Translational studies of metastatic melanoma in the era of immunotherapy

– From humanized mouse models to clinical trials

Henrik Jespersen

Department Oncology,
Institute of Clinical sciences at
Sahlgrenska Academy
University of Gothenburg

Gothenburg, Sweden, 2020

UNIVERSITY OF
GOTHENBURG

*…in this terrifying world, all we have
are the connections that we make*
– BoJack Horseman

Abstract

Immunotherapy with PD-1 inhibitors has transformed the treatment of meta-static cutaneous melanoma, and can lead to complete and durable responses in a proportion of patients. However, in around half of the patients, the treatment has little or no effect. In patients with metastatic uveal melanoma, a rare form of melanoma arising in the eye, effective treatments are lacking altogether. The overall aim of the research on which this thesis is based, is to develop and utilize mouse models to identify new immunotherapies for patients with metastatic melanoma.

In paper I we describe the development of a novel immune humanized patient derived xenograph (PDX) model. The PDX is based on sequential transplan-tation of ex vivo expanded, autologous tumor infiltrating lymphocytes (TIL), and mirror the treatment effects seen in corresponding patients. In paper II we evaluate the feasibility and preclinical efficacy of chimeric antigen receptor (CAR)-T cell therapy in melanoma and find that CAR T cells against HER2 are able to kill human cutaneous and uveal melanoma cells in vitro and in vivo. In paper III we first assess the rationale of combined epigenetic modulation and PD-1 inhibition in experimental melanoma, and show that the histone deacety-lase (HDAC) inhibitor entinostat increases expression of HLA-I and PD-1 on melanoma cell lines and enhances the effect of a PD-1-inhibitor in vivo. Next, we describe the design and preliminary results of an ongoing phase II trial eval-uating the effect of entinostat in combination with pembrolizumab (a PD-1 inhibitor) in patients with metastatic uveal melanoma.

In conclusion, this thesis shows that i) PDX models can be used to study key aspects of the human antitumoral immunity in melanoma; ii) that HER2 CAR-T cells represent a potential future treatment for metastatic melanoma refrac-tory to other immunotherapies; and iii) that entinostat increases HLA-I expres-sion and potentiates the effect of PD-1 inhibition in melanoma models, and that the same combination can result in clinical efficacy with manageable tox-icity in patients with metastatic uveal melanoma.

Keywords: Metastatic melanoma, uveal melanoma, humanized mouse models, immunotherapy, tumor infiltrating lymphocytes, chimeric antigen receptor T cells, PD-1 inhibition, epigenetics, HDAC-inhibition

Sammanfattning på svenska

Immunsystemet består av en rad olika vävnader, celler och signalmolekyler vilka skyddar kroppen från inkräktare. Att immunsystemet även kan känna igen cancerceller som främmande har varit känt länge, men under senare år har man identifierat flera bromssystem som hindrar immunsystemet från att hålla cancern under kontroll. Läkemedel som inaktiverar dessa bromsar, framförallt så kallade PD-1 hämmare, kan ge immunsystemet förmågan at döda cancerceller. Denna sorts immunterapi har varit banbrytande för en rad cancerformer, i synnerhet malignt melanom, vilken utgår från kroppens pigmentproducerande celler (melanocyter), oftast i huden. I de fall där sjukdomen har spridit sig till andra organ i kroppen (metastaserat), är melanom en mycket allvarlig cancersjukdom, men behandling med PD-1 hämmare kan hos vissa patienter få tumörerna att försvinna helt. Tyvärr har dock flertalet patienter begränsad eller ingen nytta av behandlingen. I sällsynta fall kan melanom uppstå i ögat (uvealt melanom). Uvealt melanom sprider sig hos upp mot hälften av patienterna, oftast till levern, och i regel har varken PD-1 hämmare eller andra cancerläkemedel effekt hos dessa patienter.

För att bättre kunna studera immunterapier i laboratoriet utvecklade vi en modell som gör det möjligt att undersöka cancerceller och immunceller från enskilda patienter i så kallade avatarmöss. Behandlingseffekterna hos mössen återspeglar effekten hos patienter och avatarmössen kan komma att få användning som en modell för immunterapi. Vi visade sedan att immunceller som blivit genetisk modifierade (CAR-T celler) för att känna igen ytproteinet HER2, effektivt dödar melanomceller, även från uveala melanom. Detta är således en lovande behandling för melanom där annan immunterapi inte har effekt. Genom studier av melanomceller och musmodeller i laboratoriet har vi vidare visat att entinostat (ett så kallat epigenetisk läkemedel som påverkar hur cellers olika gener uttrycks eller tystas) tycks kunna göra melanomceller mer känsliga för effekten av PD-1 hämmare. Vi initierade därför läkemedelsprövningen PEMDAC, där 29 patienter med metastaserat uvealt melanom behandlats med en kombination av entinostat och PD-1 hämmaren pembrolizumab. Preliminära data visar att det är möjligt att uppnå positiva behandlingseffekter med hanterbara biverkningar genom en kombinationsbehandling av entinostat och pembrolizumab hos patienter med metastaserat uvealt melanom. Förhoppningsvis kan fortsatta studier bidra till att klarlägga vad som särskiljer patienter med god effekt av immunterapi samt utveckla nya immunterapier som kan komma fler patienter till nytta.

iv

List of papers

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

I. **Jespersen H***, Lindberg MF*, Donia M, Söderberg EMV, Andersen R, Keller U, Ny L, Svane IM, Nilsson LM, Nilsson JA
    Clinical responses to adoptive T-cell transfer can be modelled in an autologous immune-humanized mouse model
    Nature Communications. 2017 Sep 27;8(1):707[#]

II. Forsberg EMV*, Lindberg MF*, **Jespersen H**, Alsén S, Bagge RO, Donia M, Svane IM, Nilsson O, Ny L, Nilsson LM, Nilsson JA
    HER2 CAR-T cells eradicate uveal melanoma and T-cell therapy–resistant human melanoma in IL2 transgenic NOD/SCID IL2 receptor knockout mice
    Cancer Research. 2019 Mar 1;79(5):899-904

III. **Jespersen H**, Sah V, Alsén S, Ullenhag G, Carneiro A, Helgadottir H, Ljuslinder I, Levin M, All-Eriksson C, Andersson B, Stierner U, Bagge RO, Nilsson LM, Nilsson JA, Ny L.
    Combined HDAC- and PD-1 inhibition in experimental and human melanoma.
    Manuscript

*Equal contribution

[#]Reprints were permitted according to the Creative Commons Attribution 4.0 International License

Related papers not included in thesis:

i.   Ny L, Rizzo L, Belgrano V, Karlsson J, **Jespersen H**, Carstam L, Olofsson Bagge R, Nilsson L, Nilsson J. Supporting clinical decision-making in advanced melanoma by preclinical testing in personalized immune-humanized xenograft mouse models. *Annals of Oncology, 2020, in press.*

ii.   Karlsson J, Nilsson L, Forsberg E, Mitra S, Alsén S, Shelke G, Sah V, Stierner U, All- Eriksson C, Einarsdottir B, **Jespersen H**, Ny L, Lindnér P, Larsson E, Olofsson Bagge R, Nilsson J. Molecular profiling of driver events and tumor-infiltrating lymphocytes in metastatic uveal melanoma. *Under review. Preprint available at bioRxiv.org (742023).*

iii.   Arheden A, Skalenius J, Bjursten S, Stierner U, Ny L, Levin M, **Jespersen H**. Real-world data on PD-1 inhibitor therapy in metastatic melanoma. *Acta Oncol. 2019 ;58(7):962-966.*

iv.   **Jespersen H**, Bagge RO, Ullenhag G, Carneiro A, Helgadottir H, Ljuslinder I, Levin M, All-Eriksson C, Andersson B, Stierner U, Nilsson LM, Nilsson JA, Ny L. Concomitant use of pembrolizumab and entinostat in adult patients with metastatic uveal melanoma (PEMDAC study): protocol for a multicenter phase II open label study. *BMC Cancer. 2019 2;19(1):415.*

v.   Bagge RO, Demir A, Karlsson J, Alaei-Mahabadi B, Einarsdottir BO, **Jespersen H**, Lindberg MF, Muth A, Nilsson LM, Persson M, Svensson JB, Söderberg EMV, de Krijger RR, Nilsson O, Larsson E, Stenman G, and Nilsson JA. Mutational Signature and Transcriptomic Classification Analyses as the Decisive Diagnostic Tools for a Cancer of Unknown Primary. *JCO Precision Oncology 2018 :2, 1-25*

vi.   Einarsdottir BO, Karlsson J, Söderberg EMV, Lindberg MF, Funck-Brentano E, **Jespersen H**, Brynjolfsson SF, Bagge RO, Carstam L, Scobie M, Koolmeister T, Wallner O, Stierner U, Berglund UW, Ny L, Nilsson LM, Larsson E, Helleday T, Nilsson JA. A patient-derived xenograft pre-clinical trial reveals treatment responses and a resistance mechanism to karonudib in metastatic melanoma. *Cell Death Dis. 2018 24;9(8):810.*

vii.   **Jespersen H**, Bjursten S, Ny L, Levin M. Checkpoint inhibitor-induced sarcoid reaction mimicking bone metastases. *Lancet Oncol.* 2018 Jun;19(6):e327.

viii.   Einarsdottir BO, Bagge RO, Bhadury J, **Jespersen H**, Mattsson J, Nilsson LM, Truvé K, Lopez MD, Naredi P, Nilsson O, Stierner U, Ny L, Nilsson JA. Melanoma patient- derived xenografts accurately model the disease and develop fast enough to guide treatment decisions. *Oncotarget. 2014 Oct 30;5(20):9609-18.*

Content

Selected abbreviations

ACT   Adoptive cell transfer
APC   Antigen presenting cell
B2M   Beta-2-microglobulin
BAP1   BRCA1 associated protein 1
BET   Bromodomain and extra-terminal
CAR   Chimeric antigen receptor
CDX   Cell line derived xenographs
CTLA4   Cytotoxic T-lymphocyte-associated protein 4
DNMT   DNA methyl transferases
GEMM   Genetically engineered mouse models
GNA11   Guanine Nucleotide-Binding Protein subunit Alpha-11
GNAQ   Guanine Nucleotide-Binding Protein G(q) subunit Alpha
HDAC   Histone deacetylase
HLA   Human leucocyte antigen
IGF-1   Insulin-like growth factor-1
IL-2   Interleukin-2
IFN-γ   Interferon gamma
irAE   Immune related adverse events
LAG3   Lymphocyte-activation gene-3
LDH   Lactate dehydrogenase
MHC   Major histocompatibility complex
MDSC   Myeloid derived suppressor cell
NF1   Neurofibromin-1
NOG   NOD-SCID-IL2rg knock out
OS   Overall survival
PD-1   Programmed death-1
PDX   Patient derived xenograph
PFS   Progression free survival
TAM   Tumor associated macrophage
TCR   T cell receptor
TGF-β   Transforming growth factor-β
TILs   Tumor infiltrating lymphocytes
TIM-3   T cell immunoglobulin- and mucin-domain-containing molecule 3
TMB   Tumor mutational burden
TME   Tumor micro environment
PTEN   Phosphatase and tensin homolog

x

1. Introduction

O l'oun t'awa se n'yara, Je k'abere
-Fela Kuti

Humans are all about connections. From our thoughts, feelings and consciousness arising from synapses between neurons, to the relations that make up our social lives. And then there is everything in between. Like cancer. And science. **Cancer** arise when cells start to ignore the signals and connections that govern growth and arrest in a healthy body. Fueled by the forces of natural selection, cancer cells rewire connections in the networks within to drive proliferation and get eternal life. They start to disrespect the natural boundaries of tissues, dethatch and spread to distant sites, and create new connections to favor its own invasive growth and propagation, with no regards to the health of its bodily host. **Science** is to make sense of the world through systematically establishing new connections: Between prior knowledge and future aims; between predictions and observations. Particular progress can arise from connecting different fields of research, like cancer biology and immunology. One of the biggest breakthroughs in cancer research was the laboratory finding that cancer disrupts many of the numerous connections that the immune system uses to it control it, and that amazing treatment effects can occur in patients when functioning connections are restored or created. Here lies the promise of **translational research**. To create functional connections between laboratory findings, medical needs and clinical observations. And back.

1.1 Melanoma

Malignant melanoma is a cancer originating from melanocytes, the pigment producing cells of the body. In its most common form, **cutaneous melanoma**, the tumor develops from melanocytes in the skin, but melanomas can also form in the uvea of the eye or in mucosal linings. Uveal melanoma will be covered separately due to its distinct biology and clinical characteristics. Cutaneous melanoma is increasing at an alarming rate in the fair-skinned population worldwide [1]. In Sweden the annual increase in **incidence** is around 5%, with the most recent age-adjusted incidence being 43/100 000 in men and 36/100.000 in women [2]. The mortality has not increased at the same rate, and appear to have levelled out at around 500 deaths per year in Sweden, making cutaneous melanoma the most deadly skin cancer [2].

The major environmental **risk factor** for developing cutaneous melanoma is UV radiation, recorded as self-reported, high intermittent sun exposure [3]. Other risk factors include high number of melanocytic nevi, red headedness, fair skin, low tanning ability and the propensity to freckle [4]. Between 5-10 % of cutaneous melanoma cases occur in patients with a family history of the disease; however, identification of inherited germline mutations is rare and then usually reside in the tumor suppressor CDKN2A [5].

Although the vast majority of cutaneous melanomas are cured with surgical excision of the **primary tumor**, some patients' disease metastasizes. The risk for metastatic disease can be predicted by characteristics of the primary tumor, where tumor thickness (measured as Breslow-depth) has the highest prognostic value, followed by presence of ulceration and number of mitoses (**Stage I-II**) [6]. Sentinel node biopsy is usually performed in melanomas thicker than 1 mm and provide important prognostic information; however, additional lymph node surgery has not been shown to improve survival over observation [7, 8]. If the disease has spread to the regional lymph nodes (**stage III**), a proportion of the patients will still be cured by lymph node excision. The risk of local or distant recurrence is heterogenous, and can be estimated by size and number of involved nodes, as well as the presence of in transit metastases (i.e. cutaneous or subcutaneous metastases between the primary tumor and the draining lymph node basin). Adjuvant radiation therapy effectively reduces the risk of local recurrence in high risk patients, but does not lead to improved survival [8]. Due to recent advances in medical melanoma oncology, patients with high risk stage III are now instead offered **adjuvant systemic treatment** that dramatically reduce the risk of both local and systemic recurrence.

If a melanoma has spread to distant sites of the body **(Stage IV)** the disease has generally, like most metastatic cancers, been considered incurable. Historically, **metastatic melanoma** has had a dismal prognosis, with two thirds of the patients succumbing to the disease within a year of diagnosis. All organs can be affected, but the most frequent sites of metastases include distant lymph nodes, skin and soft tissue, lungs, bone, liver and brain, with increasing impact on survival. In addition, elevated serum levels of lactate dehydrogenase (**LDH**) is associated with poor prognosis (**Table 1**) [9].

Since its approval in the 1970's, the chemotherapy dacarbazine long was the only FDA approved drug for metastatic melanoma. Although chemotherapy can obtain objective response in some 15 % of patients, the responses are rarely durable [10]. Decades of clinical trials later failed to demonstrate proven survival benefit, usually

finding a median overall survival around only 6-9 months. Recent years have, however, seen a revolution in the treatment of metastatic melanoma with the introduction of both targeted therapy and immunotherapy which has dramatically improved survival [11]. Equally important, adjuvant treatment with either modality has been shown to significantly reduce the risk of recurrence after resection of stage III or IV melanoma [12-15]. How adjuvant treatment in clinical routine will affect the long-term survival of melanoma is still unclear.

Table 1 Definition of distant metastasis (M) according to AJCC cancer staging manual 8th edition [9]

M category	Anatomic site of metastasis
M0	No evidence of distant metastasis
M1a	Skin, soft tissue and/or non regional lymph node
M1b	Lung
M1c	Non-lung, non-CNS visceral organ
M1d	CNS

Suffixes indicate whether LDH is elevated (1) or not (0)

1.1.1 Genetics and targeted therapies

Like all cancers, cutaneous melanoma develops under the accumulation of genetic alterations. Due to its typical location in sun-exposed skin, cutaneous melanoma harbors amongst the highest numbers of somatic mutations of all cancers, with an average of 14 mutations per megabase (Mb) of DNA [16]. Recurrent mutations occur predominantly in the mitogen activated protein kinase (**MAPK**)-signaling pathway, which is normally under the control of growth factors binding to their surface receptor tyrosine kinase (RTK) (**Figure 1**). Most frequently, mutations are found in the **BRAF** gene occurring in around half of patients or, in a mutually exclusive manner, *NRAS* in ca 30% [17]. These activating mutations lead to constituent MAPK-signaling through the downstream proteins MAPK/ERK kinase (**MEK**) and extracellular signal-regulated kinase (ERK), which drives cancer cell survival and proliferation. A third group of patients (14%) have inactivating mutations in the tumor suppressor gene *Neurofibromin 1 (**NF1**)*, encoding a negative regulator of RAS signaling. In the remaining group of **triple wildtype** patients, alterations or overexpression of growth factor receptors like KIT, MET or EGFR is described, underlining the crucial importance of the MAPK pathway in melanoma biology. Frequently inactivated tumor suppressor genes include *phosphatase and tensin homolog* (**PTEN***) which is

particularly frequent in *BRAF* mutated melanoma, *TP53*, most often found in *BRAF, NRAS* or *NF1* mutated cases, and *CDKN2A* which is equally distributed between the four groups [18].

Targeting mutated BRAF with small molecule **BRAF inhibitors** leads to impressive objective responses for the approximately 50% of melanoma patients harboring an activating BRAF mutation at position V600 [19, 20]. By adding a **MEK inhibitor**, the objective response rate increases, and the time to development of resistance, and consequently overall survival, is prolonged, without any increase in toxicity [21, 22]. Unfortunately, most patients will nevertheless develop resistance to the therapy within a year of treatment. The underlying mechanisms of resistance are only partly understood, but does not seem to include alteration or loss of the activating mutation itself (as seen in several targeted therapies of other malignancies). Instead reactivation of the MAPK-pathway (ERK signaling) can be restored by diverse processes like activation of parallel converging pathways, genetic alterations in other MAPK proteins, alternative splicing or amplification of the *BRAF V600* allele and upregulation of receptor tyrosine kinases [23-27]. Studies in mice have demonstrated that triple targeting of BRAF, MEK and ERK can have curative effects by suppressing the evolution of resistance; however, this treatment is yet to be tested in patients [28].
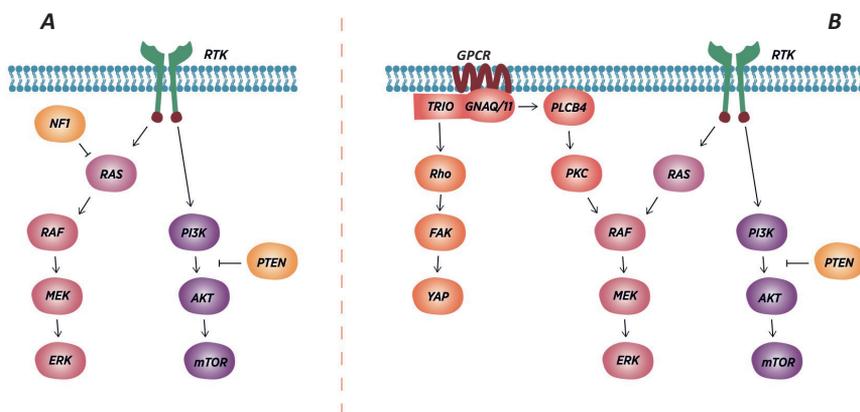
Figure 1 Key signaling pathways in cutaneous (**A**) and uveal (**B**) melanoma. RTK, receptor tyrosine kinase; GPCR, G protein-coupled receptor.

Unfortunately, there is no known way to directly inhibit mutated NRAS, and inhibiting the downstream MEK does not give meaningful effects in NRAS mutated patients [29]. Mutated KIT on the other hand is known to be directly inhibited by imatinib [30]. KIT alterations are particularly common in acral and mucosal

melanoma as well as melanomas arising on chronically sun-damaged skin, and imatinib can have efficacy in some patients with KIT mutations (but not amplifications) [31].

Apart from the obvious risk of inducing oncogenic mutations, UV radiation can also drive tumor-promoting inflammation. On the other hand, mutations may also alter the tumor cells and make them more prone to recognition by the immune system. Finally, it was increased knowledge about the interplay between cancer and the immune system that led to the biggest revolution in melanoma oncology: Effective immunotherapies.

1.1.2 Immunotherapy

The immunogenic potential of melanoma has been known for centuries, e.g. through observations of melanoma-associated vitiligo and spontaneous regressions of primary, and even in extreme cases metastatic, melanomas [32, 33]. The proposed immunogenicity of melanoma spurred long-lasting efforts to stimulate the immune system using vaccines and cytokines to achieve therapeutic effects, albeit with limited success. Instead, it was the identification of *inhibitory* receptors in the immune system (immune checkpoints), and drugs that disrupt their suppressive effect on the antitumoral immune response (immune checkpoint inhibitors), that became the long-awaited breakthrough.

In 2011, the cytotoxic T-lymphocyte-associated protein 4 (**CTLA-4**) inhibitor ipilimumab became the first **immune checkpoint inhibitor** to be approved, and at the same time the first drug ever to demonstrate a survival benefit in metastatic melanoma [34]. Although the response rate is low (10-20 %) and toxicities significant, around 20% of the patients seem to achieve long-term survival, leaving clinicians to speculate about a possible cure [35]. In the years to follow, trials with the programmed death receptor-1 (**PD-1**) inhibitory antibodies nivolumab and pembrolizumab, demonstrated a higher objective response rate (approximately 40%), unprecedented improval in survival over standard therapy, and a much more favorable toxicity profile, making them the current first line of treatment for most patients with metastatic cutaneous melanoma [36, 37]. Consequently, there has been considerable improvements in the overall survival of patients with metastatic melanoma treated in clinical routine [38, 39]. If the same very long-term benefit in survival observed with responders to ipilimumab will be achieved with PD-1 inhibitors, remains to be seen; however, recently published follow up found a 5-year survival as high as around 50% (**Figure 2**) [40].

