *5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumor immunity.* Cancer Res, 2010. **70**(8): p. 3052-61.
308. Schilling, B., et al., *Vemurafenib reverses immunosuppression by myeloid derived suppressor cells.* Int J Cancer, 2013. **133**(7): p. 1653-63.
309. Wang, Z., et al., *Association of myeloid-derived suppressor cells and efficacy of cytokine-induced killer cell immunotherapy in metastatic renal cell carcinoma patients.* J Immunother, 2014. **37**(1): p. 43-50.
310. Ries, C.H., et al., *Targeting tumor-associated macrophages with anti-CSF-1R antibody reveals a strategy for cancer therapy.* Cancer Cell, 2014. **25**(6): p. 846-59.
311. Fricke, I., et al., *Vascular endothelial growth factor-trap overcomes defects in dendritic cell differentiation but does not improve antigen-specific immune responses.* Clin Cancer Res, 2007. **13**(16): p. 4840-8.
312. Pienta, K.J., et al., *Phase 2 study of carlumab (CNTO 888), a human monoclonal antibody against CC-chemokine ligand 2 (CCL2), in metastatic castration-resistant prostate cancer.* Invest New Drugs, 2013. **31**(3): p. 760-8.
313. Ko, J.S., et al., *Sunitinib mediates reversal of myeloid-derived suppressor cell accumulation in renal cell carcinoma patients.* Clin Cancer Res, 2009. **15**(6): p. 2148-57.
314. van Cruijsen, H., et al., *Sunitinib-induced myeloid lineage redistribution in renal cell cancer patients: CD1c+ dendritic cell frequency predicts progression-free survival.* Clin Cancer Res, 2008. **14**(18): p. 5884-92.
315. Mirza, N., et al., *All-trans-retinoic acid improves differentiation of myeloid cells and immune response in cancer patients.* Cancer Res, 2006. **66**(18): p. 9299-307.
316. Kusmartsev, S., et al., *Reversal of myeloid cell-mediated immunosuppression in patients with metastatic renal cell carcinoma.* Clin Cancer Res, 2008. **14**(24): p. 8270-8.
317. Iclozan, C., et al., *Therapeutic regulation of myeloid-derived suppressor cells and immune response to cancer vaccine in patients with extensive stage small cell lung cancer.* Cancer Immunol Immunother, 2013. **62**(5): p. 909-18.
318. Lathers, D.M., et al., *Phase 1B study to improve immune responses in head and neck cancer patients using escalating doses of 25-hydroxyvitamin D3.* Cancer Immunol Immunother, 2004. **53**(5): p. 422-30.
319. Weed, D.T., et al., *Tadalafil reduces myeloid-derived suppressor cells and regulatory T cells and promotes tumor immunity in patients with head and neck squamous cell carcinoma.* Clin Cancer Res, 2015. **21**(1): p. 39-48.
320. Baur, A.S., et al., *Denileukin diftitox (ONTAK) induces a tolerogenic phenotype in dendritic cells and stimulates survival of resting Treg.* Blood, 2013. **122**(13): p. 2185-94.
321. Wobser, M., et al., *Dendritic cell based antitumor vaccination: impact of functional indoleamine 2,3-dioxygenase expression.* Cancer Immunol Immunother, 2007. **56**(7): p. 1017-24.
322. Pyonteck, S.M., et al., *CSF-1R inhibition alters macrophage polarization and blocks glioma progression.* Nat Med, 2013. **19**(10): p. 1264-72.
323. Qian, B.Z., et al., *CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis.* Nature, 2011. **475**(7355): p. 222-5.
324. Xu, J., et al., *CSF1R signaling blockade stanches tumor-infiltrating myeloid cells and improves the efficacy of radiotherapy in prostate cancer.* Cancer Res, 2013. **73**(9): p. 2782-94.
325. Zhu, Y., et al., *CSF1/CSF1R blockade reprograms tumor-infiltrating macrophages and improves response to T-cell checkpoint immunotherapy in pancreatic cancer models.* Cancer Res, 2014. **74**(18): p. 5057-69.
326. Mok, S., et al., *Inhibition of CSF-1 receptor improves the antitumor efficacy of adoptive cell transfer immunotherapy.* Cancer Res, 2014. **74**(1): p. 153-61.
327. Priceman, S.J., et al., *Targeting distinct tumor-infiltrating myeloid cells by inhibiting CSF-1 receptor: combating tumor evasion of antiangiogenic therapy.* Blood, 2010. **115**(7): p. 1461-71.