Combining the **CTLA-4 and PD-1** inhibitors ipilimumab and nivolumab (ipi-nivo), leads to an increased response rate of around 60%, and a small numeric benefit in survival (**Figure 2**), but at the cost of severe toxicities that render a general use of this regimen controversial [40]. However, it has been shown that some patients with brain metastases (a major medical need in melanoma oncology), can have excellent response to ipi-nivo [41, 42]. Future trials are needed to identify additional patient groups with a clear benefit of combined CTLA-4 and PD-1 inhibition. Hopefully even the rapidly increased knowledge about the underlying mechanisms of response and resistance to checkpoint blockade will result in biomarkers that may one day guide the selection of this and other novel immunotherapy combinations.
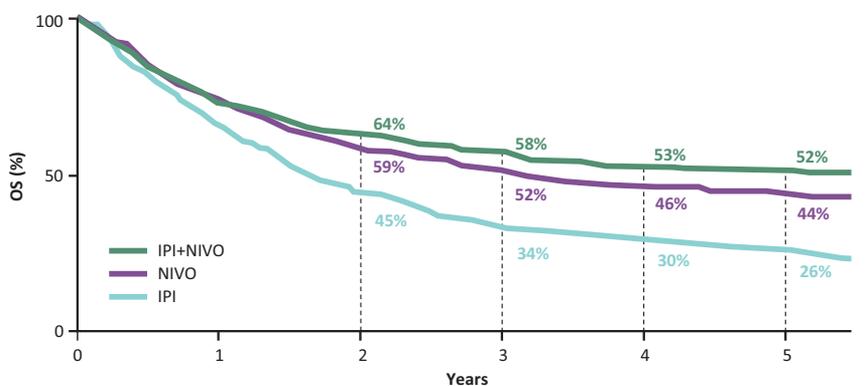
Figure 2 Overall survival estimate from the Checkmate 067 study with five years of follow up. Patients with stage IV melanoma were treated with ipilimumab (3 mg/kg) (IPI), nivolumab (3 mg/kg) (NIVO) or ipilimumab (3 mg/kg) in combination with nivolumab (1 mg/kg) (IPI+NIVO). Median survival was 3.3 years for the NIVO group and not reached for NIVO + IPI. The study was not designed to compare the two nivolumab containing arms, but the difference in survival was not found to be significant in a descriptive analysis (HR 0.83 (95% CI, 0.67 to 1.03)). Adapted from [40].

1.2 Cancer and the immune system

The idea of manipulating the immune system to fight cancer is by no means novel. A famous early attempt dates back to the 1890s when the New York-based surgeon William B. Coley noticed an apparent association between post-operative wound infections and a favorable outcome after surgery of soft tissue sarcomas. He consequently started treating tumors with "inoculation of erysipelas", i.e. live cultured bacteria, later refined to Coley's toxin, and reported spectacular cases of tumor regression [43]. Although the concept of bacterial inoculation to treat cancer still exists for treatment of bladder cancer [44], Coley's method of early immunotherapy was hampered by variable results and side effects, and soon overshadowed by modern innovations like radiotherapy and chemotherapy.

We now know that acute bacterial infections like erysipelas primarily elicit an immune response form the **innate immune system**. The innate immune system represents a rapid, first line of defense against pathogens that breach the anatomical barriers of the body. In the tissues, phagocytic cells of the innate immune system like **macrophages** and **dendritic cells** carry pattern recognition receptors (PRR) that recognize common structural components of pathogens -so called pathogen-associated molecular patterns (PAMPs)- and initiate an inflammatory response through the production of **cytokines**. Cytokines are soluble proteins that immune cells use to influence each other and include **chemokines** that direct the movement of cells to the sites where they are most needed. Effects on the capillary bed induce extravasation of cells and plasma and cause the red, warm and painful swelling that we are all familiar with. In sum, the inflammatory response results in a rapid recruitment of plasma proteins and circulating white blood cells, most notably **neutrophils,** that directly target microbes, amplify the response, and together help eliminate the intruding pathogen [45, 46]

Although inflammation is central in cancer biology and can mediate both pro- and antitumoral effects [47], the innate immune system lacks the necessary specificity to distinguish cancer cells from healthy cells. Instead it has become evident that an effective recognition and elimination of established cancer cells also require the effects of the **adaptive immune system**. Lymphocytes, named after their site of maturation in bone marrow (**B cells**) or thymus (**T cells**), are the cellular components of the adaptive immune system and themselves carry most of the features that characterize it: They have receptors of exquisite *specificity*; a huge replicative potential, enabling an *amplifiable* response; the potential of a long lifespan generating immunological *memory*; and the capacity of recirculation between blood, lymphatic and peripheral tissues

giving them *body-wide distribution*. These characteristics also happen to be very desirable features of a cancer therapy.

While B cells produce antibodies that foremost recognize and help destroy extracellular pathogens, T cells on the other hand have the unique capability of reacting upon intracellular processes in dysfunctional somatic cells. T cells can be further divided into CD4+ T cells (or T helper cells) and CD8+ T cells (or cytotoxic T cells). Each individual T cell carries a unique **T cell receptor** (TCR) capable of recognizing a peptide sequence (**antigen**) bound to a major histocompatibility complex (MHC) molecule on a cell surface through **antigen presentation**. CD4+ T cells are restricted to recognize antigens presented on **MHC class II** and respond by producing cytokines that affect surrounding immune cells. MHC-II is generally limited to professional antigen presenting cells (APC), particularly dendritic cells, that present antigens derived from internalized *extracellular proteins*. As APCs sample their surroundings for pathogen derived proteins to present, they represent a crucial link between the innate and adaptive immune response.

CD8+ T cells recognize antigens presented on **MHC class I** that is expressed on all nucleated cells. MHC-I classically presents peptides derived from *intracellular proteins* in somatic cells. A sample of all synthesized proteins are degraded by cytosolic proteasomes to peptides. These are transported to the endoplasmatic reticulium (ER) and loaded to MHC-I, and the peptide-MHC-I complex is transported to the cell surface [48]. In this way the immune system can monitor processes hidden deep in the cells interior since e.g. intracellular bacteria or vira expose themselves when their peptidome is put on display on the cell surface. Upon recognition of its cognate antigen-MHC-I complex, the T cell releases cytotoxic granules that kill the dysfunctional target cell.

The number of possible TCR target antigens greatly exceeds the number of genes in the human genome [49][1]. Instead TCR specificity is generated through stochastic reshuffling of TCR gene segments in individual precursor cells. This process, **V(D)J recombination**, generates a huge surplus of TCRs in T cell precursor clones that then undergo selection and maturation in the thymus as *thymocytes*. First, thymocytes that fail to bind MHC-I are sorted away (*positive selection*), then the developing T cells are exposed to a wide array of self-antigens, and if they bind too strongly undergo apoptosis (*negative selection*) [50]. In this way, thymic maturation of the T cell pool generates **central tolerance**. When naïve T cells leave the thymus, they are trained

---

[1] In fact, the theoretical limit of the TCR repertoire is estimated to more than 10^{13} and thus exceed even the number of nucleotides in the human genome.

to ignore self-antigens, but are collectively capable of recognizing virtually any non-self-peptide derived from e.g. virus or intracellular bacteria in infected somatic cells, as long as it is presented on MHC-I. Fortunately, even cancer cells can be sufficiently altered for some of the mature T cells to carry a TCR capable of recognizing it.

1.2.1 What the immune system sees in cancer

There are indeed other cell types that are capable of directly killing cancer cells (most particularly NK-cells [51]). However, for the purpose of brevity and focus, *antitumoral immune response* will here be used for the complex process that ends with a **CD8+ T cell killing a cancer cell**, and *immunotherapy* as an intervention that explicitly aims at increasing the chances for it to succeed.

With its TCR, the CD8+ T cell can recognize tumor-antigens presented on MHC-I on cancer cells and selectively kill it with the release of cytotoxic granules. "**Tumor antigen**" is a broad and loosely defined term describing antigens with varying degrees of cancer specificity and include *i)* aberrantly expressed peptides (e.g. tissue restricted) *ii)* peptides altered through post-translational modification *iii)* viral antigens (endogenous retroelements, or in virus associated cancers), *iv)* peptides altered through non-synonymous mutations in the parental gene, so called **neo-antigens** [52]. There is now growing acceptance in the field that neo-antigens probably are of greatest importance in the recognition of cancer cells as non-self (or rather *altered-self*) by the immune system [53].

Simply binding TCR to an antigen-MHC complex does not by itself trigger a T cell attack, instead it actually leaves the T cell in a dysfunctional, *anergic* state [54]. To acquire full effector function, naïve T cells first require activation (*priming*) by APCs, predominantly dendritic cells in the tumor draining lymph nodes. In addition to ligation of TCR to antigen-MHC complex on the APC (*signal 1*), **T cell priming** also requires engagement of **co-stimulatory** molecules (*signal 2*), of which binding of CD28 by CD80 or CD86 on the APC is best characterized [55] (**Figure 3A**). Upon activation the naïve T cell begins a massive proliferation (*clonal expansion*) that amplifies the immune response towards the encountered antigen. Furthermore, it undergoes differentiation to obtain effector functions and in parallel generate a population of long-lived *memory T cells* that can ensure a more rapid expansion and response in future encounters with the antigen. In addition to direct cell-cell interactions, even cytokines secreted by the APC and surrounding immune cells shape the differentiation pathways of the activated T cell and is required for effective proliferation (*signal 3*).

The necessity of T cell activation underlines the crucial role of APCs in connecting the innate and the adaptive immune response. In cancer, antigens are released by dead cancer cells and internalized by activated APCs. The APC then migrates to the tumor draining lymph node where it can present the cancer antigen to T cells carrying the corresponding TCR. Since CD8+ T cell TCRs are restricted to binding MHC-I, and internalized proteins are generally processed for presentation on MHC-II, priming of CD8+ T cells requires alternative processing. In a process called **cross presentation**, certain subsets of dendritic cells in particular, are able to direct internalized proteins for cytosolic degradation and subsequent loading onto MHC-I [48]. Once activated, the CD8+ effector T cell leaves the lymph node and enter the blood stream. It has now gained the capacity to mount a cytotoxic response and produce interferon gamma (**IFN-γ**) when encountering its cognate antigen. Due to upregulation of chemokine receptors and adhesion molecules it can now home to the site of cancer associated inflammation, extravasate into the tumor and lyse its target cell upon recognition of its tumor antigen, presented on MHC-I on cancer cells (**Figure 3B**). Although this response has been speculated to regularly eliminate premalignant lesions (**immunosurveillance**), it is obviously not sufficient to eradicate established tumors under normal conditions. Indeed, one of the hallmarks of established cancer is the acquired ability to evade the immune system (**immune evasion**) [56].
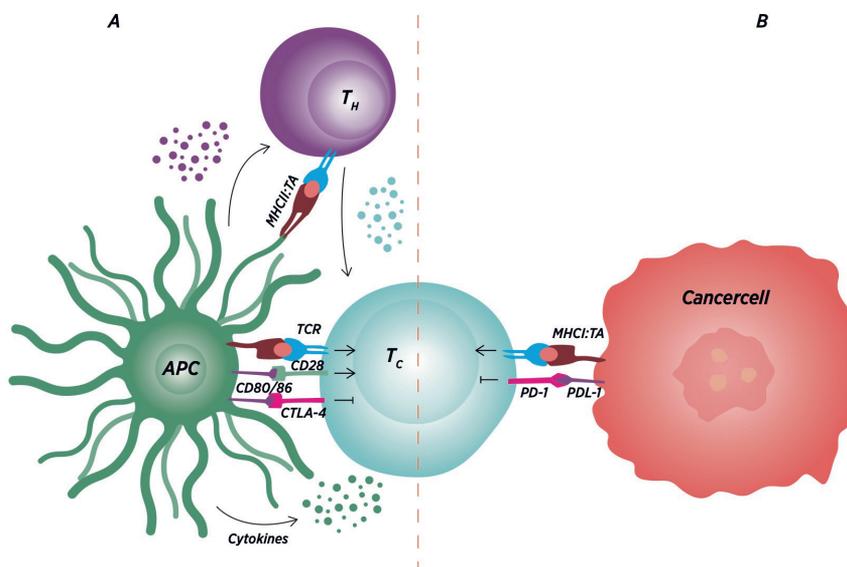

Figure 3 Schematic illustration of the T cell priming phase (**A**) and effector phase (**B**). Arrows indicate stimulatory signals, lines with a bar indicate inhibitory signals. APC, Antigen Presenting Cell; T_C, CD8+ cytotoxic T Cell; T_H, CD4+ T helper cell; MHC:TA, MHC:Tumor antigen complex; TCR, T cell receptor.

Central tolerance is not perfect. In part because of cross-reactivity towards a large number of similar peptides, there is an overlap in TCR affinity between pathogenic- and self-antigens, particularly in antigens of self-origin such as in cancer. However, it is also becoming increasingly clear that the T cell response is not a simple dichotomous discrimination of self vs. nonself, predetermined by its development and nature of the antigen. Rather, the T cell response is highly dependent on context, and can be viewed as the sum of the highly integrated input of several stimulatory *and* inhibitory signals during all stages of T cell activation. This is one of the mechanisms of **peripheral tolerance** that balance the risk of autoimmunity against the risk of chronic infection. Cancer immune evasion involves tipping this balance towards increased peripheral tolerance. Of particular importance seems to be the dysregulation of ligands on APCs or cancer cells that bind to regulatory receptors on the T cells, collectively described as **immune checkpoints**. As blocking inhibitory immune checkpoints with monoclonal antibodies skew the balance towards an improved and prolonged antitumoral immune response, immune checkpoints have become amongst the hottest targets of cancer drug development [57].

1.2.2 Immune checkpoint inhibition

It was first described in chronic viral infections that prolonged antigen stimulation generates T cell populations with decreased capacity for renewed stimulation. Likely part of an evolutionary important mechanism to avoid autoreactivity during chronic infections, these **exhausted T cells** have reduced effector functions, proliferation, cytotoxicity and cytokine production and express high levels of several inhibitory receptors on their surface [58]. It was later shown that a similar phenotype is often present amongst tumor infiltrating lymphocytes (**TILs**), particularly in the small subset of TILs that actually show reactivity towards cancer antigens, but, despite their presence, obviously lack the effector function to control tumor growth [59]. Although the prevailing model of T cell exhaustion as a linear "wearing out" of once functional cells is being challenged, it has been a fruitful concept in informing the hunt for receptors and ligands that govern T cell activation and function [60].

CTLA-4 is one of the first negative regulators of T cell activation to be induced, and becomes expressed upon TCR binding on both CD4+ and CD8+ T cells already during priming. CTLA-4 outcompetes the costimulation provided by CD28 due to its higher affinity to their shared ligands CD80 (B7-1) and CD86 (B7-2) on the APC, and consequently attenuate the T cell response (**Figure 3A**) [61, 62]. In pioneering experiments in the late 1990s, James P Alison and colleagues showed that inhibition of CTLA-4 with antibodies caused rejection of several kinds of tumor types in mice [63]. Despite decades long experience with its effects in mice (and later humans), the

exact mechanism of action of CTLA-4 inhibition is still not clear. Translational studies suggest that the CTLA-4 inhibitor ipilimumab enhances T cell priming by allowing expansion of new clones and phenotypes, and imply a critical role of CD4+ T cells [64-67]. At the end of the millennium, the novel concept of "releasing the brakes" of the immune system to treat cancer gained little interest from the pharmaceutical industry. Hence the translation from lab to clinic was slow, and the definitive breakthrough first came more than a decade later: In 2010 ipilimumab became the first drug ever to show a survival benefit in metastatic melanoma and the first demonstration of the potential of immune checkpoint inhibition in humans [34]. Even though only a minority of patients benefited from treatment, the effects were unlike anything seen before in medical oncology: The responses could be delayed by months, sometimes following an initial significant tumor growth (*pseudoprogression*); the side effects mimicked autoimmune disease; and most importantly, the occasional responses proved exceptionally durable and spurred talk about a "tail of the survival curve", suggestive of a cure. In fact, many patients from the very first trials are still alive and well today, more than 15 years later [35]. Although ipilimumab has failed to demonstrate a meaningful effect in most cancer types, its success in melanoma represented a true paradigm shift: Pharmacological targeting of a common target in the immune system could cure metastatic cancer. In the following decade the field of immunotherapy exploded, new targets were identified and tested, and ipilimumab soon became overshadowed by the success of PD-1 inhibition[2].

PD-1 come into play later during T cell activation. Although expressed by all activated T cells upon ligation of TCR, a high PD-1 expression is sustained only during prolonged antigen stimulation [68, 69]. Its ligands PD-L1 (B7-H1) and PD-L2 (B7-DC) are widely expressed by both immune cells and some non-hematologic cells, but of particular relevance is that PD-L1 is commonly expressed by cancer cells and stromal cells in the tumor infiltrate [70, 71]. PD-1 attenuates TCR signaling through inhibition of its intracellular messengers, which maintains the T cell in the dysfunctional state that characterizes an exhausted phenotype [72]. As PD-L1 expression is inducible by inflammatory cytokines, most notably IFN-γ, PD-1 appear to be particularly important in the feedback loop of **adaptive resistance** that limit the T cell attack against peripheral tissues, including cancer (**Figure 3B**) [73]. Indeed, seminal experiments in the mid 2000s showed that inhibiting PD-1 appeared more effective, and less toxic, than CTLA-4 in mouse models [74, 75]. When the first study of efficacy in humans were published in 2012 it became clear that these findings translated well: PD-1 inhibition had greater efficacy in more diseases and less side effects than ipilimumab [76]. In 2014 the first randomized trial of a PD-1 inhibitor (nivolumab)

---

[2] In the following text, *PD-1 inhibition* will refer to any treatment that disrupt the inhibitory signaling by PD-1 and thus include treatment with inhibitory antibodies to either PD-1 or PD-L1.

showed a 40% response rate and an unprecedented improvement in survival over standard therapy in melanoma, leading to its approval the same year [36]. In the following years several inhibitors of PD-1 or PD-L1 have been approved for a large number of diseases in various settings, and have cemented immunotherapy as a fourth modality for cancer treatment alongside surgery, radiation therapy and cytotoxic drugs [77]. However, the response rates are usually much lower that the 40% seen in melanoma (with some rare notable exceptions such as Hodgkin's lymphoma, Merkel cell carcinoma and advanced squamous cell carcinoma of the skin) [78]. Due to the spatial and temporal separation of CTLA-4 and PD-1 in T cell activation, there is a rationale for dual inhibition. Indeed, combined CTLA-4 and PD-1 inhibition with ipilimumab and nivolumab gives a numerically higher response rate and overall survival in cutaneous melanoma, but at the expense of drastically increased toxicities [40]. A lower dose of ipilimumab may significantly lower the toxicity and is evaluated in melanoma and other diagnoses [79] (NCT02714218; NCT03302234). At the same time several other checkpoint inhibitors are being investigated that hopefully have a more acceptable toxicity profile.

Lymphocyte-activation gene-3 (**LAG-3**) is commonly co-expressed with PD-1 in CD8+ TILs with an exhausted phenotype both in mouse models and patients [80, 81], and dual blockade synergizes to inhibit tumor growth in mice [82]. LAG-3 has structural similarities to CD4, and binds MHC class II with high affinity, although other ligands have been proposed [60]. Several trials are ongoing with LAG-3 inhibitors, mostly in combination with a PD-1 inhibitor. The LAG-3 inhibitor relatinib is already in phase III (in combination with nivolumab), following demonstration of safety and an 11.5% response rate in PD-1 refractory patients in a phase I study [83], as well as satisfying results in an unpresented phase II cohort (NCT03470922). Other inhibitory checkpoints currently investigated as targets for inhibition include T cell immunoglobulin- and mucin-domain-containing molecule 3 (**TIM-3**) and T cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (**TIGIT**) as well as several other ligands of the **B7 family** (of which CD80, CD86, PD-L1 and PD-L2 are also part) [84].

While there is certainly a large number of potential "next generation" checkpoints to be explored, there are also reasons for curbed expectations. Many of the known checkpoints either show overlapping expression and function with PD-1/PD-L1, or have a complexity in ligands and biology that may make the effects of simple inhibition unpredictable. Even as more potential targets emerge, inhibition of PD-1 will likely continue to be the mainstay of immunotherapy in many years to come, due to its apparent central role in inhibition of T cell effector function, as well as excellent tolerability. Indeed, most ongoing trials of novel immunotherapies have a PD-1

inhibitor backbone and as the number of potential combinatory candidates grow, it is becoming increasingly important to decipher the underlying mechanisms of response (and lack thereof) and let basic science guide the choice of rational combinatory partners.

1.2.3 Determinants of response and resistance to immunotherapy

The accumulation of clinical experience with checkpoint inhibition has taught us that the effect of immunotherapy is diverse. First, the efficacy varies greatly between diagnoses, from no meaningful effect in general in diseases like brain tumors or pancreatic cancer, to effect in almost all patients with Hodgkin's lymphoma [85, 86]. But even within the same tumor type the effect varies, as illustrated by PD-1 inhibition in melanoma: One fifth of patients get a rapid onset of a complete **response** with the durability that has become the hallmark and promise of immunotherapy. Twice as many patients, however, seem to have no effect at all and experience an immediate progression of disease telling of an **inherent resistance**. The rest fall somewhere in between: They either have a limited period of disease stabilization, or an initial response that weans with time under the development of **acquired resistance** [40]. As of today, there is no way to determine in advance if a patient will respond to immunotherapy or not. It has therefore become a major focus of translational immunotherapy research to search for biomarkers for response and resistance. Much of what we have learned so far comes from the experience with PD-1 inhibitors in melanoma and non-small-cell lung cancer (NSCLC), as well as mouse models. Due to the complexity of the underlying biology of both cancer and the immune system, generalization between species, diseases and treatments should be done with some caution.

The commonly proposed mechanism of action of checkpoint inhibitors would require some pre-existing antitumor reactivity, evident by the existence of an immune infiltrate in the **tumor micro environment** (TME). Indeed, the crude quantification of TILs was identified as a prognostic factor in numerous cancers long before the current era of immunotherapy [87]. Furthermore, the characteristics of the TIL infiltrate was early shown to be associated with response to PD-1 inhibitors in melanoma [88]. Particularly the density of CD8+ T cells showed correlation, whereas the influence of CD4+ T cells seemed more diverse. Paradoxically, the first disease to have a validated prognostic immunohistochemistry (IHC) *immunoscore* based on characteristics of the T cell immune infiltrate, was colorectal cancer, a disease where immune checkpoint inhibitors in general have been particularly disappointing [86, 89]. Simply counting TILs does not, however, reflect the complexity of the immune infiltrate. T cells come in many flavors, and particularly CD4+ T cells undergo a polarizing differentiation during their activation thought to be mostly influenced by cytokines. The

resulting CD4+ subsets can have conflicting roles in shaping the tumor micro environment. The classical **type 1 T helper cells** (Th1) that develop under the influence of type I interferons and IL-12, are considered to have a crucial role in facilitating an anti-tumorigenic "inflamed" TME through their secretion of e.g. interleukin-2 (**IL-2**) and IFN-γ as well as chemokines that attract other inflammatory cells. CD4+ **T regulatory cells** (Tregs), on the other hand, are known through studies of autoimmune disease to have a potent immunosuppressive capacity [90]. Thought to develop under the influence of the cytokine transforming growth factor-β (**TGF-β**), Tregs' exact role in tumor biology remains elusive, but their presence has been shown to be associated with impaired outcome [91]. Unlike other T cells, Tregs constitutively express CTLA-4 and in mouse models the effects of CTLA-4-inhibition appear to depend upon depletion of Tregs, although this is of doubtful importance in humans [92, 93]. Even **B cells** may have an important role in generating an efficient immune response against tumors. Although their role is incompletely understood, recent work show that their presence in intratumoral tertiary lymphoid structures correlate with effect of CTLA-4 and PD-1 inhibition in melanoma [94-96].

According to the model of adaptive immune resistance described above, **PD-L1 expression** could be seen as a surrogate for a pre-existing immune response. If it were the dominating mechanism for immune escape, PD-L1 expression (measured in tumor biopsies by IHC) should further be predictive for response of PD-1 inhibition. Indeed, there is a correlation between PD-L1 expression and response across diseases, including melanoma [97], but it is rather weak: A high PD-L1 expression does not guarantee a response, on the other hand, even patients with no PD-L1 staining can have durable responses [98]. Hence the predictive value of PD-L1 expression in individual cases is low. In other diseases and settings, the negative predictive value of low PD-L1 expression is sufficient to exclude patients from treatment. However, different methods, antibodies and cut-offs for positivity (ranging from ≥1% to 50% of counted cells), makes comparison between studies difficult [99]. Furthermore, the use of archival tissue raises concerns, e.g.: Does a tumor sample, often acquired by needle biopsy, months or years earlier, represent the current state of a tumor with a heterogenous and changing PD-L1 expression? As discussed above, PD-L1 expression is a dynamic response to an ongoing T cell attack, therefore PD-L1 expression in a biopsy taken after start of treatment seems to correlate better with response [100]. However, the value of repeated on-treatment biopsies in clinical routine is limited by the impracticality and invasiveness of a biopsy, and the fact that a clinical/radiological evaluation often gives a definitive answer only weeks later. A promising, more feasible way of assessing PD-L1 expression in real time, is by positron emission tomography (PET). By labelling anti-PD-L1 antibodies with zirconium-89 (^{89}Zr) isotopes, PD-L1 expression can be visualized and quantified in vivo. In a pilot study, pre-

treatment **PD-L1 PET** signal was a strong predictor of response to subsequent PD-1 inhibition, but this still needs to be validated [101].

PD-L1 is not only expressed on tumor cells, but can even be upregulated on cells in the **immune infiltrate**. The added value of separating expression on tumor and immune cells in PD-L1-testing is still uncertain, but is being explored in several trials [99]. Macrophages are myeloid cells of the innate immune system that often make up a major component of the immune infiltrate, frequently express high levels of PD-L1, and may be a significant contributor to the adaptive immune resistance [102-104]. However, the role of **tumor associated macrophages** (TAMs) is diverse. During activation, macrophages undergo a polarizing differentiation, classically divided into proinflammatory M1 or anti-inflammatory M2 phenotypes. Whereas classical (M1) macrophages are usually considered antitumoral, TAMs more often have a M2-like phenotype and mediate immune suppression and resistance to checkpoint inhibition through e.g. secretion of TGF-β and IL-10, and expression of PD-L1 [105-107]. It has, however, become clear that activated macrophages are in fact highly plastic and adapt their phenotype on a continuum between (and beyond) the M1-like and M2-like extremes [108]. Several novel approaches, in various stages of clinical testing, aims to manipulate TAMs and skew (*repolarize*) the population towards an anti-tumorigenic phenotype, inhibit the recruitment of TAMs monocytic precursor, or to target the survival of TAMs in the TME [109]. Even other myeloid cells can have a suppressive effect in the TME. In the recent decade, particular interest has been devoted to the elusive **myeloid derived suppressor cell (MDSC)**. With a morphology and phenotype similar to monocytes or neutrophils, MDSCs are now thought to represent pathologic activation states of these cell types [110]. The monocytic MDSC (M-MDSC) has even been proposed to be a precursor cell of TAMs, and high numbers of M-MDSCs in blood has been associated with impaired survival and inherent resistance to checkpoint inhibition in melanoma [111, 112]. Consequently, MDSCs have become an attractive target for novel immunotherapies [113]. Even **dendritic cells** share hematopoietic precursor with macrophages and neutrophils. Due to their crucial role in T cell activation, dendritic cells in the immune infiltrate are subject to intense study. It has been shown in mice that a rare subset of migratory BATF3-driven/CD103+ DCs are particularly good at cross-priming T-cells in the tumor draining lymph node [114-116]. Additionally, their continued presence in the TME appears to be required for recruitment of CD8+ effector T cells through their production of ligands to chemokine receptor CXCR3, which is highly expressed on activated T cells [116]. Strategies to enhance DC activation with agonists of the innate immune response are being evaluated in clinical trials.

An obvious prerequisite for effective T cells in the TME, is that there is something there for the T cell to recognize: **Antigens** presented on MHC. As random somatic mutations can be the source of immunogenic neoantigens, a high number of somatic mutations should imply a greater chance of generating an antigen for which there is a pre-existing T cell clone [117-121]. Indeed, a high **tumor mutational burden** (TMB) is associated with benefit of checkpoint inhibition across cancer types [122-124]. A particular high TMB is found in cancers with alterations in genes involved in **mismatch repair** (MMR) [125]. As predicted, a trial of PD-1 inhibition in MMR deficient cancers showed an exceptionally high response rate (53%) across 12 different tumor types, many of which are otherwise unresponsive to PD-1 inhibition [126]. In addition to support for the hypothesized importance of neoantigens, these findings led to FDA-approval of pembrolizumab for all MMR deficient cancers, making it the first ever tissue agnostic cancer drug [127]. However, TMB may be confounded by several other factors [128, 129], and in MMR proficient cancer, TMB lack predictive value in individual cases [130]. This may be partly because all neoantigens are not created equal. For instance, small insertions and deletions (indels) that cause frameshifts, have a much greater impact on the amino acid sequence in the translated peptide, than non-synonymous single nucleotide variants (SNV). As T cells specific to highly altered neoantigens are more likely to have avoided central tolerance, a high amount of indels may in part explain why some cancers like renal cell carcinoma respond better than predicted by their rather low TMB [131]. Furthermore, a high TMB may be counteracted by high **intra tumor heterogeneity** that seems to impair immunogenicity, suggesting that clonal neoantigens may be more immunogenic than subclonal events [128, 132, 133].

An altered peptide first becomes an antigen for a CD8+ T cell when presented on MHC-I. Human **MHC-I** is encoded by the genes human leucocyte antigen (**HLA**)-*A, HLA-B* and *HLA-C*. The *HLA* genes are the most polymorphic genes known, and each HLA allele only binds a restricted repertoire of peptides [134]. The finding that maximal heterozygosity at the HLA-I genes is associated with better outcome of checkpoint inhibition, may therefore be explained by presentation of a broader repertoire of neoantigens [135]. Conversely, downregulation of *HLA*, or other parts of the antigen presenting machinery, is a well described mechanism for immune evasion and may also cause resistance to immunotherapy through e.g.: Loss of a single *HLA* allele [135-137]; transcriptional suppression of *HLA* expression [138]; mutations and loss of heterozygosity (LOH) of beta-2-microglobulin (B2M) (a protein needed for the MHC-I complex to be stably bound at the cell surface) [139]. Alterations in the antigen presenting machinery is actually one of the few identified mechanisms of acquired resistance to checkpoint inhibition; however, it seems limited to very few cases [139]. Furthermore, the data on MHC expression in primary resistance to

checkpoint inhibition is conflicting and also implies a role of MHC-II that is incompletely understood [129, 140].

Cancer is a genetic disease, so ultimately even the immune response is shaped by the cancer genome, including its **driver mutations**. For instance, *PTEN* loss is associated to resistance to PD-1 inhibition [141]. In melanoma mouse models, oncogenic WNT/β-catenin signaling reduces T cell infiltration and abrogates checkpoint inhibition [114] and the common oncogenic *BRAF V600E* mutation drives degradation of MHC-I [142]. The clinical relevance and actionability of these mechanisms are being investigated. Just as cancer genetics influence the immune system, the immune system also seems to influence cancer genetics and shape the evolution of oncogenes in a *HLA* dependent manner, evidence of the bidirectional nature of **immunoediting** and subsequent **immunevasion** [143].

A tumor is more than just cancer cells and immune cells. The tumor stroma even comprises a connective tissue of **cancer associated fibroblasts** (CAF) and blood- and lymph vessels entangled in an extracellular matrix of proteins and extracellular fluid (ECF). Both CAF and endothelial cells of the tumor vasculature have been shown to directly impair trafficking and function of T cells [144-146]. Furthermore, they contribute in making the ECF a very hostile environment for T cells: Hypoxic and low in pH; low in glucose and crucial amino acids like tryptophan and arginine; and high in immunosuppressive metabolites like adenosine and lactate [147-152].

Due to its complexity in structure and function, there is a need to characterize the TME beyond what is possible with traditional IHC. A now widely used approach is global transcriptomic analyses to characterize **gene expression profiles** (GEPs) associated with response or resistance to checkpoint inhibition. Not surprisingly, such GEPs generally show enrichment of genes associated with T cell function or genes expressed in response to IFN-γ [153-156]. Although GEPs are showing impressive predictive value for response to checkpoint inhibition in some cases, their usefulness in clinical routine has not been prospectively validated, and their reproducibility in multiple patient cohorts has been challenged [157]. At the same time, novel sequencing techniques that allows for deep sequencing of single cells in the TME, as well as dramatic improvements in imaging technologies and bioinformatic tools, are rapidly increasing the detail with which the TME can be studied [158-161]. Hopefully, coming years will see an increased mechanistic understanding in how the components of the TME influence response and resistance to immunotherapies.

A tumor is not an isolated system. Both the cancer and the immune system are influenced by the organism they inhabit. In a surprising example, several independent

preclinical, translational and clinical studies have revealed an association between response to checkpoint inhibition and the composition of the **gut microbiome** in both melanoma and NSCLC [162-166]. The underlying mechanism is still unknown, and the complexity of the issue is illustrated by the fact that different studies imply importance of different microbial taxa [167]. Other host factors associated with improved outcome of checkpoint inhibition include higher age, male gender and obesity [168-170]. However, these (hardly modifiable) factors are difficult to isolate from possible confounders, and their generalizability is uncertain.

As of now, **biomarkers** have very limited relevance in melanoma. Although many correlates to response have been identified, they lack the predictive value to guide treatment decisions in individual cases. Instead classical clinical markers of poor prognosis, such as impaired performance status, elevated LDH and brain metastases, unfortunately are also predictors of poor response to PD-1 inhibition [39, 171]. Owing to the immense heterogeneity within, and between patients and cancer types, it is unlikely that any single marker will have the same predictive value as we have become used to with targeted therapies. Instead we might hopefully in the not too distant future rather use an array of different biomarkers to tailor combinations of immunotherapy in a more individualized manner.

1.2.4 Beyond Immune Checkpoint Inhibitors

Although checkpoint inhibition has transformed the treatment of metastatic melanoma, more than half of the patients still die from their disease within five years, clearly underlining the need for enhancing immunotherapies further. A very direct approach is to enhance, activate, expand or modulate T cells in vitro: **Adoptive cell transfer** (ACT)**.** In fact, one of the first successful attempts at restoring an efficient antitumoral immunity was pioneered already in the 1980s by Dr. Steven Rosenberg and colleagues at the NIH surgery branch, USA, using ACT. By culturing tumor biopsies in high doses of IL-2, TILs can be extracted. Subsequent ex vivo stimulation massively expands the TILs before transfusing them back to the patient together with IL-2, following lymphodepleting chemotherapy. This method of TIL therapy can achieve impressive durable responses in as many as 50% of the patients with metastatic melanoma in non-randomized trials [172-174]. However, it is labor intensive, costly and has significant toxicities (mainly attributed to high dose IL2), making it unlikely to ever become a standard of care in its present form (particularly considering the success of immune checkpoint inhibitors). However, it has proven an invaluable platform for translational research, particularly in the fields of antigen selection and prediction [136]. But maybe most importantly: The occasional cures of individual

patients treated with TIL therapy have served as powerful demonstrations of the unique potential of immunotherapies.
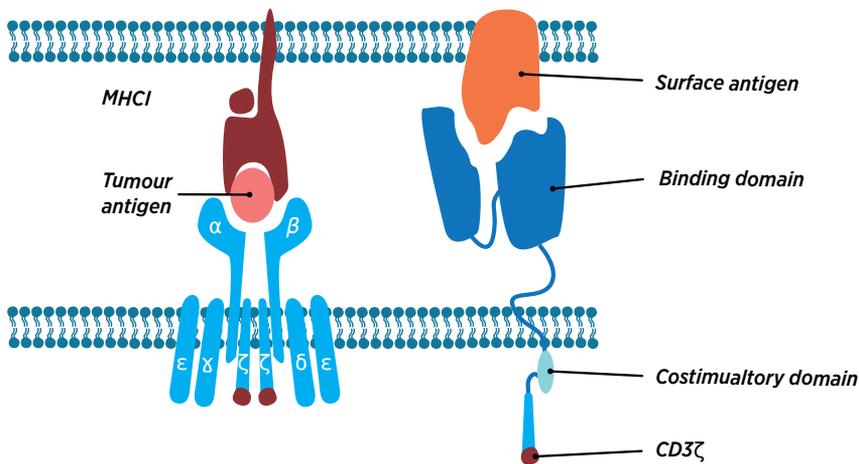

Figure 4 Schematic illustration of the T cell receptor (left) and chimeric antigen receptor (right).

Further development of cell-based immunotherapies include selecting TILs for antigen specificity, or providing T cells with exogenous receptors against known antigens in a patient's cancer by transgenic expression of **TCRs** or **chimeric antigen receptors (CAR)** [175]. The CAR is a synthetic receptor with a binding domain (usually derived from a monoclonal antibody) fused to the intracellular signaling domain (CD3ζ) of the TCR (*signal one*) as well as costimulatory domains (*signal two*) (**Figure 4**). Since the CAR provides both signal one and two, the CAR T cell does not require priming by an APC. Furthermore, the antibody-derived binding domain makes it independent of MHC, so that the cytolytic capacity of T cells can be unleashed against cancer cells bearing any specific surface protein. The first major clinical breakthrough came with CAR T cells against the common B cell marker CD19. Two commercial CAR T cell products have shown impressive durable responses in large fractions of patients with certain adult and pediatric B cell malignancies, and are now part of the standard medical care [176-178]. So far, no CAR T-cell therapy has demonstrated convincing effects in solid tumors. Challenges include finding an appropriate target (as truly cancer specific surface markers are scarce) as well as enhancing trafficking, function and survival of the CAR T cell in the hostile TME of solid tumors. Drawing from experience with TIL therapy, addition of lymphodepletion and cytokine stimulation are currently investigated approaches that aim to overcome some of these hurdles [179, 180].

A major disadvantage of current cell therapies is that they are produced in a personalized manner and require advanced biotechnological techniques, making them labor intensive and costly. This may also be an obstacle for feasible **cancer vaccines**. While early cancer vaccines targeted common cancer associated antigens and probably failed due to central tolerance, current promising approaches utilize platforms to identify and target personal neoantigens with individualized mRNA- or peptide vaccines [181, 182]. However, cancer vaccines have yet to demonstrate efficacy in clinical trials and, like cell therapies, still face challenges in obtaining scalability. Advances in molecular engineering has enabled novel concepts for redirecting T cells to specific cancer antigens, using "off-the-rack" mass produced therapeutic agents. One such example is that of **immune-mobilizing monoclonal TCRs against cancer** (ImmTACs), that combine a soluble epitope-specific TCR fused with an antibody derived (single chain variable fraction (scFv)) binding site against CD3. The ImmTAC tebentafusp has a TCR domain against a fragment of gp100 presented on HLA A*02:01 on melanoma cells, and recruits adjacent non-specific T cells through crossbinding with CD3. Trials with tebentafusp are ongoing in both cutaneous and uveal melanoma and show promising effects [183].

Early attempts to stimulate the immune response with cytokines had very limited success, maybe because of the strong inhibitory signals of immune checkpoints (in analogy to stepping on the gas with the parking brakes engaged). Now that important breaks have been identified and uncoupled, we see an awakened interest in augmenting the immune response by cotreatment with stimulatory agents. Furthermore, improved knowledge in cytokine biology, as well as modern molecular techniques, has enabled modification of **cytokines** that may have a better risk-benefit profile than earlier. One such example is bempegaldesleukin, a modified IL-2 agonist [184], that recently demonstrated encouraging response rates in combination with nivolumab in metastatic melanoma [185]. Additionally, several trials are ongoing with agonists of **costimulatory receptors** in T cell activation, such as 41-BB, OX40, ICOS, GITR and CD40 [186]. Even costimulators of the **innate immune** response have great potential to enhance T cell mediated therapies through improved T cell activation and recruitment by APCs. The stimulator of interferon genes (**STING**) pathway appears to be critical for initiation of the type I interferons that activate BATF3+ dendritic cells capable of cross priming CD8+ T cells [187]. Once initiated, the innate response is further propagated by PRRs like toll like receptors (**TLR**s), and several agonists of STING and TLRs are in clinical development and testing (NCT02680184; NCT03956680; NCT04096638).

One goal of novel immunotherapies is to modulate the TME to become a less hostile milieu for immune cells. Already mentioned approaches include targeting of macrophages and MDSCs. As dysfunctional cancer vasculature contributes to the immune suppressive environment, there is also a strong rationale to target the drivers of angiogenesis (e.g. vascular endothelial growth factor (**VEGF**) or its receptor **VEGFR**) to improve the effect of immunotherapy and several trials are ongoing [147] (NCT03820986). The first clinical attempt to modify metabolites in the TME targeted degradation of tryptophan by inhibiting the enzyme indoleamine-pyrrole 2,3-dioxygenase (**IDO**). After promising early phase data, the following trial unfortunately broke a remarkably long streak of positive phase III trials in melanoma, when the addition of the IDO-inhibitor epacadostat to pembrolizumab failed show benefit over pembrolizumab alone [188]. Currently developing approaches include inhibiting the degradation of arginine, and inhibition of adenosine signaling (NCT02903914) [189]

Finally, even traditional treatment modalities in oncology may synergize with immunotherapy. For instance, both **chemotherapy** and **radiation therapy** have the potential to induce immunogenic mutations, antigen spread, immunogenic cell death and T cell chemoattraction, and are already in use in combination or sequence with PD-1 inhibition in NSCLC [190, 191]. In *BRAF V60*0 mutant melanoma, **BRAF and MEK inhibition** synergizes with immunotherapy in mouse models and early phase trials [192-197]. Ongoing phase III trials will elucidate whether combined BRAF/MEK/PD-1 inhibition will result in a survival benefit (and reasonable toxicity) compared to sequential treatment (NCT02908672; NCT02967692; NCT-02902029; NCT02224781).

Considering the vast number of possible immunotherapy combinations, trials will soon risk to outnumber the eligible patients. It is therefore becoming increasingly important to develop preclinical models that can guide rational combinations, and mitigate the risk of exposing patients to ineffective and potentially harmful treatments. Because when you unleash the force of the immune system, healthy tissue may end up in harm's way.

1.2.5 Adverse events associated with immunotherapies

Due to their unique mode of action immunotherapies can induce side effects that are nothing like what we are used to from other cancer therapies. Considering the roles of immune checkpoints in peripheral tolerance, it should be no surprise that **checkpoint inhibition** may lead to the immune system attacking healthy tissues, and give rise to autoimmune-like, **immune related adverse events** (irAEs). Although any

organ can be affected, some are much more frequently involved than others. Highest risk of irAE is seen the first weeks and months of treatment, but irAE may even occur months after cessation of treatment. In recently reported five-year follow-up data, there were no new late stage AEs observed [40].

In monotherapy for metastatic melanoma **CTLA-4 inhibition** with ipilimumab (at 3 mg/kg) give rise to irAEs in approximately 75% of patients, with around 25% of patients developing irAEs graded as severe (common terminology criteria for adverse events (CTCAE) grade ≥3) [34, 198]. The most common severe irAE is diarrhea and/or colitis, which can be life threatening. Other irAE include skin reactions, endocrinopathies (hypophysitis and thyroiditis), hepatitis and neurologic disorders. Even though around 50% of patients treated with **PD-1 inhibition** for metastatic melanoma develop an irAE, the rate of grade ≥3 irAE is less than 10% [199]. The most common irAEs are skin reactions, followed by diarrhea and hypothyroidism, which is more common than with ipilimumab. Although PD-1 inhibition is generally very well tolerated, rare irAE like pneumonitis and myocarditis can be life threatening [200]. Combined **CTLA-4 and PD-1 inhibition** with ipilimumab and nivolumab, leads to irAEs in almost all treated patients, with around 50% developing grade 3-4 irAEs. As with ipilimumab monotherapy, diarrhea/colitis is the most common severe irAE, but combined checkpoint inhibition is also associated with particularly high rates of hepatitis and hypophysitis.

Several consensus guidelines for **management of irAEs** have been published [201-203] and generally include: Assessments for diagnosis and to rule out other causes (e.g. infection or progression of disease); grading of severity; symptomatic and supportive care; immunosuppressive agents (primarily corticosteroids) in severe or persistent cases. Checkpoint inhibition may be continued, withheld until irAE is resolved or permanently discontinued, depending on severity and type of irAE, as well as benefit of treatment. As irAEs mimic autoimmune disease in their presentation, management guidelines draw heavily on experience from autoimmunity in organ specific specialties. However, irAEs often respond much better to immunosuppressive therapy than their autoimmune counterpart, underlining that extrapolation between the two should be done with some caution. Instead, prospective studies of optimal handling of irAEs are warranted to fill current evidence gaps. Another imminent need is to improve the consistency in assessment and reporting of irAE in clinical trials [204]. The commonly used grading system (CTCAE) have weaknesses in capturing the type, severity and duration of irAEs, which has probably resulted in underreporting of several common irAEs, as well as a substantial difficulty in assessing true incidence of irAEs in the pivotal clinical trials [205-207]. Ongoing efforts for harmonization of immunotherapy trial reporting, will hopefully result in a terminology that better

separate assumed etiology from symptoms and signs; increase reporting of the use of immunosuppression; and better capture the incidence, grade, timing and duration of irAEs [208].

The underlying mechanisms of irAEs are only partly understood, and probably diverse. Some evidence exists for tissue specific antibody-effects, as well as unmasking of pre-existing autoimmunity [209, 210]. Other possible mechanisms imply a more direct bystander effect from antitumoral T cells that would link **irAEs and response** to treatment [211, 212]. There is indeed cumulating evidence that the occurrence of irAE is associated with treatment benefit of PD-1 inhibition across tumor types [39, 199, 213-216]. Conversely, concerns may be raised that treatment effects could be hampered by immunosuppression in the management of irAEs. Although most studies show that patients who receive immunosuppressive treatment for irAEs have comparable outcomes to those who do not, it is impossible to rule out or that immunosuppression might have blunted an otherwise superior outcome in this group. Hence the impact of high dose corticosteroids remains controversial [217-219]. Hopefully, ongoing and future work will identify targetable, non-overlapping mechanisms that can uncouple the relationship between irAE and effect, and improve the risk-benefit of checkpoint inhibition.