328. Ozao-Choy, J., et al., *The novel role of tyrosine kinase inhibitor in the reversal of immune suppression and modulation of tumor microenvironment for immune-based cancer therapies.* Cancer Res, 2009. **69**(6): p. 2514-22.
329. Qin, H., et al., *Generation of a new therapeutic peptide that depletes myeloid-derived suppressor cells in tumor-bearing mice.* Nat Med, 2014. **20**(6): p. 676-81.
330. Wu, J., et al., *Dopamine inhibits the function of Gr-1+CD115+ myeloid-derived suppressor cells through D1-like receptors and enhances anti-tumor immunity.* J Leukoc Biol, 2014.
331. Shen, L., et al., *Tasquinimod modulates suppressive myeloid cells and enhances cancer immunotherapies in murine models.* Cancer Immunol Res, 2014.
332. Meyer, C., et al., *Chronic inflammation promotes myeloid-derived suppressor cell activation blocking antitumor immunity in transgenic mouse melanoma model.* Proc Natl Acad Sci U S A, 2011. **108**(41): p. 17111-6.
333. Serafini, P., et al., *Phosphodiesterase-5 inhibition augments endogenous antitumor immunity by reducing myeloid-derived suppressor cell function.* J Exp Med, 2006. **203**(12): p. 2691-702.
334. Carlson, L.M., et al., *Low-dose aspirin delays an inflammatory tumor progression in vivo in a transgenic mouse model of neuroblastoma.* Carcinogenesis, 2013. **34**(5): p. 1081-8.
335. Fujita, M., et al., *COX-2 blockade suppresses gliomagenesis by inhibiting myeloid-derived suppressor cells.* Cancer Res, 2011. **71**(7): p. 2664-74.
336. Veltman, J.D., et al., *COX-2 inhibition improves immunotherapy and is associated with decreased numbers of myeloid-derived suppressor cells in mesothelioma. Celecoxib influences MDSC function.* BMC Cancer, 2010. **10**: p. 464.
337. Terabe, M., et al., *Transforming growth factor-beta production and myeloid cells are an effector mechanism through which CD1d-restricted T cells block cytotoxic T lymphocyte-mediated tumor immunosurveillance: abrogation prevents tumor recurrence.* J Exp Med, 2003. **198**(11): p. 1741-52.
338. Highfill, S.L., et al., *Disruption of CXCR2-mediated MDSC tumor trafficking enhances anti-PD1 efficacy.* Sci Transl Med, 2014. **6**(237): p. 237ra67.
339. Jamieson, T., et al., *Inhibition of CXCR2 profoundly suppresses inflammation-driven and spontaneous tumorigenesis.* J Clin Invest, 2012. **122**(9): p. 3127-44.
340. Solomon, S.D., et al., *Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention.* N Engl J Med, 2005. **352**(11): p. 1071-80.
341. Ahmetaj-Shala, B., et al., *Evidence That Links Loss of Cyclo-Oxygenase-2 With Increased Asymmetric Dimethylarginine: Novel Explanation of Cardiovascular Side Effects Associated With Anti-inflammatory Drugs.* Circulation, 2014.
342. Nakanishi, M., et al., *mPGES-1 as a target for cancer suppression: A comprehensive invited review "Phospholipase A2 and lipid mediators".* Biochimie, 2010. **92**(6): p. 660-4.
343. Katoh, H., et al., *CXCR2-expressing myeloid-derived suppressor cells are essential to promote colitis-associated tumorigenesis.* Cancer Cell, 2013. **24**(5): p. 631-44.
344. Huang, M.E., et al., *Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia.* Blood, 1988. **72**(2): p. 567-72.
345. Mohty, M., et al., *All-trans retinoic acid skews monocyte differentiation into interleukin-12-secreting dendritic-like cells.* Br J Haematol, 2003. **122**(5): p. 829-36.
346. Kusmartsev, S., et al., *All-trans-retinoic acid eliminates immature myeloid cells from tumor-bearing mice and improves the effect of vaccination.* Cancer Res, 2003. **63**(15): p. 4441-9.
347. Nefedova, Y., et al., *Mechanism of all-trans retinoic acid effect on tumor-associated myeloid-derived suppressor cells.* Cancer Res, 2007. **67**(22): p. 11021-8.
348. Holash, J., et al., *VEGF-Trap: a VEGF blocker with potent antitumor effects.* Proc Natl Acad Sci U S A, 2002. **99**(17): p. 11393-8.
349. Wennerberg, E., et al., *Doxorubicin sensitizes human tumor cells to NK cell- and T-cell-mediated killing by augmented TRAIL receptor signaling.* Int J Cancer, 2013. **133**(7): p. 1643-52.