Even adoptive **cell therapies** have adverse events. In the case of TIL therapy, toxicity is dominated by those of the lymphodepleting regimen and subsequent IL-2 treatment. High dose IL-2 leads to sepsis-like symptoms of fever, capillary leak and hypotension, frequently requiring management at an intensive care unit [220]. A related clinical presentation is seen in **cytokine release syndrome** (CRS), the dominating acute toxicity of CAR T-cell therapy in B cell malignancies [221]. CRS is characterized by high levels of inflammatory cytokines, like IL-1 and IL-6, and usually responds well to IL-6 inhibition. In addition to CRS, CAR T cell-therapy is associated with reversible neurological toxicities, that can be severe [176-178]. Furthermore, all cell therapies may even potentially have on-target/off-tumor effects due to shared antigens in healthy tissues, as exemplified by vitiligo and anterior uveitis with TIL or TCR based therapy for melanoma, and B cell aplasia in CD19 CAR T-cell therapy [220].

1.3 Uveal Melanoma

Unfortunately, the progress in treatment of cutaneous melanoma has so far not translated to metastatic **uveal melanoma**. In fact, uveal and cutaneous melanoma have very little in common other than in name and melanocytic origin, and their disparities may actually be traced all the way to the developing embryo. All melanocytes originate from highly migratory progenitor cells in the neural crest (thus sharing origin with e.g. peripheral neurons and their supporting glia) [222]. However, as they migrate throughout the developing embryo, these melanoblasts are subject to different intrinsic and extrinsic factors on their separate routes that likely give rise to site specific heterogeneity in different melanocyte populations [223]. As carcinogenesis often include dedifferentiation and reverting to embryonic traits [224], it is fair to hypothesize that their developmental origin may contribute to making uveal and cutaneous melanoma biologically and clinically distinct entities.
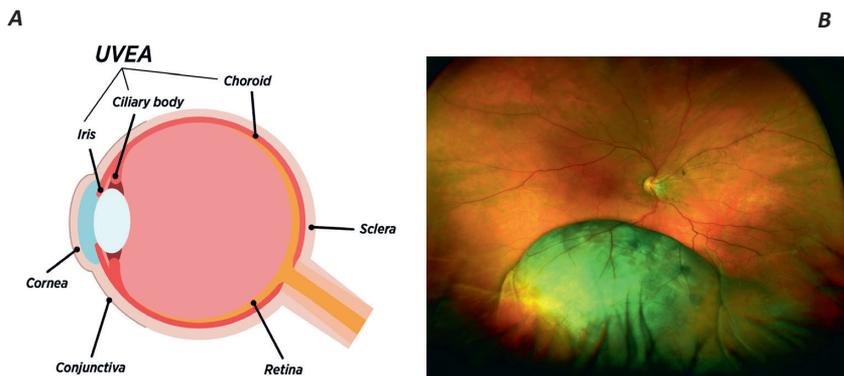
Figure 5 (**A**) Gross anatomy of the eye. (**B**) Wide-angle image of a choroidal melanoma in the inferior aspect of the macula region in the right eye. Secondary retinal detachment inferonasally. Courtesy of St. Erik Eye Hospital.

The terms *ocular melanoma* or *eye melanoma* are sometimes used interchangeably with *uveal melanoma* or *choroidal melanoma*. While the vast majority of uveal melanomas (90%) indeed arise from the choroid, the latter term would, however, exclude melanomas from the ciliary body and iris which are also part of the uveal tract (**Figure 5**) [225]. *Ocular-* or *eye melanoma* on the other hand, would anatomically include even conjunctival melanomas which have more in common with cutaneous or mucosal melanomas (possibly due to a related embryonic origin) and should be treated accordingly [226].

Uveal melanoma is the most frequent intraocular malignancy in adults. However, with an annual **incidence** of only approximately 8 new cases per million in Sweden, it is a very rare cancer and make up only 2 % of all melanoma cases [227]. Known **host risk factors** include fair skin, a propensity to sunburn and freckle, cutaneous nevi, ocular melanosis and choroidal nevi, [228]. The incidence is fairly equal between the genders and increase with age with a median age at diagnosis around 60 years [229].

Little is known about the etiology and **environmental risk factors** of uveal melanoma. Epidemiological evidence seems to contradict a role of sun exposure: Unlike the case for cutaneous melanoma, the incidence of uveal melanoma is stable, or even decreasing in certain populations and the latitude gradient in incidence appears to be inverted [227, 230-232]. However, several of the mentioned host risk factors are indeed associated to photosensitivity. Furthermore, an increased risk of uveal melanoma in occupational welders has been attributed to artificial light exposure [233]. On the other hand, a recent metanalysis found no significant impact of common surrogates for UV-exposure, despite their strong associations to *cutaneous* melanoma [228]. Most importantly, modern molecular profiling should now have provided definitive evidence in a decades long debate: Independent whole genome sequencing efforts show an unambiguous absence of a UV-light induced mutational signature in a large number of sequenced primary and metastatic uveal melanomas [234, 235]. The sole exception seems to be iris melanomas (which only constitutes 1% of all uveal melanomas and hence had not been sequenced before), where we recently demonstrated a UV-light induced mutational signature, explained by its sun exposed origin in the anterior parts of the eye [235]. With the rare exception of iris melanoma, the above-mentioned host risk factors are thus likely due to confounding factors other than UV-exposure. For instance, traits like fair skin, blue eyes and a propensity to freckle are caused by production of relatively more yellow-reddish pheomelanin pigment than black or brown eumelanin which may itself contribute to carcinogenesis in melanocytes [236]. Even the increased risk in welders may well be attributed to several uncontrolled exposures in an industrial working environment.

Although usually being only millimeters in size, most uveal melanomas give rise to **symptoms** like blurred vision or visual field defects that lead to the diagnosis [229]. Other cases may be incidentally found by an ophthalmologist or optician. Treatment modalities for the primary tumor include enucleation, transpupillary thermal therapy, brachytherapy with radioisotopes (ruthenium-106 or iodine-125), external proton beam radiation or observation. An aggressive approach has not been shown to give superior survival over less invasive modalities, suggesting that metastatic seeding is a very early event in the disease [237]. Consequently, most patients in Sweden are now

treated with brachytherapy which spares the eye and often some degree of vision. The choice of treatment is, however, highly personalized based on size and location of the tumor as well as comorbidities, age and preference of the patient.

Metastases are rare at the time of diagnosis. But despite successful treatment of the primary tumor and excellent local control in most cases, up to half of the patients will nevertheless develop **metastatic disease** and die from the disease in the following years [238]. The risk of relapse is associated with several clinicopathological characteristics of the primary tumor: Size (diameter or protrusion), the presence of epithelioid cells, extraocular tumor extension, presence of orange pigment and tumor location in the anterior uvea, all confer a higher risk of metastatic relapse [239].

In addition to clinicopathological characteristics, **cytogenetic analyses** can provide further prognostic information: It has long been known that the presence of **monosomy of chromosome 3** in primary uveal melanomas is strongly correlated with metastatic relapse and subsequent death [240]. Monosomy 3 often occur with other **copy number alterations** such as gain of the long arm of chromosome 8 (8q+), whereas the gain of the short arm of chromosome 6 (6p+) is correlated to disomy 3 and good prognosis [241]. More recently, gene expression profiling has identified signatures that can define two distinctive classes strongly associated with excellent (class 1) or poor (class 2) survival [242, 243]. Although developed into a patented, commercially available and internationally validated qPCR kit of only 12 discrimination genes, the lack of therapies to modify risk of recurrence renders its value in clinical practice limited [244].

For unknown reasons, the primary site of metastases in the vast majority (90%) of patients is the liver [238]. For patients with hepatic metastases only, liver directed therapies like surgery, stereotactic radiotherapy, radiofrequency ablation, isolated hepatic perfusion (IHP) with chemotherapy or transarterial embolization with chemotherapy or radioisotopes, may benefit well selected patients, although prospective data demonstrating this is lacking. An ongoing trial of IHP with chemotherapy versus best supportive care will demonstrate if promising retrospective data translates to improved survival in a randomized phase III setting (NCT01785316) [245, 246]. For patients presenting with extrahepatic metastases or progression of disease following liver directed therapies, empirical treatment with chemotherapy can be considered, but responses are few and seldom durable. Consequently, the prognosis of metastatic uveal melanoma is poor, with a median survival of less than a year in most trials, and only around 10 % of the patients surviving two years after diagnosis, making it a major unmet medical need in melanoma oncology [247, 248].

1.3.1 Genetics, molecular profiling and targeted therapies

In contrast to its cutaneous counterpart, uveal melanomas generally have an exceptionally low mutational burden (0.24/Mb). Furthermore, recurrent mutations reside in different **oncogenes** and tumor suppressors than in cutaneous melanoma, underlining that the diseases are distinct entities [234]. Uveal melanoma lacks recurrent mutations in *BRAF, NRAS, NF1* or *KIT*. Instead, 80-90% of uveal melanomas harbor activating mutations in the *Guanine Nucleotide-Binding Protein G(q) subunit Alpha (**GNAQ**)* gene or, in a mutually exclusive manner, *Guanine Nucleotide-Binding Protein subunit Alpha-11 (**GNA11**)* [249, 250]. In most remaining cases, recurrent mutations can be found in *PLCB4* or *CYSLTR2* [251, 252]. *GNAQ* and *GNA11* are paralogs that encode for an alpha subunit downstream of several G protein-coupled receptors that are important for melanocyte homeostasis. The recurring mutations at codon Q209 (or in some cases R183) lead to blocked GTPase activity and lock the G protein in a GTP-bound active state that constitutively stimulates downstream signaling. Like the recurring *BRAF* or *NRAS* mutations in cutaneous melanoma, *GNAQ/GNA11* mutations appear to be early or initiating events in uveal melanoma and similarly lead to increased signaling in the MAPK pathway [253, 254]. Mutant *GNAQ/11* drives MAPK through PLCβ-mediated activation of protein kinase C (PKC) and RAS guanyl-releasing protein-3 (RasGRP3) (**Figure 1B**).

Drugs selectively inhibiting mutated GNAQ/11 are lacking, and may in fact be challenging to develop due to the nature of the mutations and the ubiquity of G-proteins in normal cell functions. Instead attempts have been directed at inhibiting downstream signaling molecules such as PKC or MEK. Indeed, the selective MEK 1/2 inhibitor selumetinib showed pre-clinical activity against GNAQ mutant uveal melanoma cell lines [255] and an objective response rate of 14% as well as improved progression free survival over chemotherapy in a phase II trial in metastatic uveal melanoma [256]. However, in a following randomized phase III study, adding selumetinib to dacarbazine, did not significantly improve progression free survival nor overall survival [257]. Even PKC inhibitors have demonstrated anti-cancer effects in vitro, but reports from early phase clinical trials indicate modest efficacy in monotherapy [258, 259].

The **PI3K/AKT/mTOR pathway** is also commonly activated in uveal melanoma, often in conjunction with MAPK hyperactivity [260, 261]. PI3K/AKT/mTOR activation appears to be independent of GNAQ/11 signaling in uveal melanoma. Instead, PI3K/AKT/mTOR activation can be driven by loss of *PTEN* (**Figure 1B**). Although rarely mutated in uveal melanoma, LOH of the *PTEN* locus is common, and reduced cytoplasmatic expression of PTEN is associated with impaired prognosis [262]. Furthermore, PI3K/AKT/mTOR signaling can also be activated by the

binding of ligands to receptor tyrosine kinases (**RTK**) such as **MET**, **KIT** or the insulin-like growth factor 1 receptor (IGF-1R), which are all frequently overexpressed in uveal melanoma [263-265]. RTKs signal through numerous intracellular pathways, so downstream inhibition may be challenging. There is therefore a rationale for inhibiting multiple targets in the signaling cascade, nevertheless, combined inhibition of IGF-1R and mTOR showed limited efficacy in a phase II trial [266]. Other trials are ongoing evaluating the effect of mulitkinase inhibitors (e.g. NCT02223819, NCT02068586). In one study, simultaneous inhibition of both the PI3K/AKT/mTOR and MAPK pathways was required for apoptotic cell death in uveal melanoma cell lines [267]. However, in a randomized phase II trial the addition of an AKT inhibitor to a MEK inhibitor did not to improve progression free survival [268].

Part of the reason for disappointing results in trials targeting MAPK-signaling in uveal melanoma, may be because *GNAQ/GNA11* mutations lead to signaling in parallel pathways other than MAPK, most notably leading to hippo-independent activation of the potent oncogene yes associated protein (YAP) via the proteins TRIO, Rho and focal adhesion kinase (FAK) (**Figure 1B**) [269, 270]. Furthermore, sole inhibition of targets downstream of oncogenes has so far not been very successful in other diagnoses. In analogous examples, MEK inhibition in NRAS mutated metastatic cutaneous melanoma failed to yield meaningful effects [29], and the effect of MEK inhibitor monotherapy in BRAF mutated melanoma is modest compared to that of direct BRAF inhibition [20, 271]. It may be speculated that the approach of indirect, downstream targeting may be particularly susceptible to selection of resistant clones. Combined inhibition of several targets in the same or related pathways may be more effective, but all attempts so far have been disappointing (although a trial assessing the effect of combined PI3K and PKC inhibition is still ongoing (NCT02273219)). Intriguingly, recent data have raised new hope for directly targeting GNAQ/GNA11 in uveal melanoma when it was shown that the cyclic depsipeptide FR900359 inhibits GNAQ/11 and 14 (but not other mammalian Gα isoforms) and has in vitro and in vivo efficacy against GNAQ/11 driven uveal melanoma [272, 273]. As it is not specific for mutated GNAQ/11 it remains to be seen if the on-target/off-tumor effects of compounds like FR900359 allows for use in patients.

While *GNAQ/GNA11* mutations appear to be initiating events in uveal melanoma, they are roughly equally distributed between the low risk class I and high-risk class II tumors, and can also be found in benign melanocytic lesions [249, 250]. Consequently, additional alterations are required for a uveal melanoma to obtain metastatic behavior. Monosomy of chromosome 3 has been known for decades to be strongly correlated with poor risk [240]. Eventually, sequencing efforts revealed frequent alterations in the gene for BRCA1 associated protein 1 (**BAP1**) on the remaining copy

of chromosome 3, consistent with the Knudsen two hit hypothesis for a **tumor suppressor** [274]. While mutated in a less than half of primary uveal melanomas, loss of function mutations in *BAP1* has subsequently been found in over 90% of metastatic tumors [235, 275]. Additionally, the majority of *BAP1* mutations are truncal, suggesting a crucial role in the metastatic process [235, 254]. Indeed, knocking out or restoring BAP1 in cell lines is sufficient to initiate a switch between a Class I and II-like gene expression pattern [235, 276]. The mechanisms by which loss of BAP1 induces metastases is still not clear; however, a recent study demonstrated an association with vast epigenetic changes including downregulation of several loci on the remaining chromosome 3 [276].

In cases with disomy 3, single nucleotide substitutions in *EIF1AX* or *SF3B1* can be seen in an almost non-overlapping pattern [277, 278]. Consequently, these alterations are both associated with class I tumors; however, SF3B1 mutations seem to infer an intermediate metastatic risk. By integrating copy number data, methylation patterns and RNA expression with DNA analyses, a further subdivision of primary uveal melanomas into four molecularly distinct subsets with strong correlation to prognosis has now been suggested (as summarized in **Figure 6**) [275]. While the clinical value of such pure prognostic markers is questionable, this and similar efforts have greatly increased our molecular mechanistic understanding of uveal melanoma and provide a framework for continued research that will hopefully one day soon result in a therapeutic breakthrough for patients in dire need for treatment options.

Gene Alterations	Disomy 3				Monosomy 3			
	GNAQ, GNA11, PLCB4 or CYSLTR2							
	EIF1AX		*SF3B1*		*BAP1* abberancy			
Copy Number	6p+		6p+, 8q+		8q+, ++		8q+, 6q-, ++	
DNA methylation	1		2/3		4			
Transcription	1	2	1	2	3	4	3	4
Metastatic risk	LOW						HIGH	

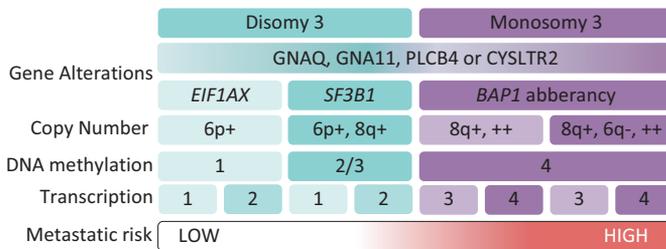**Figure 6** Four molecularly and clinically distinct subsets of uveal melanoma can be identified by integrating copy number data, methylation patterns and RNA expression profiles with DNA analyses. Adapted from [234, 275]

1.3.2 Uveal melanoma and the immune system

Uveal melanoma is a very rare disease compared to skin melanoma, but the uvea is also very small compared to the skin. Actually, the "tumor density" (i.e. incidence

adjusted for tissue surface area) is almost 50 times higher for uveal than for skin melanoma, which is puzzling since uveal melanocytes are shielded from UV radiation and other known carcinogens [227]. One may hypothesize that efficient immunosurveillance is lacking. The eye is frequently described as a site of **immune privilege**, often defined by delayed rejection of tissue grafts in tissues with a limited capacity to regenerate (e.g. gonads and brain). Rather than a passive "lack of an immune system", immune privilege actually involves several active mechanisms that dampen inflammation and suppress adaptive immune responses, many of which may be highjacked by uveal melanoma [279]. For instance, primary tumors often have a low expression of MHC-I, which has been associated with impaired survival, maybe implicating a role of NK cells in immunosurveillance [280]. Furthermore, and in contrast to most other cancers, a rich immune infiltrate is correlated with a higher mortality rate in uveal melanoma, suggesting a presence of suppressive immune cells [281].

While the exclusively hematogenic spread of uveal melanoma can be explained by lack of lymphatics in the uvea, there is no anatomical basis for liver tropism. A possible explanation may be high levels of liver-synthesized growth factors like hepatocyte growth factor (HGF) and IGF-1 whose receptors MET and IGF-1R are both frequently expressed on uveal melanoma cells [264, 265]. An alternative, albeit speculative, explanation, may be that cancer cells developed without immunoediting will have difficulties surviving in tissues with an efficient immunosurveillance. Perhaps the liver represents an immunologic niche that is suitable for the uveal melanoma cells? It has a natural population of MDSCs, and the liver's Kupffer cells can suppress CD8+ T cells through expression of PD-L1 [282]. However, the liver is extremely rich in NK cells, and metastasized uveal melanoma cells with low expression of MHC would need to adapt to avoid NK cell killing. Indeed, metastatic uveal melanoma cells have been shown to lose expression of ligands for the NK-cell activating receptor NKG2D [283]. The immunogenic potential of uveal melanoma was demonstrated when a non-randomized trial of adoptive TIL therapy demonstrated an objective response rate of 35% [284]. However, the toxicity and inaccessibility of TIL therapy makes it unlikely to become standard of care in its current form.

Patients with uveal melanoma were excluded from all phase III trials of checkpoint inhibition in melanoma and no prospective trial of PD-1 inhibition in uveal melanoma has been completed to date. There are reasons to suspect that metastatic uveal melanoma may be rather unresponsive to checkpoint inhibition: It has among the lowest number of somatic mutations of all cancers, and generally a low expression of PD-L1 on tumor cells (and of PD-1 on TILs), factors associated with a low response rate to PD-1 directed therapy across tumor types [234, 285]. Indeed, two large early, retrospective multicenter studies of PD-1 inhibition in patients with metastatic uveal

melanoma both found an objective response rate below 5% [286, 287]. In addition, a later multicenter, retrospective study, evaluated 100 patients with uveal melanoma treated with checkpoint-inhibitors. The cohort included 68 patients treated with PD-1-inhibitors in first line (n=37) or following ipilimumab (n=31), among which not a single objective response was observed [288]. Studies are ongoing to evaluate if combined CTLA-4 and PD-L1 inhibition have better efficacy, but no study has been published. However, preliminary data from a phase II trial of ipi-nivo, showed an objective response rate of 11.5% as well as an overall survival and progression free survival that compare favorably to historic data [289]. Before widespread use of ipi-nivo, the indication of modest efficacy should be carefully balanced against the significant associated toxicities. A more promising and seemingly less toxic alternative is tebentafusp, a biological drug bispecific for CD3 and gp100 (as briefly described under 1.2.4) [183].

1.4 Epigenetics

All cells of the body have practically identical genomes. Yet cells can take on a myriad of shapes to serve vastly different functions, and pass their identity on to their daughter cells if dividing. The nuclear processes that govern cellular development and differentiation are called epigenetics, and can be defined as mechanisms responsible for **heritable and reversible changes in gene expression** that are not due to any alteration in the DNA sequence [290].

1.4.1 Epigenetic modifiers

The best described mechanisms of epigenetic modification involve covalent changes to DNA or the associated histone proteins. Additionally, even changes in the **higher order chromatin structure** and the expression of **non-coding RNAs** influence gene expression, although the underlying mechanisms are less well characterized [291, 292].

DNA methylation (addition of methyl groups to cytosine residues) in promotor regions, generally suppresses the expression of the associated gene. While methylation is catalyzed by DNA methyl transferases (**DNMT**), mechanisms for active demethylation are more complex, and traditionally, DNA methylation has been assumed to lead to a rather persistent repression of gene expression [293].

In eukaryotic organisms, DNA is effectively packed in nucleosome units. Each nucleosome consists of a section of the duplex DNA molecule, wrapped almost two turns around an octamer of histone proteins. The enzymatic addition and removal of small molecules to specific sites and residues in the histone tails influences the accessibility of DNA-binding complexes and consequently the expression of genes. The most extensively studied **post-translational modification (PTM) of histones** is acetylation, but histone PTM also includes methylation, phosphorylation, ubiquitylation and others less well described [294]. In general, acetylation of lysine residues within the histone tails by histone acetyltransferases (**HATs**, "writers"), leaves the DNA more accessible to transcription, whereas histone deacetylation by histone deacetylases (**HDACs**, "erasers"), decreases gene expression (**Figure 7**). To date, 18 different HDACs have been discovered and are grouped in four classes (I-IV) based on sequence similarities and diversity in substrates, tissue specific expression and cellular location. In addition to histones, many HDACs have been shown to also have significant non-histone targets [295].

In addition to "writers" and "erasers", there are also a large group of proteins that serve as "readers" of histone modifications. For instance, 46 human proteins contain bromodomains that can bind acetylated lysine residues in histones (and other proteins) and, via mechanisms only partly understood, regulate transcription and chromatin remodeling [296]. A particularly well studied family of epigenetic readers in cancer, are the bromodomain and extra-terminal (**BET**) bromodomains. There are now several available potent **BET inhibitors** that alter the transcriptional programs of treated cells and show broad anti-cancer activity in preclinical studies [297].
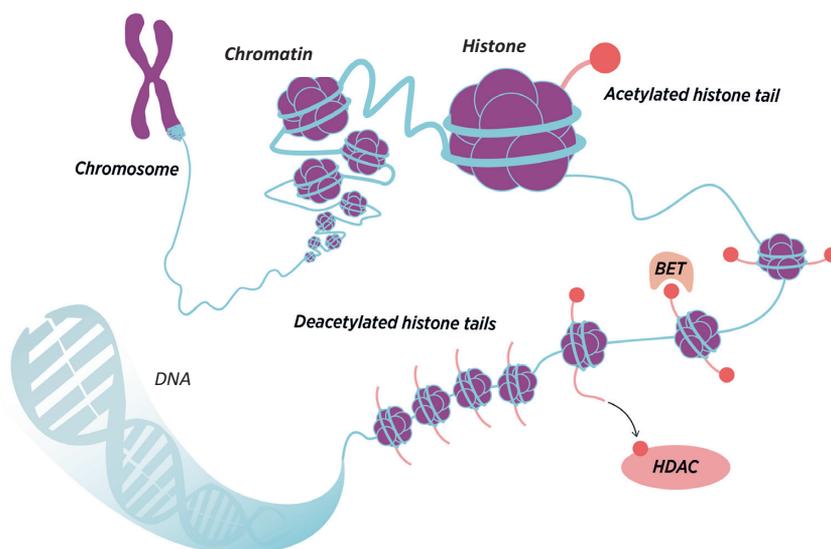
Figure 7 HDAC and BET in chromatin remodeling. Acetylated histones are associated with a transcriptionally permissive chromatin state. HDAC, histone deacetylase; BET, bromodomain and extra-terminal bromodomain

1.4.1 Epigenetics and cancer

In addition to somatic mutations involving oncogenes and tumor suppressor genes, it is now widely accepted that epigenetic alterations contribute to oncogenesis [298]. Tell-tales of epigenetic involvement in cancer include abnormal patterns of DNA methylation, disrupted patterns of histone modifications and changes in chromatin organization (visible to the pathologist in IHC as changes in nuclear morphology). In the absence of specific mutations identified to drive hallmark malignant behavior like invasiveness and metastases, it can be argued that the **instability of the epigenome** is central in cancer development, as it can lead to vast changes in cancer cell

differentiation and changed expression of oncogenes and tumor suppressors. Conversely, strikingly many recurrent mutations in cancer affect genes with known roles in the epigenetic machinery, telling of an inextricable relationship between genetics and epigenetics in cancer biology [294].

Epigenetic contribution to oncogenesis is believed to be of particular importance in cancers with few somatic mutations such as certain pediatric cancers, with which uveal melanoma shares genetic similarities [234, 299, 300]. Like developing tissues, cancer cells undergo switches in phenotype that are under epigenetic regulation. For instance, the common aberration of *BAP-1* in high risk uveal melanoma was recently shown to be associated with vast changes in methylation of genes implicated in melanocyte differentiation, suggesting activation of a latent epigenetic program leading to the aggressive class 2 phenotype [276]. Focal hypermethylation of promoters is a well described method for inactivation of tumor suppressor genes in cancer. However, a more general hypomethylation, which is often seen in cancer, is thought to lead to increased genetic instability [301]. Although inhibitors of DNMT exist (e.g. azacitidine and decitabine) and may restore expression of tumor suppressors, their global effect also risks inducing oncogenes, DNA damage and genetic instability in healthy tissues; gives rise to considerable side effects, and thus far DNMT inhibitors are only proven effective for treatment of myelodysplastic syndrome and not for solid cancer [301-304].

Dysregulation of HDACs has been demonstrated in a large number of solid tumors and is probably involved in silencing of tumor suppressor genes. Although deacetylation generally leads to reduced transcription, HDACs can paradoxically also be necessary for the formation of super-enhancers of core regulatory transcription factors that drive tumor growth [305]. Histone PTM is a highly dynamic process and reflects the balanced effects of HDACs and HATs. Pharmacological inhibition of HDACs has the potential of reversing some of the epigenetic alterations acquired during cancer development and progression [306, 307]. Different **HDAC inhibitors** have varying selectivity for different HDAC classes, which may make their effects diverse. In particular, dysregulation of class I HDACs has been implied for solid tumors. Of note, class I HDACs also have a well described non-histone target in the tumor suppressor protein p53, which is suppressed by deacetylation [308].

In vitro studies show that HDAC inhibition can induce growth arrest and differentiation in for instance uveal melanoma [309]. However, a phase II trial of the HDAC inhibitor vorinostat in melanoma (including uveal melanoma) reported modest efficacy [310], and HDAC inhibitors are yet to be proven effective as monotherapy for

solid tumors. There is, however, emerging optimism of synergistic effects of HDAC inhibitors with other cancer treatment modalities, including immunotherapy [311].

1.4.3 Epigenetics and immunotherapy

Through their key role in cell development, epigenetic mechanisms even regulate immune cell differentiation and exhaustion states [312, 313]. Multiple HDACs are involved in the regulation of cytokine production, and some HDAC inhibitors have anti-inflammatory effects [314]. However, different HDACs may have opposing roles, and HDAC inhibition may have diverse effects on different immune cell populations. The net effect of HDAC inhibition on immune cells is therefore hard to predict, with reports of both reduced viability of T cells, as well as improved cytokine production and cell function [315, 316]. In contrast to other HDAC inhibitors, selective class I HDAC inhibitors seem to impair inhibitory immune cells like Tregs and MDSCs, more than CD8+ T cells or activated CD4+ T cells [317-319]. Furthermore, inhibitors of class I HDACs have been shown to increase the function of both CD8+ T cells and NK cells [316, 320]. Considering the unpredictable effects of HDACs on immune cells, it is reasonable to hypothesize that narrow spectrum class I HDAC inhibitors may be most beneficial in enhancing the antitumoral immune response.

Epigenetic mechanisms are also likely to be important to the cancer cells for effective immune evasion. Probable mechanisms include suppressing the expression of immunogenic retroelements and germline specific genes, downregulation of antigen presentation and impaired interferon responsiveness [321-323]. Therefore, reversal of a dysregulated epigenome may lead to improved expression of tumor antigens and sensitivity to the antitumoral immune response. Indeed, preclinical studies have shown that DNMT inhibition gives rise to immunogenic viral mimicry, increased interferon responsiveness and synergistic effects with immunotherapy [324]. Even HDAC inhibitors may enhance immunogenicity of cancer cells by enhancing the expression of *HLA*, possibly leading to enhanced antigen presentation [325], trigger immunogenic cell death [326, 327] and induce the expression of ligands to activating NK cell receptors [320].

Synergistic effects of HDAC inhibition with different modalities of immunotherapy have been demonstrated in preclinical models [316, 318]. HDAC inhibitors generally have acceptable side effects and are currently evaluated in combination with PD-1 inhibitors in several ongoing trials (e.g. NCT01928576; NCT02619253; NCT-02393794; NCT02638090), with promising early results in NSCLC [328]. Even inhibitors of BET bromodomains can induce transcriptional programs that partly overlap with those induced by HDAC inhibition [329]. The potential for synergy between

BET inhibitors and immunotherapy in solid tumors is more poorly characterized, although some preclinical evidence exists [330, 331].

The combined complexity of the epigenetic machinery and the human antitumoral immune response, makes it next to impossible to predict the net outcome of particular interventions and illustrate the necessity of improved preclinical models to guide the continued development of novel immunotherapies.

2. Aims

Stay on target
-Gold Leader

The overall aim of the research on which this thesis is based, is to develop and utilize mouse models to identify new immunotherapies for patients with metastatic melanoma.

2.1 Specific aims

The specific aims of the thesis are:

- To develop and validate a humanized mouse model for the study of anti-tumoral T-cell responses (Paper I)

- To use the animal model described in Paper I in the development of a CAR-T cell therapy for melanoma (Paper II)

- To investigate if HDAC-inhibitors can enhance the effect of PD-1 inhibitors in experimental melanoma models and in patients with metastatic uveal melanoma (Paper III)

3. Methods

Is it real, son, is it really real, son?
Let me know it's real son, if it's really real
-Method man

3.1 Preclinical methods

3.1.1 Mouse models

Due to advantages in housing and breeding, as well as reasonable homology to human genetics and disease, the house mouse (*mus musculus*) has long been the mainstay animal model in biomedical research. Amongst the oldest in vivo cancer models are **syngeneic models** that utilize transplantable murine cancer cell lines in histocompatibile strains of inbred mice, typically C57Bl/6 or BALB/c. The cancer cell lines can be derived from spontaneous tumors (such as the melanoma cell line B16) or be carcinogen induced, and are usually subcutaneously injected at the flank. As syngeneic mouse models are fully immunocompetent, well characterized, reproducible and easy to use, they have been the most used model in early studies of cancer immunology and immunotherapy. However, they have several major limitations: For instance, the B16 model of melanoma lacks the recurrent driver mutations in human cutaneous (or uveal) melanoma. Furthermore, the fast-growing cell lines develop tumors without a representative TME. Modern molecular techniques have facilitated the development of **genetically engineered mouse models** (GEMMs) where tumorigenesis is driven by relevant genetic events and tumors develop spontaneously on a germline background, or by selective inducible expression [332]. Although GEMMs allow for better mechanistic studies of the molecular underpinnings of cancer and their autochronous development lead to a more representative TME, GEMMs usually have relatively low immunogenicity, possibly owning to a simple genetic background with few neoantigens. Moreover, both syngeneic mouse models and GEMMs share the major limitation that they do not represent the complexity and heterogeneity of *human* cancer.

The transplantation of established human cancer cell lines to mice, cell line derived xenographs (**CDX**), was demonstrated in athymic *nude mice* already in the 1960's. However, CDX models have been shown to poorly represent human cancer, and also

fail to capture the considerable heterogeneity between, and within, individual patients [333]. Successful transplantation of tumor samples from individual patients requires a more severe immune deficiency. Severe combined immune-deficiency (**SCID**) mice, first described in the early 80's, have a spontaneous mutation in a protein kinase (*Prkdc*) necessary for B and T cell development. Crossing the SCID mutation on a non-obese diabetic (**NOD**) background, introduces a polymorphism in the *Sirpa* gene which encodes the CD47 receptor. This polymorphism impairs the macrophages' ability to engulf human cancer cells, leading to massively improved engraftment of human tissues. In the early 2000's a further improvement was made by knocking out the gene encoding the common γ-chain of the IL-2 receptor (*Il2rg*) leading to defective binding (or signaling) of several interleukins and loss of murine NK cells. The resulting **NOD-SCID-IL2rg knock out** (NSG or NOG) mouse has since revolutionized the ability to grow patient derived tissues in mice [334, 335]. Although the use of **patient derived xenographs** (PDX) is limited in some diseases by poor engraftment, cutaneous melanoma shows a near complete take rate, possibly owning to the orthotopic nature of subcutaneous implantation [336].

Our group and others have shown that **PDX** models carry the possibility of capturing the genetic complexity and heterogeneity that characterize human cancers, and model patient responses to several targeted therapies [24, 336-338]. However, serial passaging in PDX applies a selective pressure and drives the accumulation of copy number alterations that may influence the efficacy of tested compounds [339]. Furthermore, the human tumor stroma gradually gets replaced with murine stroma which may significantly influence cancer biology [340, 341]. Paradoxically, the severe immunodeficiency that ultimately enabled the success of these mouse strains, represents a major disadvantage in the current era of cancer immunotherapy, but at the same time also allows for engraftment of human immune cells, e.g. from peripheral blood mononuclear cells (PBMCs), bone marrow or fetal hematopoietic tissues. Such **immune humanized models** have been shown to successfully reconstitute several niches of the human immune system, but have thus far not been able to induce complete responses in autologous cancer models. Furthermore, the use of current immune humanized models is limited by frequent development of severe graft-versus-host disease (GvDH) [342, 343].

In paper I we describe the development of a novel immune humanized PDX model based on sequential transplantation of ex vivo expanded, autologous TILs in PDX models. Tumors were grown and expanded in NOG or human IL-2 transgenic NOG (hIL-2 NOG) mice in parallel with TIL expansion, before allocation of mice to treatment groups and adoptive transfer of TILs by tail vein injection. To support TIL persistence, NOG mice were treated with regular s.c. injections of human IL-2.

Cancer cells and corresponding TILs were derived from patients with metastatic cutaneous melanoma enrolled in a phase II trial of ACT TIL therapy. Tumor volumes were assessed by caliper measurements or in vivo luminescence. In paper II a similar approach is used for the study of allogeneic CAR T cells against cutaneous and uveal melanoma PDX and CDX.

One of the many obstacles for translational research on uveal melanoma is the lack of representable mouse models. Spontaneous uveal melanoma has not been described in mice, hence there are no syngeneic mouse models. Human cell lines can generate CDX, but have the same limitations discussed above. Recently developed GEMMs driven by the canonical *GNAQ* or *GNA11* mutations, do form melanocytic tumors, but fails to mimic the human disease as they do not metastasize to the liver [344, 345]. Unfortunately, even PDX models have limited utility in uveal melanoma due to a very low take rate (10% from liver metastases) and slow growth [346]. Consequently, the in vivo studies in paper III use the common melanoma model B16-F10 cell line in B57Bl/6 mice to study combination immunotherapy aimed at metastatic uveal melanoma.

3.1.2 In vitro methods

Cancer cell lines derived from human or murine cancers are arguably the most widely used tool for cancer research. Cancer cell lines are cheap, and particularly useful in functional studies through pharmacological inhibition or genetic manipulation of defined targets. For instance, the function of individual genes can be studied by transgenic introduction or knock out. Recently, the CRISPR/Cas9 system has dramatically increased the precision and ease with which the human genome can be edited.

Cancer cell lines are usually grown attached to plastic, at ambient oxygen pressure and in a cell culture medium high in glucose and supplemented with essential amino acids. These artificial conditions are far from the harsh TME where oxygen, glucose and other nutrients are limited. Cell culture thus applies a significant selective pressure that over time dramatically alters the cell lines, that eventually may poorly represent the cancer tissue they were derived from [333].

Even immune cells can be cultured in vitro, provided the right stimulation of cytokines. In the work presented here, TILs were extracted by culturing pieces of patient metastases in a medium supplemented with high doses of IL-2 as previously described [347-349]. For further use in experiments these TILs were expanded using a standard small-scale rapid expansion protocol (**REP**) [348]. In short this includes

stimulating the TILs with CD3 antibodies in the presence of high dose IL-2 and feeder cells (pooled donor PBMCs, inactivated through radiation). After a 14-day REP cycle, the TILs have typically expanded by a 1000-fold or more and can be harvested, resuspended in buffered saline and intravenously injected in mice. To enable in vivo tracking of TILs, they were transduced with lentiviral GFP-firefly luciferase immediately before initiating the REP.

In vitro assays of TIL **cytotoxicity and reactivity** were performed by co-culturing TILs with autologous cancer cells for 24 hours, followed by measuring the fraction of surviving cancer cells (by relative bioluminescence or cell count) as well as medium concentrations of IFN-γ (by ELISA) and TIL surface expression of the degranulation marker CD107a (by flow cytometry).

Immune cell identity and function can be characterized by the presence and levels of several surface (or intracellular) markers using monoclonal antibodies labelled with fluorochromes. **Flow cytometry** allows for analysis of multiple markers at a rate of several thousand cells per second and has long been a crucial technique in both preclinical and clinical immunology. In the work presented here, we used flow cytometry to characterize the phenotype, differential status and expression of immune checkpoints in PBMCs, expanded TILs as well as TILs extracted from the mouse models. Furthermore, flow cytometry was used in the studies of tumor cell viability and PD-L1 expression, T cell reactivity and to enrich populations for successfully transduced cells.

3.1.3 Statistical analyses

The effects of interventions in all mouse studies in papers I-III were evaluated using tumor growth curves and survival curves. Mouse experiments generally contained five mice per group based on a pre-determined ability to detect a >30% suppression of tumors growth with power of 0.8. However, following the curative effects of adoptive TIL or CAR-T cells in the hIL-2 NOG models, the sample size was normally decreased to three. In grouped experiments, values are shown as mean ± standard error and growth curves are compared using multiple *t*-test (with Sidak corrections). No randomization or blinding was used. Survival curves were generated with the Kaplan-Meier method and compared using the log-rank test.

3.2 Clinical investigations

Preclinical findings from the lab were extensively analyzed in order to design a clinical trial to explore key results in the treatment of metastatic melanoma. Findings suggestive of a synergistic effect between HDAC inhibition and PD-1 inhibition in experimental melanoma models, added to a strong exciting rationale in the literature for testing this combination in patients. Combining studies of both metastatic cutaneous and uveal melanoma was found not feasible and led to a study focusing on metastatic uveal melanoma, an area of high medical need and much less interest from the pharmaceutical industry. The study, PEMDAC, is conducted in collaboration within the Swedish Melanoma Study Group (SMSG) at four university hospitals (in Lund, Stockholm, Uppsala and Gothenburg), with the potential to include all eligible patients in Sweden.

The study protocol is available in an open access publication [350]. Below is a brief summary of key aspects of the trial.

3.2.1 Patients

The investigated cohort of patients includes both untreated and previously treated patients with histologically (or cytologically) confirmed metastatic uveal melanoma. Other key **inclusion criteria** include: Age above 18; ECOG Performance status 0-1; measurable disease by computed tomography (CT) or Magnetic resonance imaging (MRI) per RECIST 1.1 criteria. Key **exclusion criteria** include: Active brain metastases; previous treatment with anticancer immunotherapy; active autoimmune disease; immune deficiency or treatment with systemic corticosteroids; life expectancy of less than 3 months

3.2.2 Study design

The PEMDAC study is an investigator initiated, prospective multicenter, non-randomized, open label study. Patients with metastatic uveal melanoma are concomitantly treated with pembrolizumab 200 mg administered intravenously every third week and entinostat 5 mg administered orally once weekly. Planned sample size is 29 patients, allocated using Simon's Optimal Two Stage Design. Radiological assessment is scheduled every 9 weeks. Treatment is continued until documented disease progression, intolerable side effects, patient's withdrawal of consent, decision of the investigating physician to end treatment, or to a maximum of 2 years. Treatment beyond progression is allowed if the patient is clinically stable according to criteria

specified in the study protocol. Adverse events (AEs) are registered and graded according to CTCAE v4.03 Blood and tissue for biomarker analyses is collected throughout the study. **Primary endpoint** is objective response rate (ORR) according to RECIST v1.1 [351]. Secondary endpoints include clinical benefit rate (CBR) at week 18, progression free survival (PFS), overall survival (OS) as well as incidence and severity of AEs.

3.2.3 Statistical analysis

All analyses are based on all patients who received at least one dose of study drug.

The sample size and power estimation are based on the primary endpoint only. Power is required to be 80%, significance is generally set to 5%. We assume that an ORR of 5% is not a clinically relevant treatment effect, whereas 20% is sufficient to consider the treatment useful. Enrollment will continue until the required sample size has been reached. Patients will be enrolled in two batches, the first consisting of 10 patients and the second group of 19. The second group will not be recruited if the result from the 10 first is considered inadequate. This is the optimal allocation according to Simon's Optimal Two Stage Design (significance level = 5% (one-sided)) [352]. If no objective response is reported after the first stage of 10 patients, the study is interrupted early for futility. Outcome measures that are proportions will be reported using a 95% confidence interval. Since the sample size is small an exact method will be used. If applicable, tests are conducted versus zero or highest non-efficient value. Outcome measures that are times to various events will be analyzed using non-parametric methods. Time is summarized using medians, together with 95% confidence intervals. If applicable, tests are conducted versus zero or highest non-efficient value. Results are graphically presented using Kaplan-Meier survival curves. The study is considered positive when at least 4 patients of the total of 29 have a confirmed objective response.

3.3 Ethical considerations

All patient samples used in the research on which this thesis is built, were obtained after informed consent and approval by the institutional review board. All animal experiments were performed in accordance with EU directive 2010/63 and approved by the regional animal ethics committee of Gothenburg.

The study protocol and all amendments for the trial described in paper III were approved by the institutional review board and the Swedish Medical Products Agency (EudraCT registration number: 2016–002114-50). ClinicalTrials.gov registration number: NCT02697630 (Registered 3 March 2016). Signed and dated informed consent was obtained from each patient in accordance with the principles of ICH-GCP and the latest version of the Declaration of Helsinki.

4. Results

-It's like this and like that
and like this and uh
-Dr. Dre

4.1 Paper I

This paper describes the development of a novel immune humanized mouse model where autologous TILs are transplanted to tumor bearing PDX models in mice.

4.1.1 TILs cumulate in autologous tumors in NOG mice

For our first test of adoptive TIL transfer in PDX models, we chose a patient sample with high *in vitro* TIL cytotoxicity and reactivity against autologous cancer cells, as well as a feasible growth rate in NOG mice. Nevertheless, transplanting 20 x 10⁶ autologous TILs to the tumor-bearing NOG mice, led to no significant suppression of tumor growth, compared to untreated controls. A tumor from one treated mouse was examined by flow cytometry and IHC which demonstrated presence of human CD3+ cells in the tumor as well as upregulation of PD-1 and PD-L1 on T cells and tumor cells respectively. However, treating the remaining mice with a PD-1 inhibitor failed to induce tumor regression. We repeated the experiments with samples from a patient with a known response to TIL ACT with the addition of a PD-1 inhibitor from the start, and observed only slightly protracted tumor growth but no regression.

4.1.3 IL-2 is essential for TIL persistence and tumor eradication

We hypothesized that s.c. injections of human IL-2 failed to provide sufficient plasma levels to support TIL persistence and effect in vivo. This was confirmed when repeat measurements of IL-2 plasma levels showed a peak after two hours, followed by a rapid and complete elimination the following few hours. To circumvent this, we obtained a strain of NOG mice that produce human IL-2 (hIL-2 NOG), with constitutive high plasma levels of IL-2 (although varying between individual mice). By transducing TILs with a luciferase expressing lentivirus, we could track the proliferation and distribution of the injected cells in NOG and hIL2-NOG by repeat in vivo bioluminescence imaging. We observed that the signal disappeared from the NOG

mice within days. In the hIL2 NOG mice on the other hand, the signal persisted for several weeks. In mice with the highest plasma levels of IL-2, the signal even increased over time with an apparent accumulation in bone marrow, spleen and liver. However, when injected in tumor-bearing hIL2 NOG mice, the labelled TILs exclusively accumulated in the tumor and in the following weeks the tumor started shrinking and eventually disappeared completely.