350. Dovedi, S.J., et al., *Acquired resistance to fractionated radiotherapy can be overcome by concurrent PD-L1 blockade.* Cancer Res, 2014. **74**(19): p. 5458-68.
351. Lesterhuis, W.J., et al., *Synergistic effect of CTLA-4 blockade and cancer chemotherapy in the induction of anti-tumor immunity.* PLoS One, 2013. **8**(4): p. e61895.
352. Zeng, J., et al., *Anti-PD-1 blockade and stereotactic radiation produce long-term survival in mice with intracranial gliomas.* Int J Radiat Oncol Biol Phys, 2013. **86**(2): p. 343-9.
353. Demaria, S., et al., *Immune-mediated inhibition of metastases after treatment with local radiation and CTLA-4 blockade in a mouse model of breast cancer.* Clin Cancer Res, 2005. **11**(2 Pt 1): p. 728-34.
354. Hanahan, D., G. Bergers, and E. Bergsland, *Less is more, regularly: metronomic dosing of cytotoxic drugs can target tumor angiogenesis in mice.* J Clin Invest, 2000. **105**(8): p. 1045-7.
355. Dewan, M.Z., et al., *Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody.* Clin Cancer Res, 2009. **15**(17): p. 5379-88.
356. Butt, A.Q. and K.H. Mills, *Immunosuppressive networks and checkpoints controlling antitumor immunity and their blockade in the development of cancer immunotherapeutics and vaccines.* Oncogene, 2014. **33**(38): p. 4623-31.
357. Steitz, J., et al., *Depletion of CD25(+) CD4(+) T cells and treatment with tyrosinase-related protein 2-transduced dendritic cells enhance the interferon alpha-induced, CD8(+) T-cell-dependent immune defense of B16 melanoma.* Cancer Res, 2001. **61**(24): p. 8643-6.
358. Jarnicki, A.G., et al., *Suppression of antitumor immunity by IL-10 and TGF-beta-producing T cells infiltrating the growing tumor: influence of tumor environment on the induction of CD4+ and CD8+ regulatory T cells.* J Immunol, 2006. **177**(2): p. 896-904.
359. Rech, A.J. and R.H. Vonderheide, *Clinical use of anti-CD25 antibody daclizumab to enhance immune responses to tumor antigen vaccination by targeting regulatory T cells.* Ann N Y Acad Sci, 2009. **1174**: p. 99-106.
360. Liu, X., et al., *Selective inhibition of IDO1 effectively regulates mediators of antitumor immunity.* Blood, 2010. **115**(17): p. 3520-30.
361. Goel, S., et al., *Normalization of the vasculature for treatment of cancer and other diseases.* Physiol Rev, 2011. **91**(3): p. 1071-121.
362. Hamzah, J., et al., *Vascular normalization in Rgs5-deficient tumours promotes immune destruction.* Nature, 2008. **453**(7193): p. 410-4.
363. Huang, Y., et al., *Vascular normalizing doses of antiangiogenic treatment reprogram the immunosuppressive tumor microenvironment and enhance immunotherapy.* Proc Natl Acad Sci U S A, 2012. **109**(43): p. 17561-6.
364. Voron, T., et al., *VEGF-A modulates expression of inhibitory checkpoints on CD8+ T cells in tumors.* J Exp Med, 2015. **212**(2): p. 139-48.
365. Gavalas, N.G., et al., *VEGF directly suppresses activation of T cells from ascites secondary to ovarian cancer via VEGF receptor type 2.* Br J Cancer, 2012. **107**(11): p. 1869-75.
366. Ohm, J.E., et al., *VEGF inhibits T-cell development and may contribute to tumor-induced immune suppression.* Blood, 2003. **101**(12): p. 4878-86.
367. Yang, D.H., et al., *The dysfunction and abnormal signaling pathway of dendritic cells loaded by tumor antigen can be overcome by neutralizing VEGF in multiple myeloma.* Leuk Res, 2009. **33**(5): p. 665-70.
368. Shrimali, R.K., et al., *Antiangiogenic agents can increase lymphocyte infiltration into tumor and enhance the effectiveness of adoptive immunotherapy of cancer.* Cancer Res, 2010. **70**(15): p. 6171-80.
369. Hurwitz, H., et al., *Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer.* N Engl J Med, 2004. **350**(23): p. 2335-42.
370. Hodi, F.S., et al., *Bevacizumab plus ipilimumab in patients with metastatic melanoma.* Cancer Immunol Res, 2014. **2**(7): p. 632-42.