4.1.4 Responses to ACT can be modelled in hIL-2 NOG

Subsequent experiments demonstrated a relationship between response to ACT and the level of IL-2 in plasma as well as number of injected cells, and helped us define a minimum plasma IL-2 level for use in following experiments. Next, we wanted to see if the responses in our PDX model correlated with the effects in the clinic. Therefore, we repeated the experiments with six different patient TIL and tumor samples: Three from patients responding to TIL ACT, and three from patients who had no effect of the treatment. We found that ACT in our model caused durable regression in all samples from responding patients, whereas if the samples came from a non-responding patient, no effect was seen of ACT in the PDX. Finally, we even demonstrated that ACT is effective in eradicating metastases that developed in PDX models after resecting large implanted flank tumors in untreated mice.

4.2 Paper II

In paper II we evaluate the feasibility and in vitro efficacy of CAR-T cell therapy in melanoma, before testing CAR-T cell therapy in the model described in paper I.

4.2.1 HER2 is expressed in melanoma

To identify potential targets for CARs in melanoma, we searched the The Cancer Genome Atlas (TCGA) database for expression of mRNA for surface proteins against which there are commercially available CAR T cells. Among seven evaluated targets, we found that only HER2 (*ERBB2*) was expressed at a considerable level in the majority of both uveal and cutaneous melanomas. The finding was validated in a local biobank of melanoma biopsies and corresponding PDXs that showed constituent expression of HER2 mRNA. The expression in commercially available cell lines of cutaneous and uveal melanoma was on the other hand more variable.

4.2.3 HER2 CAR-T cells can kill melanoma cell lines

We tested the in vitro cytotoxicity and reactivity of commercially available allogeneic HER2 CAR T cells against two melanoma cell lines, and found that killing and reactivity was greater in the cell line with highest HER2 expression. To further ensure target specificity, we used the CRISPR/Cas9 system to knock out HER2 in cell lines of uveal and cutaneous melanoma which abrogated both killing and reactivity of the CAR T cell.

4.2.3 HER2 CAR T cells can kill T cell resistant melanoma *in vivo*

The in vivo efficacy of HER2 CAR-T cells was tested against five patient derived samples growing in NOG or hIL2-NOG mice. Adoptive transfer of HER2 CAR T cells caused durable deep or complete regression of all tumors growing in hIL2 NOG mice, but showed little or no effect in NOG mice. Three of the tested samples were derived from patients that were resistant to TIL therapy. Moreover, we also demonstrated in vivo regression in two models of uveal melanoma (one CDX and one PDX).

4.3 Paper III

In paper III we assess the rationale of combined epigenetic modulation and PD-1 inhibition in experimental melanoma in vitro and in vivo. Next, we describe the rationale, design and preliminary results of an ongoing phase II trial evaluating the effect of HDAC inhibitor entinostat in combination with pembrolizumab, a PD-1 inhibitor, in patients with metastatic uveal melanoma.

4.3.1 Preclinical studies

BET inhibition suppressed the expression of PD-L1 and MHC in B16-F10 melanoma cell lines, and abrogated the curative effects of combined checkpoint inhibition *in vivo*. HDAC inhibition, on the other hand, increased both MHC-I and PD-L1 expression in B16-F10 cells as well as in human uveal melanoma cell lines. Combined treatment with HDAC inhibition and PD-1 inhibition showed superior inhibition of tumor growth of B16-F10 *in vivo* compared to either agent alone.

4.3.2 Clinical investigations

Twenty-nine patients were enrolled between February 2018 and December 2018. Data cut off for the analysis in paper III was June 21, 2019, i.e. 6 months after the last enrolled patient received the first dose. Median follow up for OS was 7.7 months. Median age was 70 years (range, 34 - 83). Ninety percent had liver metastases. Twelve patients (41%) had received no previous treatment for metastatic disease and only 8 patients (24%) had received previous systemic therapy for uveal melanoma.

Twenty-eight patients had at least one follow-up radiological evaluation. One patient was excluded the first week following the first dose due to a protocol violation. A partial response (PR) was confirmed in three patients resulting in an ORR of 10% (95% CI, 2.2 to 27). All responses were ongoing at data cut off, with a duration of 1.5, 6.3 and 13.9 months respectively. Nine patients (31%) had a best overall response of stable disease (SD). Clinical benefit for 18 or more weeks was observed in 7 patients (CBR = 24%). Treatment was ongoing in seven patients (24%) at the time of data cut off.

Adverse events (regardless of assessed causality) were reported in 28 patients (97%). Eighteen patients (62%) had an AE of grade ≥3, the most common being increased blood alkaline phosphatase, followed by neutropenia, increased aspartate/alanine aminotransferase and rash. Twenty-three patients (79%) experienced an immune related adverse event (irAE) and 7 patients (24%) had an irAE of grade ≥3 or greater: Two events each of hepatitis and skin toxicity and one event of colitis, hypophysitis and stomatitis respectively. Three patients (10%) had an AE leading to treatment discontinuation. There were no treatment related deaths.

5. Discussion

Was ist ist was nicht ist ist möglich
-Einstürzende Neubauten

5.1 Paper I

To overcome some of the limitations of current in vivo models, we developed a humanized PDX model inspired by TIL ACT in humans. When the TILs used in our model came from patients who had experienced a response to TIL ACT in the clinic, ACT also caused curative effects in hIL2-NOG mice bearing their tumors. This is, to the best of our knowledge, the first publication to demonstrate durable complete responses of human tumors to autologous immune cells in mice. Equally important, tumors from patients that had no effect of TIL ACT, were resistant to autologous TILs even in mice. The truthful recapitulation of clinical responses, makes the PDX model a promising biomarker for response to TIL therapy. Another important finding of this study is the necessity of IL-2 to achieve an effect. As treatment with high dose IL-2 has severe adverse event, there have been attempts to reduce, or even omit, concomitant IL-2 in ACT trials (NCT01995344; NCT01468818). Based on our findings, completely omitting IL-2 in ACT would be unadvisable. Instead, novel and potentially less toxic IL2 analogues could hopefully make systemic IL-2 treatment better tolerated [184, 353]

Despite its potential usefulness in translational immunotherapy research, the presented model has some considerable limitations. First, the general limitation of PDX models lacking human stroma applies even to this model and may even be more relevant as the cancer stroma has been shown to greatly influence immune function [354]. As the TILs used are expanded by a T cell directed method, the immune reconstitute in the model further fails to represent the large spectrum of immune cell types involved in the antitumoral immune response. Most notably the model lacks all myeloid cell lineages, which are crucial e.g. for T cell activation and it does therefore not capture T cell priming. Instead, it should be seen as a model for studying the effector function of activated T cells, which is underlined by the fact that the infused TILs almost exclusively consist om T effector memory cells. An outstanding question is how well (if at all) the described PDX model can imitate PD-1 inhibition or other immunotherapies currently in use or development in the clinic. In the model, PD-1 inhibition was unable to enhance the effect of ACT in responders, and did not reverse

resistance in non-responders. This, however, may be due to several factors. First, in responding patients the effect was curative, making it difficult to improve further. The complete lack of effect of PD-1 inhibition in non-responding samples, could be because IL-2 has been shown to override the inhibitory effect of PD-1 in vitro, making further inhibition redundant and pointing to other mechanisms of immune evasion in these particular samples [355]. It is therefore conceivable that the mode of action of PD-1 inhibition is partly overlapping with that of IL-2 and can be represented in the model. Furthermore, it may be a tool to investigate immune checkpoint inhibitors beyond PD-1, other T cell directed therapies, and to study how other treatment modalities may interfere with, or enhance, the antitumoral T cell response. It also remains to see how our TIL humanized PDX model compares to other models in use or development. Recently, organoid cultures have shown promise in predicting response to immunotherapy [356-358]. Furthermore, the efforts to humanize immunocompromised mice with human CD34+ stem cells are gaining progress [359]. Although these have the promise of recapitulating more niches of the immune system, they so far fail to induce autologous antitumoral responses and are hampered by frequent GvHD, which was not a limitation in our model. Despite its limitations, we believe that the described PDX model have potential usefulness in the search for the molecular mechanisms that underly inherent or acquired resistance to immunotherapy.

5.2 Paper II

The shortcomings of CAR-T cell therapies in solid tumors have generally been mirrored by few curative effects by single injections in PDX models. Inspired by the responses of TIL ACT in our newly developed humanized PDX model, we wanted to see if resistance to CAR-T cell therapy could be overcome by similar means. We found appreciable levels of HER2 mRNA expression in melanoma samples in the TCGA, a local biobank, as well as in most commercial melanoma cell lines. HER2 expression in melanoma has previously mainly been assessed by ICH and found at very low rates, and more recent panel sequencing efforts of large cohorts have revealed targetable HER2 amplifications only in a small group of acral and mucosal melanomas [360]. While HER2 aberrations and high surface expression may be rare events in melanoma, the expression levels of mRNA found in paper II were comparable to that seen in sarcoma, a disease where HER-2 CAR-T cell therapy is currently investigated in a phase I trial with encouraging preliminary efficacy and safety [361]. We further show effective and target specific killing of human cutaneous and uveal melanoma with allogeneic HER2 CAR T cells in both cell lines and hIL-2 expressing

CDXs and PDXs. Particularly encouraging is the finding of effect against cutaneous melanoma resistant to T cell therapy as well as uveal melanoma, both settings with scarce treatment options. As in paper I, the effect of CAR T cells in vivo was dependent of IL-2. This suggests that exhaustion or depletion of IL-2 in the TME could be contributing to the relative resistance of CAR-T cell therapy in solid tumors. Our findings thus support the attempts to develop CAR T cells that are self-sufficient in IL-2 production or signaling [180]. Alternatively, novel engineered IL-2 analogues may make concomitant systemic IL-2 treatment more feasible [184, 353].

5.3 Paper III

The results from the preclinical experiments strengthens the rationale to combine inhibition of HDAC, but not BET, with PD-1 inhibition in uveal melanoma. The presented phase II study PEMDAC, is the first to investigate combined HDAC- and PD-1 inhibition in uveal melanoma, and the first study to investigate the combination of pembrolizumab and entinostat in a cohort that include treatment-naive patients, and where no patients have previously received immunotherapy. However, an ongoing phase I/II study of the same combination recently reported encouraging efficacy in PD-1 refractory metastatic cutaneous melanoma [362].

The observed ORR of 10% may be modest, but compares favorably with the very low ORRs reported in most representative series of PD-1 inhibition in uveal melanoma [286-288]. However, the small sample size makes the estimate uncertain, and it will require one more response to meet the prespecified primary endpoint. There are data that challenge ORR as the most appropriate primary endpoint for phase II studies of immunotherapy in cancer as it risks underestimating the treatment benefit [363]. However, ORR is still the primary endpoint recommended by the European Medicines Agency (EMA) in exploratory single armed studies such as PEMDAC. Many immunotherapy trials have now adopted one of several modified RECIST criteria that have been developed to account for the unconventional response characteristics associated with immunotherapies [364-366]. Our experience with cutaneous melanoma is that responses to PD-1 inhibitors usually occur quite early, without previous pseudoprogression, and are generally captured with conventional RECIST criteria. In the present study however, late onset of response was observed, as well as shrinkage of target lesions after an initial increase in one patient, indicating different response kinetics from PD-1 inhibition in cutaneous melanoma. Response (ORR, CBR and PFS) according to immune related RECIST (irRECIST) criteria will be evaluated as an exploratory endpoint.

Retrospective data suggest that combined CTLA-4 and PD-1 inhibition may be more effective than PD-1 inhibition alone in uveal melanoma, but no prospective trial investigating this has been published [38, 287]. In preliminary results from an ongoing phase II trial of ipi-nivo, Piulats et al reported an ORR of 11.5%, comparable to that found in the PEMDAC trial [289]. Furthermore ipi-nivo seems associated with a favorable PFS and OS compared to historic data, having led to a quite widespread use of the regimen in metastatic uveal melanoma. The possible benefit of ipi-nivo should, however, be carefully balanced against a very high rate of severe (grade 3-4) irAEs. With a more favorable toxicity profile, combined entinostat and pembrolizumab could be a feasible alternative to ipi-nivo in uveal melanoma if efficacy is confirmed in an upcoming analysis. In any case, the preliminary results from the PEMDAC trial adds to the growing body of evidence that at least a subset of patients with uveal melanoma may have considerable benefit of immunotherapy. Hopefully, future work will identify biomarkers to better predict who may respond to treatment.

6. Conclusions and future work

Roads? Where we going, we don't need roads.
-Dr. Emmett Brown

Paper I demonstrates that it is possible to achieve durable complete responses to immunotherapy in humanized PDX models. The model has since been applied in testing interactions between targeted therapies and the immune system [194, 367]. In a recently published paper, we used a modified version of the model to screen for immunogenicity of patient samples from a retrospective biobank by transplanting melanoma metastasis biopsies directly into NOG or hIL-2 NOG mice. While the take rate was almost complete in NOG mice, growth patterns in hIL-2 NOG were variable and appear to reflect the inter- and intrapatient heterogeneity of response patterns to immunotherapy in a large number of patient samples. Furthermore, lack of engraftment in hIL-2 NOG mice appears to be correlated with survival in patients previously treated with PD-1 inhibition [194]. Although prospective validation is required, the model thus shows promising utility in predicting response to PD-1 inhibition, and in the study of inherent or acquired resistance to checkpoint inhibition. In Paper II we show that the model described can be utilized in the study of genetically modified T-cells. This work has continued and may in the future also include TCR engineered T cells. We believe that the described model will contribute to filling the gap between preclinical and clinical immunotherapy research and facilitate the development of novel immunotherapeutic strategies.

Paper II presents encouraging in vivo efficacy for HER2 CAR-T cell therapy in human melanoma, including subgroups with few or no treatment options such as uveal melanoma. Our continued work has therefore focused on trying to bring HER2 CAR T cells towards a clinical application. We have demonstrated scalability in automated production according to Good Manufacturing Practice (GMP) (Forsberg et al, *unpublished*). Toxicity studies are ongoing in other animal models and a protocol for a future phase I trial in humans is in development.

Paper III describes the first results of combined HDAC and PD-1 inhibition in metastatic uveal melanoma and shows signs of clinical efficacy with manageable toxicities. The trial is ongoing and at the time of data cut of several patients were still receiving treatment. The next update (scheduled for late December 2019), will provide an updated ORR, ORR as per irRECIST, toxicity data, as well as mature

estimates of PFS and overall survival. Although several patients achieved durable benefit, and the final analysis is pending, it is clear that a majority of the patients had little or no effect of the treatment. It will be our utmost priority to try and identify biomarkers associated with a treatment benefit. For this purpose, we have established a comprehensive biobank and extensive exploratory analyses are in progress: Whole exome sequencing (WES) of germline and tumor DNA; tumor RNAseq; multiplex immunohistochemistry; paired scRNAseq of PBMC in selected patients; serial analyses of circulating tumor DNA (ctDNA), serum cytokines and detailed phenotyping of PBMC. In this way the PEMDAC trial will hopefully contribute to bringing new insight into the biology of uveal melanoma, and maybe help uncover what characterizes the small subset of patients with uveal melanoma that can achieve durable responses to immunotherapy.

Acknowledgements

A great number of people have had a role in the making of this thesis. I would like to mention some in particular:

First and foremost, **Lars Ny**, my main supervisor, dear colleague and mentor in all aspects of my professional life. Your knowledge, ambition and authority, combined with kindness and a genuine wish to lift the people around you, are supervisor-super-powers. I am so grateful that you chose to lead me on this journey. Thank you for always making time for discussions and advice, and for many memorable moments on our travels together.

My co-supervisor **Jonas Nilsson**, for your unrivaled enthusiasm for science, curiosity and great ideas: It is truly contagious. Thank you for trusting me with this exciting project (and all the fancy equipment in your lab) and for creating such a creative, friendly, yet competitive, environment around you. Thank you also to my second co-supervisor **Peter Naredi**, for your important role in initiating this project.

Past and present members of the **Jonas Nilsson group**: First of all, **Mattias** for brilliantly executing the PDX v2.0 project at the times when I was busy at the hospital. Thanks for all the laughs and good talks in and outside of the lab, for the water of life and for a friendship that will last. **Lisa** for all the fruitful discussions and for putting up with me when I never find stuff in the lab. **Berglind**, for your patience and kindness when introducing me at the lab, and for a great trip to Iceland. **Elin** for the nice talks and for leading the way through the administrative jungle in the final stages of the dissertation process. **Samuel, Joakim, Vasu**, **Ganesh, Som, Joy, Eliza, Lydia, Sofia, Carina** for your nice company, help and collaboration.

All the other **staff and researchers at Sahlgrenska Cancer Center** (particularly 6th floor) for creating such a stimulating and friendly environment.

Roger (the most oncological surgeon there is) for your key role in our translational melanoma group and for your inspiring enthusiasm for clinical science.

Our collaborators at the Center for Cancer Immune Therapy in Herlev, Denmark: **Marco**, **Rikke**, and **Inge Marie**, for your hospitality when I was visiting, and for teaching me the fine art of growing TILs.

Everyone involved in the **PEMDAC study**. Particularly: **Gustav, Ana** and **Hildur**, the principal investigators. **Bengt, Henrik, Anders** and **Carola** for help with explorative analyses, **Martin** for radiological support, and **Maria** for deciphering the numbers. **Annika** and **Erén** at the clinical trials unit for your help in making this unique study happen. A special thanks to our eminent study nurse **Anette**: This study would not have been possible without your dedication, knowledge and good spirit.

All the **patients** involved in this research for your generous contributions to science.

Friends and **colleagues at Jubileumskliniken**. I specifically would like to thank my kind, clever and funny colleagues at the melanoma unit, **Max** and **Sara**: Thank you for taking so good care of our patients (sorry for being gone so much), for our inspiring discussions and for always keeping science on the agenda. You are the best colleagues I could wish for. A special thanks to **Ulrika** for noticing my interest in immunotherapy and melanoma early on, for introducing me to the field in Paris, and for always sharing your immense knowledge. Thank you to our excellent nurses **Lise-Lotte**, **Marie** and (alumni) **Mia** for outstanding teamwork. I would also like to thank the head of clinic **Johanna Svensson** for truly acknowledging the importance of research in the everyday life of a busy clinic, and former head of clinic **Marie Lindh** for your support in getting my project started.

Friends outside work, for giving life other perspectives and making our years here in Gothenburg among the best in our lives. A special thanks to **Johan**: I hope people judge this book by its cover.

Takk til familien i Norge og svigerforeldre i Falun for all kjærlig støtte. En særlig takk mamma **Kari** og pappa **Jørn-Ove** for all hjelp med å få vårt hektiske liv til å gå rundt de seneste årene, tross at vi bor så langt unna. Dere er utrolige. Det har også vært veldig fint å komme av gårde til hytta på fjellet iblant og glemme alt om forskning og kreft for en stund. Jeg ser fram til å komme nærmere den og dere.

Signe, Lisa, **Emil** og **Linnéa**. Takk for att dere finns der og gjør livet mitt rikt. Takk for at dere har hold ut med meg den siste tiden når hodet mitt ofte har vært et annet sted. Takk for at dere påminner meg om hva som egentlig betyr noe: Båndene mellom oss. Dere er mitt alt.

References

1. **World Cancer Report 2014** [http://publications.iarc.fr/Non-Series-Publications/World-Cancer-Reports]
2. **SweMR - Svenska Melanomregistret. Nationell årsrapport för hudmelanom.** [https://www.cancercentrum.se/globalassets/om-rcc/sydost/pdf/swemr-1990-2018.pdf]
3. Chang YM, Barrett JH, Bishop DT, Armstrong BK, Bataille V, Bergman W, Berwick M, Bracci PM, Elwood JM, Ernstoff MS, et al: **Sun exposure and melanoma risk at different latitudes: a pooled analysis of 5700 cases and 7216 controls.** *Int J Epidemiol* 2009, **38:**814-830.
4. **Clinical Practice Guidelines for the Management of Melanoma in Australia and New Zealand 2008.**
5. Soura E, Eliades PJ, Shannon K, Stratigos AJ, Tsao H: **Hereditary melanoma: Update on syndromes and management: Genetics of familial atypical multiple mole melanoma syndrome.** *Journal of the American Academy of Dermatology* 2016, **74:**395.
6. Homsi J, Kashani-Sabet M, Messina JL, Daud A: **Cutaneous melanoma: prognostic factors.** *Cancer control : journal of the Moffitt Cancer Center* 2005, **12:**223-229.
7. Faries MB, Thompson JF, Cochran AJ, Andtbacka RH, Mozzillo N, Zager JS, Jahkola T, Bowles TL, Testori A, Beitsch PD, et al: **Completion Dissection or Observation for Sentinel-Node Metastasis in Melanoma.** *New England Journal of Medicine* 2017, **376:**2211-2222.
8. Leiter U, Stadler R, Mauch C, Hohenberger W, Brockmeyer N, Berking C, Sunderkotter C, Kaatz M, Schulte KW, Lehmann P, et al: **Complete lymph node dissection versus no dissection in patients with sentinel lymph node biopsy positive melanoma (DeCOG-SLT): a multicentre, randomised, phase 3 trial.** *Lancet Oncol* 2016, **17:**757-767.
9. Gershenwald JE, Scolyer RA: **Melanoma Staging: American Joint Committee on Cancer (AJCC) 8th Edition and Beyond.** *Annals of Surgical Oncology* 2018, **25:**2105-2110.
10. Middleton MR, Grob JJ, Aaronson N, Fierlbeck G, Tilgen W, Seiter S, Gore M, Aamdal S, Cebon J, Coates A, et al: **Randomized phase III study of temozolomide versus dacarbazine in the treatment of patients with advanced metastatic malignant melanoma.** *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2000, **18:**158-166.
11. Ugurel S, Röhmel J, Ascierto PA, Flaherty KT, Grob J, Hauschild A, Larkin J, Long GV, Lorigan P, McArthur GA, et al: **Survival of patients with advanced metastatic melanoma: the impact of novel therapies–update 2017.** *European Journal of Cancer* 2017, **83:**247-257.
12. Eggermont A, Blank CU, Mandala M, Long GV, Atkinson V, Dalle S, Haydon A, Lichinitser M, Khattak A, Carlino MS, et al: **Adjuvant Pembrolizumab versus Placebo in Resected Stage III Melanoma.** *The New England Journal of Medicine* 2018.
13. Eggermont AMM, Chiarion-Sileni V, Grob J-J, Dummer R, Wolchok JD, Schmidt H, Hamid O, Robert C, Ascierto PA, Richards JM, et al: **Adjuvant ipilimumab versus placebo after complete resection of high-risk stage III melanoma (EORTC 18071): a randomised, double-blind, phase 3 trial.** *The Lancet Oncology* 2015, **16:**522-530.
14. Long GV, Hauschild A, Santinami M, Atkinson V, Mandala M, Chiarion-Sileni V, Larkin J, Nyakas M, Dutriaux C, Haydon A, et al: **Adjuvant Dabrafenib plus Trametinib in Stage III BRAF-Mutated Melanoma.** *N Engl J Med* 2017, **377:**1813-1823.
15. Weber J, Mandala M, Del Vecchio M, Gogas HJ, Arance AM, Cowey CL, Dalle S, Schenker M, Chiarion-Sileni V, Marquez-Rodas I, et al: **Adjuvant Nivolumab versus Ipilimumab in Resected Stage III or IV Melanoma.** *N Engl J Med* 2017.
16. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, Bignell GR, Bolli N, Borg A, Borresen-Dale AL, et al: **Signatures of mutational processes in human cancer.** *Nature* 2013, **500:**415-421.
17. Cancer Genome Atlas N: **Genomic Classification of Cutaneous Melanoma.** *Cell* 2015, **161:**1681-1696.

18. Hodis E, Watson IR, Kryukov GV, Arold ST, Imielinski M, Theurillat JP, Nickerson E, Auclair D, Li L, Place C, et al: **A landscape of driver mutations in melanoma.** *Cell* 2012, **150:**251-263.

19. Hauschild A, Grob JJ, Demidov LV, Jouary T, Gutzmer R, Millward M, Rutkowski P, Blank CU, Miller WH, Jr., Kaempgen E, et al: **Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial.** *Lancet* 2012, **380:**358-365.

20. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, Dummer R, Garbe C, Testori A, Maio M, et al: **Improved survival with vemurafenib in melanoma with BRAF V600E mutation.** *N Engl J Med* 2011, **364:**2507-2516.

21. Long GV, Stroyakovskiy D, Gogas H, Levchenko E, de Braud F, Larkin J, Garbe C, Jouary T, Hauschild A, Grob JJ, et al: **Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma.** *N Engl J Med* 2014, **371:**1877-1888.

22. Larkin J, Ascierto PA, Dreno B, Atkinson V, Liszkay G, Maio M, Mandala M, Demidov L, Stroyakovskiy D, Thomas L, et al: **Combined vemurafenib and cobimetinib in BRAF-mutated melanoma.** *N Engl J Med* 2014, **371:**1867-1876.

23. Shaffer SM, Dunagin MC, Torborg SR, Torre EA, Emert B, Krepler C, Beqiri M, Sproesser K, Brafford PA, Xiao M, et al: **Rare cell variability and drug-induced reprogramming as a mode of cancer drug resistance.** *Nature* 2017, **546:**431-435.

24. Krepler C, Xiao M, Sproesser K, Brafford PA, Shannan B, Beqiri M, Liu Q, Xu W, Garman B, Nathanson KL, et al: **Personalized Preclinical Trials in BRAF Inhibitor-Resistant Patient-Derived Xenograft Models Identify Second-Line Combination Therapies.** *Clin Cancer Res* 2016, **22:**1592-1602.

25. Poulikakos PI, Persaud Y, Janakiraman M, Kong X, Ng C, Moriceau G, Shi H, Atefi M, Titz B, Gabay MT, et al: **RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF(V600E).** *Nature* 2011, **480:**387-390.

26. Shi H, Hugo W, Kong X, Hong A, Koya RC, Moriceau G, Chodon T, Guo R, Johnson DB, Dahlman KB, et al: **Acquired resistance and clonal evolution in melanoma during BRAF inhibitor therapy.** *Cancer Discov* 2014, **4:**80-93.

27. Shi H, Moriceau G, Kong X, Lee MK, Lee H, Koya RC, Ng C, Chodon T, Scolyer RA, Dahlman KB, et al: **Melanoma whole-exome sequencing identifies (V600E)B-RAF amplification-mediated acquired B-RAF inhibitor resistance.** *Nat Commun* 2012, **3:**724.

28. Xue Y, Martelotto L, Baslan T, Vides A, Solomon M, Mai TT, Chaudhary N, Riely GJ, Li BT, Scott K, et al: **An approach to suppress the evolution of resistance in BRAF(V600E)-mutant cancer.** *Nat Med* 2017, **23:**929-937.

29. Dummer R, Schadendorf D, Ascierto PA, Arance A, Dutriaux C, Di Giacomo AM, Rutkowski P, Del Vecchio M, Gutzmer R, Mandala M, et al: **Binimetinib versus dacarbazine in patients with advanced NRAS-mutant melanoma (NEMO): a multicentre, open-label, randomised, phase 3 trial.** *Lancet Oncol* 2017, **18:**435-445.

30. Jiang X, Zhou J, Yuen NK, Corless CL, Heinrich MC, Fletcher JA, Demetri GD, Widlund HR, Fisher DE, Hodi FS: **Imatinib targeting of KIT-mutant oncoprotein in melanoma.** *Clin Cancer Res* 2008, **14:**7726-7732.

31. Hodi FS, Corless CL, Giobbie-Hurder A, Fletcher JA, Zhu M, Marino-Enriquez A, Friedlander P, Gonzalez R, Weber JS, Gajewski TF, et al: **Imatinib for Melanomas Harboring Mutationally Activated or Amplified KIT Arising on Mucosal, Acral, and Chronically Sun-Damaged Skin.** *Journal of Clinical Oncology* 2013, **31:**3182-3190.

32. Naveh HP, Rao UN, Butterfield LH: **Melanoma-associated leukoderma - immunology in black and white?** *Pigment cell & melanoma research* 2013, **26:**796-804.

33. Kalialis LV, Drzewiecki KT, Klyver H: **Spontaneous regression of metastases from melanoma: review of the literature.** *Melanoma Res* 2009, **19:**275-282.

34. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, et al: **Improved survival with ipilimumab in patients with metastatic melanoma.** *N Engl J Med* 2010, **363:**711-723.

35. Schadendorf D, Hodi FS, Robert C, Weber JS, Margolin K, Hamid O, Patt D, Chen TT, Berman DM, Wolchok JD: **Pooled Analysis of Long-Term Survival Data From Phase II and Phase III Trials of Ipilimumab in Unresectable or Metastatic Melanoma.** *J Clin Oncol* 2015, **33:**1889-1894.

36. Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, Hassel JC, Rutkowski P, McNeil C, Kalinka-Warzocha E, et al: **Nivolumab in previously untreated melanoma without BRAF mutation.** *N Engl J Med* 2015, **372:**320-330.
37. Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, Daud A, Carlino MS, McNeil C, Lotem M, et al: **Pembrolizumab versus Ipilimumab in Advanced Melanoma.** *N Engl J Med* 2015, **372:**2521-2532.
38. Donia M, Ellebaek E, Øllegaard TH, Duval L, Aaby JB, Hoejberg L, Køhler UH, Schmidt H, Bastholt L, Svane IM: **The real-world impact of modern treatments on the survival of patients with metastatic melanoma.** *European journal of cancer (Oxford, England : 1990)* 2018, **108:**25-32.
39. Arheden A, Skalenius J, Bjursten S, Stierner U, Ny L, Levin M, Jespersen H: **Real-world data on PD-1 inhibitor therapy in metastatic melanoma.** *Acta Oncol* 2019, **58:**962-966.
40. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Rutkowski P, Lao CD, Cowey CL, Schadendorf D, Wagstaff J, Dummer R, et al: **Five-Year Survival with Combined Nivolumab and Ipilimumab in Advanced Melanoma.** *N Engl J Med* 2019, **381:**1535-1546.
41. Tawbi HA, Forsyth PA, Algazi A, Hamid O, Hodi FS, Moschos SJ, Khushalani NI, Lewis K, Lao CD, Postow MA, et al: **Combined Nivolumab and Ipilimumab in Melanoma Metastatic to the Brain.** *New England Journal of Medicine* 2018, **379:**722-730.
42. Long GV, Atkinson V, Lo S, Sandhu S, Guminski AD, Brown MP, Wilmott JS, Edwards J, Gonzalez M, Scolyer RA, et al: **Combination nivolumab and ipilimumab or nivolumab alone in melanoma brain metastases: a multicentre randomised phase 2 study.** *The Lancet Oncology* 2018, **19:**672-681.
43. Coley WB: **The treatment of malignant tumors by repeated inoculations of erysipelas. With a report of ten original cases. 1893.** *Clin Orthop Relat Res* 1991:3-11.
44. Morales A, Eidinger D, Bruce AW: **Intracavitary Bacillus Calmette-Guerin in the treatment of superficial bladder tumors.** *J Urol* 1976, **116:**180-183.
45. Janeway CA, Jr., Medzhitov R: **Innate immune recognition.** *Annu Rev Immunol* 2002, **20:**197-216.
46. Chertov O, Yang D, Howard OM, Oppenheim JJ: **Leukocyte granule proteins mobilize innate host defenses and adaptive immune responses.** *Immunol Rev* 2000, **177:**68-78.
47. Greten FR, Grivennikov SI: **Inflammation and Cancer: Triggers, Mechanisms, and Consequences.** *Immunity* 2019, **51:**27-41.
48. Blum JS, Wearsch PA, Cresswell P: **Pathways of antigen processing.** *Annu Rev Immunol* 2013, **31:**443-473.
49. Nikolich-Zugich J, Slifka MK, Messaoudi I: **The many important facets of T-cell repertoire diversity.** *Nat Rev Immunol* 2004, **4:**123-132.
50. Starr TK, Jameson SC, Hogquist KA: **Positive and negative selection of T cells.** *Annu Rev Immunol* 2003, **21:**139-176.
51. Shimasaki N, Jain A, Campana D: **NK cells for cancer immunotherapy.** *Nat Rev Drug Discov* 2020.
52. Fritsch EF, Rajasagi M, Ott PA, Brusic V, Hacohen N, Wu CJ: **HLA-binding properties of tumor neoepitopes in humans.** *Cancer Immunol Res* 2014, **2:**522-529.
53. Efremova M, Finotello F, Rieder D, Trajanoski Z: **Neoantigens Generated by Individual Mutations and Their Role in Cancer Immunity and Immunotherapy.** *Frontiers in immunology* 2017, **8:**1679.
54. Schwartz RH: **A cell culture model for T lymphocyte clonal anergy.** *Science* 1990, **248:**1349-1356.
55. Chen L, Flies DB: **Molecular mechanisms of T cell co-stimulation and co-inhibition.** *Nat Rev Immunol* 2013, **13:**227-242.
56. Hanahan D, Weinberg RA: **Hallmarks of cancer: the next generation.** *Cell* 2011, **144:**646-674.
57. Tang J, Pearce L, O'Donnell-Tormey J, Hubbard-Lucey VM: **Trends in the global immuno-oncology landscape.** *Nat Rev Drug Discov* 2018, **17:**783-784.
58. Wherry EJ, Kurachi M: **Molecular and cellular insights into T cell exhaustion.** *Nat Rev Immunol* 2015, **15:**486-499.
59. Scheper W, Kelderman S, Fanchi LF, Linnemann C, Bendle G, de Rooij MAJ, Hirt C, Mezzadra R, Slagter M, Dijkstra K, et al: **Low and variable tumor reactivity of the intratumoral TCR repertoire in human cancers.** *Nat Med* 2019, **25:**89-94.

60. Huard B, Mastrangeli R, Prigent P, Bruniquel D, Donini S, El-Tayar N, Maigret B, Dreano M, Triebel F: **Characterization of the major histocompatibility complex class II binding site on LAG-3 protein.** *Proc Natl Acad Sci U S A* 1997, **94:**5744-5749.

61. Walunas TL, Lenschow DJ, Bakker CY, Linsley PS, Freeman GJ, Green JM, Thompson CB, Bluestone JA: **CTLA-4 can function as a negative regulator of T cell activation.** *Immunity* 1994, **1:**405-413.

62. Krummel MF, Allison JP: **CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation.** *J Exp Med* 1995, **182:**459-465.

63. Leach DR, Krummel MF, Allison JP: **Enhancement of antitumor immunity by CTLA-4 blockade.** *Science* 1996, **271:**1734-1736.

64. Kvistborg P, Philips D, Kelderman S, Hageman L, Ottensmeier C, Joseph-Pietras D, Welters MJ, van der Burg S, Kapiteijn E, Michielin O, et al: **Anti-CTLA-4 therapy broadens the melanoma-reactive CD8+ T cell response.** *Sci Transl Med* 2014, **6:**254ra128.

65. Wei SC, Sharma R, Anang NAS, Levine JH, Zhao Y, Mancuso JJ, Setty M, Sharma P, Wang J, Pe'er D, Allison JP: **Negative Co-stimulation Constrains T Cell Differentiation by Imposing Boundaries on Possible Cell States.** *Immunity* 2019, **50:**1084-1098 e1010.

66. Wei SC, Levine JH, Cogdill AP, Zhao Y, Anang NAS, Andrews MC, Sharma P, Wang J, Wargo JA, Pe'er D, Allison JP: **Distinct Cellular Mechanisms Underlie Anti-CTLA-4 and Anti-PD-1 Checkpoint Blockade.** *Cell* 2017, **170:**1120-1133 e1117.

67. Robert L, Tsoi J, Wang X, Emerson R, Homet B, Chodon T, Mok S, Huang RR, Cochran AJ, Comin-Anduix B, et al: **CTLA4 blockade broadens the peripheral T-cell receptor repertoire.** *Clin Cancer Res* 2014, **20:**2424-2432.

68. Agata Y, Kawasaki A, Nishimura H, Ishida Y, Tsubata T, Yagita H, Honjo T: **Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes.** *Int Immunol* 1996, **8:**765-772.

69. Ishida Y, Agata Y, Shibahara K, Honjo T: **Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death.** *EMBO J* 1992, **11:**3887-3895.

70. Dong H, Zhu G, Tamada K, Chen L: **B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion.** *Nat Med* 1999, **5:**1365-1369.

71. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, Fitz LJ, Malenkovich N, Okazaki T, Byrne MC, 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:**1027-1034.

72. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, Freeman GJ, Ahmed R: **Restoring function in exhausted CD8 T cells during chronic viral infection.** *Nature* 2006, **439:**682-687.

73. Lee SJ, Jang BC, Lee SW, Yang YI, Suh SI, Park YM, Oh S, Shin JG, Yao S, Chen L, Choi IH: **Interferon regulatory factor-1 is prerequisite to the constitutive expression and IFN-gamma-induced upregulation of B7-H1 (CD274).** *FEBS Lett* 2006, **580:**755-762.

74. Iwai Y, Terawaki S, Honjo T: **PD-1 blockade inhibits hematogenous spread of poorly immunogenic tumor cells by enhanced recruitment of effector T cells.** *Int Immunol* 2005, **17:**133-144.

75. Hirano F, Kaneko K, Tamura H, Dong H, Wang S, Ichikawa M, Rietz C, Flies DB, Lau JS, Zhu G, et al: **Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity.** *Cancer Res* 2005, **65:**1089-1096.

76. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, et al: **Safety, activity, and immune correlates of anti-PD-1 antibody in cancer.** *N Engl J Med* 2012, **366:**2443-2454.

77. **Cancer Research Institute** [https://www.cancerresearch.org/scientists/immuno-oncology-landscape/pd-1-pd-l1-landscape#timelines]

78. Ribas A, Wolchok JD: **Cancer immunotherapy using checkpoint blockade.** *Science* 2018, **359:**1350-1355.

79. Long GV, Atkinson V, Cebon JS, Jameson MB, Fitzharris BM, McNeil CM, Hill AG, Ribas A, Atkins MB, Thompson JA, et al: **Standard-dose pembrolizumab in combination with reduced-dose ipilimumab for patients with advanced melanoma (KEYNOTE-029): an open-label, phase 1b trial.** *Lancet Oncol* 2017, **18:**1202-1210.

80. Grosso JF, Goldberg MV, Getnet D, Bruno TC, Yen HR, Pyle KJ, Hipkiss E, Vignali DA, Pardoll DM, Drake CG: **Functionally distinct LAG-3 and PD-1 subsets on activated and chronically stimulated CD8 T cells.** *J Immunol* 2009, **182:**6659-6669.

81. Matsuzaki J, Gnjatic S, Mhawech-Fauceglia P, Beck A, Miller A, Tsuji T, Eppolito C, Qian F, Lele S, Shrikant P, et al: **Tumor-infiltrating NY-ESO-1-specific CD8+ T cells are negatively regulated by LAG-3 and PD-1 in human ovarian cancer.** *Proc Natl Acad Sci U S A* 2010, **107:**7875-7880.

82. Woo SR, Turnis ME, Goldberg MV, Bankoti J, Selby M, Nirschl CJ, Bettini ML, Gravano DM, Vogel P, Liu CL, et al: **Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape.** *Cancer Res* 2012, **72:**917-927.

83. Ascierto PA, Bono P, Bhatia S, Melero I, Nyakas MS, Svane I-M, Larkin J, Gomez-Roca C, Schadendorf D, Dummer R, et al: **LBA18Efficacy of BMS-986016, a monoclonal antibody that targets lymphocyte activation gene-3 (LAG-3), in combination with nivolumab in pts with melanoma who progressed during prior anti–PD-1/PD-L1 therapy (mel prior IO) in all-comer and biomarker-enriched populations.** *Annals of Oncology* 2017, **28**.

84. Andrews LP, Yano H, Vignali DAA: **Inhibitory receptors and ligands beyond PD-1, PD-L1 and CTLA-4: breakthroughs or backups.** *Nat Immunol* 2019, **20:**1425-1434.

85. Ansell SM, Lesokhin AM, Borrello I, Halwani A, Scott EC, Gutierrez M, Schuster SJ, Millenson MM, Cattry D, Freeman GJ, et al: **PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma.** *N Engl J Med* 2015, **372:**311-319.

86. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, et al: **Safety and activity of anti-PD-L1 antibody in patients with advanced cancer.** *N Engl J Med* 2012, **366:**2455-2465.

87. Fridman WH, Zitvogel L, Sautes-Fridman C, Kroemer G: **The immune contexture in cancer prognosis and treatment.** *Nat Rev Clin Oncol* 2017, **14:**717-734.

88. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, Chmielowski B, Spasic M, Henry G, Ciobanu V, et al: **PD-1 blockade induces responses by inhibiting adaptive immune resistance.** *Nature* 2014, **515:**568-571.

89. Pages F, Mlecnik B, Marliot F, Bindea G, Ou FS, Bifulco C, Lugli A, Zlobec I, Rau TT, Berger MD, et al: **International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study.** *Lancet* 2018, **391:**2128-2139.

90. Vignali DA, Collison LW, Workman CJ: **How regulatory T cells work.** *Nat Rev Immunol* 2008, **8:**523-532.

91. Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, Evdemon-Hogan M, Conejo-Garcia JR, Zhang L, Burow M, et al: **Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival.** *Nat Med* 2004, **10:**942-949.

92. Simpson TR, Li F, Montalvo-Ortiz W, Sepulveda MA, Bergerhoff K, Arce F, Roddie C, Henry JY, Yagita H, Wolchok JD, 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:**1695-1710.

93. Sharma A, Subudhi SK, Blando J, Scutti J, Vence L, Wargo J, Allison JP, Ribas A, Sharma P: **Anti-CTLA-4 Immunotherapy Does Not Deplete FOXP3(+) Regulatory T Cells (Tregs) in Human Cancers.** *Clin Cancer Res* 2019, **25:**1233-1238.

94. Cabrita R, Lauss M, Sanna A, Donia M, Skaarup Larsen M, Mitra S, Johansson I, Phung B, Harbst K, Vallon-Christersson J, et al: **Tertiary lymphoid structures improve immunotherapy and survival in melanoma.** *Nature* 2020.

95. Petitprez F, de Reyniès A, Keung EZ, Chen TW-W, Sun C-M, Calderaro J, Jeng Y-M, Hsiao L-P, Lacroix L, Bougoüin A, et al: **B cells are associated with survival and immunotherapy response in sarcoma.** *Nature* 2020.

96. Helmink BA, Reddy SM, Gao J, Zhang S, Basar R, Thakur R, Yizhak K, Sade-Feldman M, Blando J, Han G, et al: **B cells and tertiary lymphoid structures promote immunotherapy response.** *Nature* 2020.

97. Taube JM, Klein A, Brahmer JR, Xu H, Pan X, Kim JH, Chen L, Pardoll DM, Topalian SL, Anders RA: **Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy.** *Clin Cancer Res* 2014, **20:**5064-5074.

98. Daud AI, Wolchok JD, Robert C, Hwu WJ, Weber JS, Ribas A, Hodi FS, Joshua AM, Kefford R, Hersey P, et al: **Programmed Death-Ligand 1 Expression and Response to the Anti-Programmed Death 1 Antibody Pembrolizumab in Melanoma.** *J Clin Oncol* 2016, **34:**4102-4109.

99. Davis AA, Patel VG: **The role of PD-L1 expression as a predictive biomarker: an analysis of all US Food and Drug Administration (FDA) approvals of immune checkpoint inhibitors.** *J Immunother Cancer* 2019, **7:**278.

100. Vilain RE, Menzies AM, Wilmott JS, Kakavand H, Madore J, Guminski A, Liniker E, Kong BY, Cooper AJ, Howle JR, et al: **Dynamic Changes in PD-L1 Expression and Immune Infiltrates Early During Treatment Predict Response to PD-1 Blockade in Melanoma.** *Clin Cancer Res* 2017, **23:**5024-5033.

101. Bensch F, van der Veen EL, Lub-de Hooge MN, Jorritsma-Smit A, Boellaard R, Kok IC, Oosting SF, Schroder CP, Hiltermann TJN, van der Wekken AJ, et al: **(89)Zr-atezolizumab imaging as a non-invasive approach to assess clinical response to PD-L1 blockade in cancer.** *Nat Med* 2018, **24:**1852-1858.

102. Krieg C, Nowicka M, Guglietta S, Schindler S, Hartmann FJ, Weber LM, Dummer R, Robinson MD, Levesque MP, Becher B: **High-dimensional single-cell analysis predicts response to anti-PD-1 immunotherapy.** *Nat Med* 2018, **24:**144-153.

103. Liu Y, Zugazagoitia J, Ahmed FS, Henick BS, Gettinger S, Herbst RS, Schalper KA, Rimm DL: **Immune cell PD-L1 co-localizes with macrophages and is associated with outcome in PD-1 pathway blockade therapy.** *Clin Cancer Res* 2019.

104. Cassetta L, Pollard JW: **Targeting macrophages: therapeutic approaches in cancer.** *Nat Rev Drug Discov* 2018, **17:**887-904.

105. Neubert NJ, Schmittnaegel M, Bordry N, Nassiri S, Wald N, Martignier C, Tille L, Homicsko K, Damsky W, Maby-El Hajjami H, et al: **T cell-induced CSF1 promotes melanoma resistance to PD1 blockade.** *Sci Transl Med* 2018, **10**.

106. Noy R, Pollard JW: **Tumor-associated macrophages: from mechanisms to therapy.** *Immunity* 2014, **41:**49-61.

107. Riaz N, Havel JJ, Makarov V, Desrichard A, Urba WJ, Sims JS, Hodi FS, Martin-Algarra S, Mandal R, Sharfman WH, et al: **Tumor and Microenvironment Evolution during Immunotherapy with Nivolumab.** *Cell* 2017, **171:**934-949 e916.