371. Motz, G.T., et al., *Tumor endothelium FasL establishes a selective immune barrier promoting tolerance in tumors.* Nat Med, 2014. **20**(6): p. 607-15.
372. Chames, P. and D. Baty, *Bispecific antibodies for cancer therapy: the light at the end of the tunnel?* MAbs, 2009. **1**(6): p. 539-47.
373. Burges, A., et al., *Effective relief of malignant ascites in patients with advanced ovarian cancer by a trifunctional anti-EpCAM x anti-CD3 antibody: a phase I/II study.* Clin Cancer Res, 2007. **13**(13): p. 3899-905.
374. Eissler, N., et al., *Potential of the trifunctional bispecific antibody surek depends on dendritic cells: rationale for a new approach of tumor immunotherapy.* Mol Med, 2013. **19**: p. 54-61.
375. Liddy, N., et al., *Monoclonal TCR-redirected tumor cell killing.* Nat Med, 2012. **18**(6): p. 980-7.
376. Li, Y., et al., *Directed evolution of human T-cell receptors with picomolar affinities by phage display.* Nat Biotechnol, 2005. **23**(3): p. 349-54.
377. Haile, S.T., L.A. Horn, and S. Ostrand-Rosenberg, *A soluble form of CD80 enhances antitumor immunity by neutralizing programmed death ligand-1 and simultaneously providing costimulation.* Cancer Immunol Res, 2014. **2**(7): p. 610-5.
378. Haile, S.T., et al., *Soluble CD80 restores T cell activation and overcomes tumor cell programmed death ligand 1-mediated immune suppression.* J Immunol, 2013. **191**(5): p. 2829-36.
379. Yang, R., et al., *CD80 in immune suppression by mouse ovarian carcinoma-associated Gr-1+CD11b+ myeloid cells.* Cancer Res, 2006. **66**(13): p. 6807-15.
380. Ribas, A., et al., *Hepatotoxicity with combination of vemurafenib and ipilimumab.* N Engl J Med, 2013. **368**(14): p. 1365-6.
381. Lei, C., et al., *Local release of highly loaded antibodies from functionalized nanoporous support for cancer immunotherapy.* J Am Chem Soc, 2010. **132**(20): p. 6906-7.
382. Kwong, B., et al., *Localized immunotherapy via liposome-anchored Anti-CD137 + IL-2 prevents lethal toxicity and elicits local and systemic antitumor immunity.* Cancer Res, 2013. **73**(5): p. 1547-58.
383. Zheng, Y., et al., *In vivo targeting of adoptively transferred T-cells with antibody- and cytokine-conjugated liposomes.* J Control Release, 2013. **172**(2): p. 426-35.
384. Stephan, M.T., et al., *Therapeutic cell engineering with surface-conjugated synthetic nanoparticles.* Nat Med, 2010. **16**(9): p. 1035-41.
385. Xiang, X., et al., *Induction of myeloid-derived suppressor cells by tumor exosomes.* Int J Cancer, 2009. **124**(11): p. 2621-33.
386. Chalmin, F., et al., *Membrane-associated Hsp72 from tumor-derived exosomes mediates STAT3-dependent immunosuppressive function of mouse and human myeloid-derived suppressor cells.* J Clin Invest, 2010. **120**(2): p. 457-71.
387. Viaud, S., et al., *Dendritic cell-derived exosomes for cancer immunotherapy: what's next?* Cancer Res, 2010. **70**(4): p. 1281-5.
388. Zitvogel, L., et al., *Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes.* Nat Med, 1998. **4**(5): p. 594-600.
389. Gehrmann, U., et al., *Synergistic induction of adaptive antitumor immunity by codelivery of antigen with alpha-galactosylceramide on exosomes.* Cancer Res, 2013. **73**(13): p. 3865-76.
390. Qazi, K.R., et al., *Antigen-loaded exosomes alone induce Th1-type memory through a B-cell-dependent mechanism.* Blood, 2009. **113**(12): p. 2673-83.
391. Naslund, T.I., et al., *Dendritic cell-derived exosomes need to activate both T and B cells to induce antitumor immunity.* J Immunol, 2013. **190**(6): p. 2712-9.
392. Finn, O.J., *Cancer vaccines: between the idea and the reality.* Nat Rev Immunol, 2003. **3**(8): p. 630-41.
393. Li, W.A. and D.J. Mooney, *Materials based tumor immunotherapy vaccines.* Curr Opin Immunol, 2013. **25**(2): p. 238-45.
394. Cho, N.H., et al., *A multifunctional core-shell nanoparticle for dendritic cell-based cancer immunotherapy.* Nat Nanotechnol, 2011. **6**(10): p. 675-82.

395. Moon, J.J., et al., *Interbilayer-crosslinked multilamellar vesicles as synthetic vaccines for potent humoral and cellular immune responses.* Nat Mater, 2011. **10**(3): p. 243-51.
396. Ali, O.A., et al., *Infection-mimicking materials to program dendritic cells in situ.* Nat Mater, 2009. **8**(2): p. 151-8.
397. Kim, J., et al., *Injectable, spontaneously assembling, inorganic scaffolds modulate immune cells in vivo and increase vaccine efficacy.* Nat Biotechnol, 2015. **33**(1): p. 64-72.
398. Cheung, R.K. and P.J. Utz, *Screening: CyTOF-the next generation of cell detection.* Nat Rev Rheumatol, 2011. **7**(9): p. 502-3.
399. Bendall, S.C., et al., *A deep profiler's guide to cytometry.* Trends Immunol, 2012. **33**(7): p. 323-32.
400. Bendall, S.C., et al., *Single-cell mass cytometry of differential immune and drug responses across a human hematopoietic continuum.* Science, 2011. **332**(6030): p. 687-96.
401. Newell, E.W., et al., *Cytometry by time-of-flight shows combinatorial cytokine expression and virus-specific cell niches within a continuum of CD8+ T cell phenotypes.* Immunity, 2012. **36**(1): p. 142-52.
402. Giesen, C., et al., *Highly multiplexed imaging of tumor tissues with subcellular resolution by mass cytometry.* Nat Methods, 2014. **11**(4): p. 417-22.
403. Marraffini, L.A. and E.J. Sontheimer, *CRISPR interference: RNA-directed adaptive immunity in bacteria and archaea.* Nat Rev Genet, 2010. **11**(3): p. 181-90.
404. Mali, P., et al., *RNA-guided human genome engineering via Cas9.* Science, 2013. **339**(6121): p. 823-6.
405. Mandal, P.K., et al., *Efficient ablation of genes in human hematopoietic stem and effector cells using CRISPR/Cas9.* Cell Stem Cell, 2014. **15**(5): p. 643-52.
406. Hou, Z., et al., *Efficient genome engineering in human pluripotent stem cells using Cas9 from Neisseria meningitidis.* Proc Natl Acad Sci U S A, 2013. **110**(39): p. 15644-9.
407. Mauti, L.A., et al., *Myeloid-derived suppressor cells are implicated in regulating permissiveness for tumor metastasis during mouse gestation.* J Clin Invest, 2011. **121**(7): p. 2794-807.
408. Li, H., et al., *Cancer-expanded myeloid-derived suppressor cells induce anergy of NK cells through membrane-bound TGF-beta 1.* J Immunol, 2009. **182**(1): p. 240-9.
409. Nausch, N., et al., *Mononuclear myeloid-derived "suppressor" cells express RAE-1 and activate natural killer cells.* Blood, 2008. **112**(10): p. 4080-9.
410. Bronte, V. and M.J. Pittet, *The spleen in local and systemic regulation of immunity.* Immunity, 2013. **39**(5): p. 806-18.
411. Chesler, L. and W.A. Weiss, *Genetically engineered murine models--contribution to our understanding of the genetics, molecular pathology and therapeutic targeting of neuroblastoma.* Semin Cancer Biol, 2011. **21**(4): p. 245-55.
412. Zirath, H., et al., *MYC inhibition induces metabolic changes leading to accumulation of lipid droplets in tumor cells.* Proc Natl Acad Sci U S A, 2013. **110**(25): p. 10258-63.
413. Puissant, A., et al., *Targeting MYCN in neuroblastoma by BET bromodomain inhibition.* Cancer Discov, 2013. **3**(3): p. 308-23.
414. Marigo, I., et al., *Tumor-induced tolerance and immune suppression depend on the C/EBPbeta transcription factor.* Immunity, 2010. **32**(6): p. 790-802.
415. Weiss, W.A., et al., *Targeted expression of MYCN causes neuroblastoma in transgenic mice.* EMBO J, 1997. **16**(11): p. 2985-95.
416. Banerjee, S.A., et al., *5' flanking sequences of the rat tyrosine hydroxylase gene target accurate tissue-specific, developmental, and transsynaptic expression in transgenic mice.* J Neurosci, 1992. **12**(11): p. 4460-7.
417. Rasmuson, A., et al., *Tumor development, growth characteristics and spectrum of genetic aberrations in the TH-MYCN mouse model of neuroblastoma.* PLoS One, 2012. **7**(12): p. e51297.