108. Xue J, Schmidt SV, Sander J, Draffehn A, Krebs W, Quester I, De Nardo D, Gohel TD, Emde M, Schmidleithner L, et al: **Transcriptome-based network analysis reveals a spectrum model of human macrophage activation.** *Immunity* 2014, **40:**274-288.

109. Pathria P, Louis TL, Varner JA: **Targeting Tumor-Associated Macrophages in Cancer.** *Trends Immunol* 2019, **40:**310-327.

110. Veglia F, Perego M, Gabrilovich D: **Myeloid-derived suppressor cells coming of age.** *Nat Immunol* 2018, **19:**108-119.

111. Weber J, Gibney G, Kudchadkar R, Yu B, Cheng P, Martinez AJ, Kroeger J, Richards A, McCormick L, Moberg V, et al: **Phase I/II Study of Metastatic Melanoma Patients Treated with Nivolumab Who Had Progressed after Ipilimumab.** *Cancer Immunol Res* 2016, **4:**345-353.

112. Kitano S, Postow MA, Ziegler CG, Kuk D, Panageas KS, Cortez C, Rasalan T, Adamow M, Yuan J, Wong P, et al: **Computational algorithm-driven evaluation of monocytic myeloid-derived suppressor cell frequency for prediction of clinical outcomes.** *Cancer Immunol Res* 2014, **2:**812-821.

113. Liu Y, Wei G, Cheng WA, Dong Z, Sun H, Lee VY, Cha SC, Smith DL, Kwak LW, Qin H: **Targeting myeloid-derived suppressor cells for cancer immunotherapy.** *Cancer Immunol Immunother* 2018, **67:**1181-1195.

114. Spranger S, Bao R, Gajewski TF: **Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity.** *Nature* 2015, **523:**231-235.

115. Broz ML, Binnewies M, Boldajipour B, Nelson AE, Pollack JL, Erle DJ, Barczak A, Rosenblum MD, Daud A, Barber DL, et al: **Dissecting the tumor myeloid compartment reveals rare activating antigen-presenting cells critical for T cell immunity.** *Cancer Cell* 2014, **26:**638-652.

116. del Rio ML, Rodriguez-Barbosa JI, Kremmer E, Forster R: **CD103- and CD103+ bronchial lymph node dendritic cells are specialized in presenting and cross-presenting innocuous antigen to CD4+ and CD8+ T cells.** *J Immunol* 2007, **178:**6861-6866.

117.   Gubin MM, Zhang X, Schuster H, Caron E, Ward JP, Noguchi T, Ivanova Y, Hundal J, Arthur CD, Krebber WJ, et al: **Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens.** *Nature* 2014, **515:**577-581.
118.   Tran E, Ahmadzadeh M, Lu YC, Gros A, Turcotte S, Robbins PF, Gartner JJ, Zheng Z, Li YF, Ray S, et al: **Immunogenicity of somatic mutations in human gastrointestinal cancers.** *Science* 2015, **350:**1387-1390.
119.   Stronen E, Toebes M, Kelderman S, van Buuren MM, Yang W, van Rooij N, Donia M, Boschen ML, Lund-Johansen F, Olweus J, Schumacher TN: **Targeting of cancer neoantigens with donor-derived T cell receptor repertoires.** *Science* 2016, **352:**1337-1341.
120.   van Rooij N, van Buuren MM, Philips D, Velds A, Toebes M, Heemskerk B, van Dijk LJ, Behjati S, Hilkmann H, El Atmioui D, et al: **Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma.** *J Clin Oncol* 2013, **31:**e439-442.
121.   Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, Lee W, Yuan J, Wong P, Ho TS, et al: **Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer.** *Science* 2015, **348:**124-128.
122.   Yarchoan M, Hopkins A, Jaffee EM: **Tumor Mutational Burden and Response Rate to PD-1 Inhibition.** *N Engl J Med* 2017, **377:**2500-2501.
123.   Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, Walsh LA, Postow MA, Wong P, Ho TS, et al: **Genetic basis for clinical response to CTLA-4 blockade in melanoma.** *N Engl J Med* 2014, **371:**2189-2199.
124.   Van Allen EM, Miao D, Schilling B, Shukla SA, Blank C, Zimmer L, Sucker A, Hillen U, Foppen MHG, Goldinger SM, et al: **Genomic correlates of response to CTLA-4 blockade in metastatic melanoma.** *Science* 2015, **350:**207-211.
125.   Germano G, Lamba S, Rospo G, Barault L, Magri A, Maione F, Russo M, Crisafulli G, Bartolini A, Lerda G, et al: **Inactivation of DNA repair triggers neoantigen generation and impairs tumour growth.** *Nature* 2017, **552:**116-120.
126.   Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, Lu S, Kemberling H, Wilt C, Luber BS, et al: **Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade.** *Science* 2017, **357:**409-413.
127.   ***FDA grants accelerated approval to pembrolizumab for first tissue/site agnostic indication*** [https://www.fda.gov/drugs/resources-information-approved-drugs/fda-grants-accelerated-approval-pembrolizumab-first-tissuesite-agnostic-indication]
128.   Miao D, Margolis CA, Vokes NI, Liu D, Taylor-Weiner A, Wankowicz SM, Adeegbe D, Keliher D, Schilling B, Tracy A, et al: **Genomic correlates of response to immune checkpoint blockade in microsatellite-stable solid tumors.** *Nat Genet* 2018, **50:**1271-1281.
129.   Liu D, Schilling B, Liu D, Sucker A, Livingstone E, Jerby-Amon L, Zimmer L, Gutzmer R, Satzger I, Loquai C, et al: **Integrative molecular and clinical modeling of clinical outcomes to PD1 blockade in patients with metastatic melanoma.** *Nature Medicine* 2019.
130.   Colli LM, Machiela MJ, Myers TA, Jessop L, Yu K, Chanock SJ: **Burden of Nonsynonymous Mutations among TCGA Cancers and Candidate Immune Checkpoint Inhibitor Responses.** *Cancer Res* 2016, **76:**3767-3772.
131.   Turajlic S, Litchfield K, Xu H, Rosenthal R, McGranahan N, Reading JL, Wong YNS, Rowan A, Kanu N, Al Bakir M, et al: **Insertion-and-deletion-derived tumour-specific neoantigens and the immunogenic phenotype: a pan-cancer analysis.** *Lancet Oncol* 2017, **18:**1009-1021.
132.   Wolf Y, Bartok O, Patkar S, Eli GB, Cohen S, Litchfield K, Levy R, Jimenez-Sanchez A, Trabish S, Lee JS, et al: **UVB-Induced Tumor Heterogeneity Diminishes Immune Response in Melanoma.** *Cell* 2019, **179:**219-235 e221.
133.   McGranahan N, Furness AJ, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, Jamal-Hanjani M, Wilson GA, Birkbak NJ, Hiley CT, et al: **Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade.** *Science* 2016, **351:**1463-1469.
134.   **Complete sequence and gene map of a human major histocompatibility complex. The MHC sequencing consortium.** *Nature* 1999, **401:**921-923.
135.   Chowell D, Morris LGT, Grigg CM, Weber JK, Samstein RM, Makarov V, Kuo F, Kendall SM, Requena D, Riaz N, et al: **Patient HLA class I genotype influences cancer response to checkpoint blockade immunotherapy.** *Science* 2018, **359:**582-587.

136. Tran E, Robbins PF, Lu YC, Prickett TD, Gartner JJ, Jia L, Pasetto A, Zheng Z, Ray S, Groh EM, et al: **T-Cell Transfer Therapy Targeting Mutant KRAS in Cancer.** *N Engl J Med* 2016, **375:**2255-2262.

137. McGranahan N, Rosenthal R, Hiley CT, Rowan AJ, Watkins TBK, Wilson GA, Birkbak NJ, Veeriah S, Van Loo P, Herrero J, et al: **Allele-Specific HLA Loss and Immune Escape in Lung Cancer Evolution.** *Cell* 2017, **171:**1259-1271 e1211.

138. Paulson KG, Voillet V, McAfee MS, Hunter DS, Wagener FD, Perdicchio M, Valente WJ, Koelle SJ, Church CD, Vandeven N, et al: **Acquired cancer resistance to combination immunotherapy from transcriptional loss of class I HLA.** *Nat Commun* 2018, **9:**3868.

139. Zaretsky JM, Garcia-Diaz A, Shin DS, Escuin-Ordinas H, Hugo W, Hu-Lieskovan S, Torrejon DY, Abril-Rodriguez G, Sandoval S, Barthly L, et al: **Mutations Associated with Acquired Resistance to PD-1 Blockade in Melanoma.** *N Engl J Med* 2016, **375:**819-829.

140. Rodig SJ, Gusenleitner D, Jackson DG, Gjini E, Giobbie-Hurder A, Jin C, Chang H, Lovitch SB, Horak C, Weber JS, et al: **MHC proteins confer differential sensitivity to CTLA-4 and PD-1 blockade in untreated metastatic melanoma.** *Sci Transl Med* 2018, **10**.

141. Peng W, Chen JQ, Liu C, Malu S, Creasy C, Tetzlaff MT, Xu C, McKenzie JA, Zhang C, Liang X, et al: **Loss of PTEN Promotes Resistance to T Cell-Mediated Immunotherapy.** *Cancer Discov* 2016, **6:**202-216.

142. Bradley SD, Chen Z, Melendez B, Talukder A, Khalili JS, Rodriguez-Cruz T, Liu S, Whittington M, Deng W, Li F, et al: **BRAFV600E Co-opts a Conserved MHC Class I Internalization Pathway to Diminish Antigen Presentation and CD8+ T-cell Recognition of Melanoma.** *Cancer Immunol Res* 2015, **3:**602-609.

143. Marty R, Kaabinejadian S, Rossell D, Slifker MJ, van de Haar J, Engin HB, de Prisco N, Ideker T, Hildebrand WH, Font-Burgada J, Carter H: **MHC-I Genotype Restricts the Oncogenic Mutational Landscape.** *Cell* 2017, **171:**1272-1283 e1215.

144. Mariathasan S, Turley SJ, Nickles D, Castiglioni A, Yuen K, Wang Y, Kadel EE, III, Koeppen H, Astarita JL, Cubas R, et al: **TGFbeta attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells.** *Nature* 2018, **554:**544-548.

145. Rodig N, Ryan T, Allen JA, Pang H, Grabie N, Chernova T, Greenfield EA, Liang SC, Sharpe AH, Lichtman AH, Freeman GJ: **Endothelial expression of PD-L1 and PD-L2 down-regulates CD8+ T cell activation and cytolysis.** *Eur J Immunol* 2003, **33:**3117-3126.

146. Turley SJ, Cremasco V, Astarita JL: **Immunological hallmarks of stromal cells in the tumour microenvironment.** *Nat Rev Immunol* 2015, **15:**669-682.

147. Fukumura D, Kloepper J, Amoozgar Z, Duda DG, Jain RK: **Enhancing cancer immunotherapy using antiangiogenics: opportunities and challenges.** *Nat Rev Clin Oncol* 2018, **15:**325-340.

148. Chang CH, Qiu J, O'Sullivan D, Buck MD, Noguchi T, Curtis JD, Chen Q, Gindin M, Gubin MM, van der Windt GJ, et al: **Metabolic Competition in the Tumor Microenvironment Is a Driver of Cancer Progression.** *Cell* 2015, **162:**1229-1241.

149. Munn DH, Mellor AL: **Indoleamine 2,3-dioxygenase and tumor-induced tolerance.** *J Clin Invest* 2007, **117:**1147-1154.

150. Geiger R, Rieckmann JC, Wolf T, Basso C, Feng Y, Fuhrer T, Kogadeeva M, Picotti P, Meissner F, Mann M, et al: **L-Arginine Modulates T Cell Metabolism and Enhances Survival and Anti-tumor Activity.** *Cell* 2016, **167:**829-842 e813.

151. Antonioli L, Blandizzi C, Pacher P, Hasko G: **Immunity, inflammation and cancer: a leading role for adenosine.** *Nat Rev Cancer* 2013, **13:**842-857.

152. Brand A, Singer K, Koehl GE, Kolitzus M, Schoenhammer G, Thiel A, Matos C, Bruss C, Klobuch S, Peter K, et al: **LDHA-Associated Lactic Acid Production Blunts Tumor Immunosurveillance by T and NK Cells.** *Cell Metab* 2016, **24:**657-671.

153. Ayers M, Lunceford J, Nebozhyn M, Murphy E, Loboda A, Kaufman DR, Albright A, Cheng JD, Kang SP, Shankaran V, et al: **IFN-γ–related mRNA profile predicts clinical response to PD-1 blockade.** *The Journal of Clinical Investigation* 2017, **127:**2930-2940.

154. Auslander N, Zhang G, Lee JS, Frederick DT, Miao B, Moll T, Tian T, Wei Z, Madan S, Sullivan RJ, et al: **Robust prediction of response to immune checkpoint blockade therapy in metastatic melanoma.** *Nature medicine* 2018, **24:**1545-1549.

155. Jiang P, Gu S, Pan D, Fu J, Sahu A, Hu X, Li Z, Traugh N, Bu X, Li B, et al: **Signatures of T cell dysfunction and exclusion predict cancer immunotherapy response.** *Nature medicine* 2018, **24:**1550-1558.

156. Cristescu R, Mogg R, Ayers M, Albright A, Murphy E, Yearley J, Sher X, Liu XQ, Lu H, Nebozhyn M, et al: **Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy.** *Science* 2018, **362**.

157. Carter JA, Gilbo P, Atwal GS: **IMPRES does not reproducibly predict response to immune checkpoint blockade therapy in metastatic melanoma.** *Nature Medicine* 2019, **25:**1833-1835.

158. Killock D: **Sequencing cells of the immune TME.** *Nat Rev Clin Oncol* 2018, **15:**531.

159. Baharlou H, Canete NP, Cunningham AL, Harman AN, Patrick E: **Mass Cytometry Imaging for the Study of Human Diseases—Applications and Data Analysis Strategies.** *Frontiers in Immunology* 2019, **10**.

160. Parra ER, Francisco-Cruz A, Wistuba, II: **State-of-the-Art of Profiling Immune Contexture in the Era of Multiplexed Staining and Digital Analysis to Study Paraffin Tumor Tissues.** *Cancers (Basel)* 2019, **11**.

161. Liu CC, Steen CB, Newman AM: **Computational approaches for characterizing the tumor immune microenvironment.** *Immunology* 2019, **158:**70-84.

162. Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM, Benyamin FW, Lei YM, Jabri B, Alegre ML, et al: **Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy.** *Science* 2015, **350:**1084-1089.

163. Vetizou M, Pitt JM, Daillere R, Lepage P, Waldschmitt N, Flament C, Rusakiewicz S, Routy B, Roberti MP, Duong CP, et al: **Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota.** *Science* 2015, **350:**1079-1084.

164. Matson V, Fessler J, Bao R, Chongsuwat T, Zha Y, Alegre ML, Luke JJ, Gajewski TF: **The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients.** *Science* 2018, **359:**104-108.

165. Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, Prieto PA, Vicente D, Hoffman K, Wei SC, et al: **Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients.** *Science* 2018, **359:**97-103.

166. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillere R, Fluckiger A, Messaoudene M, Rauber C, Roberti MP, et al: **Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors.** *Science* 2018, **359:**91-97.

167. Gharaibeh RZ, Jobin C: **Microbiota and cancer immunotherapy: in search of microbial signals.** *Gut* 2018.

168. Kugel CH, 3rd, Douglass SM, Webster MR, Kaur A, Liu Q, Yin X, Weiss SA, Darvishian F, Al-Rohil RN, Ndoye A, et al: **Age Correlates with Response to Anti-PD1, Reflecting Age-Related Differences in Intratumoral Effector and Regulatory T-Cell Populations.** *Clin Cancer Res* 2018, **24:**5347-5356.

169. Conforti F, Pala L, Bagnardi V, De Pas T, Martinetti M, Viale G, Gelber RD, Goldhirsch A: **Cancer immunotherapy efficacy and patients' sex: a systematic review and meta-analysis.** *Lancet Oncol* 2018, **19:**737-746.

170. McQuade JL, Daniel CR, Hess KR, Mak C, Wang DY, Rai RR, Park JJ, Haydu LE, Spencer C, Wongchenko M, et al: **Association of body-mass index and outcomes in patients with metastatic melanoma treated with targeted therapy, immunotherapy, or chemotherapy: a retrospective, multicohort analysis.** *Lancet Oncol* 2018, **19:**310-322.

171. Weide B, Martens A, Hassel JC, Berking C, Postow MA, Bisschop K, Simeone E, Mangana J, Schilling B, Di Giacomo AM, et al: **Baseline Biomarkers for Outcome of Melanoma Patients Treated with Pembrolizumab.** *Clin Cancer Res* 2016, **22:**5487-5496.

172. Rosenberg SA, Yang JC, Sherry RM, Kammula US, Hughes MS, Phan GQ, Citrin DE, Restifo NP, Robbins PF, Wunderlich JR, et al: **Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy.** *Clin Cancer Res* 2011, **17:**4550-4557.

173. Andersen R, Donia M, Ellebaek E, Borch TH, Kongsted P, Iversen TZ, Holmich LR, Hendel HW, Met O, Andersen MH, et al: **Long-Lasting Complete Responses in Patients with Metastatic Melanoma after Adoptive Cell Therapy with Tumor-Infiltrating Lymphocytes and an Attenuated IL2 Regimen.** *Clin Cancer Res* 2016, **22:**3734-3745.

174. Besser MJ, Shapira-Frommer R, Itzhaki O, Treves AJ, Zippel DB, Levy D, Kubi A, Shoshani N, Zikich D, Ohayon Y, et al: **Adoptive transfer of tumor-infiltrating lymphocytes in patients with metastatic melanoma: intent-to-treat analysis and efficacy after failure to prior immunotherapies.** *Clin Cancer Res* 2013, **19:**4792-4800.

175. Rosenberg SA, Restifo NP: **Adoptive cell transfer as personalized immunotherapy for human cancer.** *Science (New York, NY)* 2015, **348:**62-68.
176. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, Bader P, Verneris MR, Stefanski HE, Myers GD, et al: **Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia.** *N Engl J Med* 2018, **378:**439-448.
177. Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, Braunschweig I, Oluwole OO, Siddiqi T, Lin Y, et al: **Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma.** *N Engl J Med* 2017, **377:**2531-2544.
178. Schuster SJ, Bishop MR, Tam CS, Waller EK, Borchmann P, McGuirk JP, Jager U, Jaglowski S, Andreadis C, Westin JR, et al: **Tisagenlecleucel in Adult Relapsed or Refractory Diffuse Large B-Cell Lymphoma.** *N Engl J Med* 2019, **380:**45-56.
179. Hegde M, DeRenzo CC, Zhang H, Mata M, Gerken C, Shree A, Yi Z, Brawley V, Dakhova O, Wu M-F, et al: **Expansion of HER2-CAR T cells after lymphodepletion and clinical responses in patients with advanced sarcoma.** *Journal of Clinical Oncology* 2017, **35:**10508-10508.
180. Kagoya Y, Tanaka S, Guo T, Anczurowski M, Wang C-H, Saso K, Butler MO, Minden MD, Hirano N: **A novel chimeric antigen receptor containing a JAK–STAT signaling domain mediates superior antitumor effects.** *Nature Medicine* 2018, **24:**352.
181. Ott PA, Hu Z, Keskin DB, Shukla SA, Sun J, Bozym DJ, Zhang W, Luoma A, Giobbie-Hurder A, Peter L, et al: **An immunogenic personal neoantigen vaccine for patients with melanoma.** *Nature* 2017, **547:**217-221.
182. Sahin U, Derhovanessian E, Miller M, Kloke BP, Simon P, Lower M, Bukur V, Tadmor AD, Luxemburger U, Schrors B, et al: **Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer.** *Nature* 2017, **547:**222-226.
183. Damato BE, Dukes J, Goodall H, Carvajal RD: **Tebentafusp: T Cell Redirection for the Treatment of Metastatic Uveal Melanoma.** *Cancers (Basel)* 2019, **11**.
184. Charych D, Khalili S, Dixit V, Kirk P, Chang T, Langowski J, Rubas W, Doberstein SK, Eldon M, Hoch U, Zalevsky J: **Modeling the receptor pharmacology, pharmacokinetics, and pharmacodynamics of NKTR-214, a kinetically-controlled interleukin-2 (IL2) receptor agonist for cancer immunotherapy.** *PLoS One* 2017, **12:**e0179431.
185. Diab A, Puzanov I, Maio M, Curti BD, Bilen MA, Lewis KD, Tykodi SS, Gregory A. Daniels, Spira AI, Bernatchez C, et al: **O35: Clinical activity of BEMPEG plus NIVO in previously untreated patients with metastatic melanoma: updated results from the phase 1/2 PIVOT-02 study.** *J Immunotherapy Cancer* 2919, **7**.
186. Dempke WCM, Fenchel K, Uciechowski P, Dale SP: **Second- and third-generation drugs for immuno-oncology treatment-The more the better?** *Eur J Cancer* 2017, **74:**55-72.
187. Woo SR, Fuertes MB, Corrales L, Spranger S, Furdyna MJ, Leung MY, Duggan R, Wang Y, Barber GN, Fitzgerald KA, et al: **STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors.** *Immunity* 2014, **41:**830-842.
188. Long GV, Dummer R, Hamid O, Gajewski TF, Caglevic C, Dalle S, Arance A, Carlino MS, Grob JJ, Kim TM, et al: **Epacadostat plus pembrolizumab versus placebo plus pembrolizumab in patients with unresectable or metastatic melanoma (ECHO-301/KEYNOTE-252): a phase 3, randomised, double-blind study.** *Lancet Oncol* 2019, **20:**1083-1097.
189. Leone RD, Emens LA: **Targeting adenosine for cancer immunotherapy.** *J Immunother Cancer* 2018, **6:**57.
190. Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, Yokoi T, Chiappori A, Lee KH, de Wit M, et al: **Durvalumab after Chemoradiotherapy in Stage III Non-Small-Cell Lung Cancer.** *N Engl J Med* 2017, **377:**1919-1929.
191. Gandhi L, Rodriguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, Domine M, Clingan P, Hochmair MJ, Powell SF, et al: **Pembrolizumab plus Chemotherapy in Metastatic Non-Small-Cell Lung Cancer.** *N Engl J Med* 2018, **378:**2078-2092.
192. Hu-Lieskovan S, Mok S, Homet Moreno B, Tsoi J, Robert L, Goedert L, Pinheiro EM, Koya RC, Graeber TG, Comin-Anduix B, Ribas A: **Improved antitumor activity of immunotherapy with BRAF and MEK inhibitors in BRAF(V600E) melanoma.** *Sci Transl Med* 2015, **7:**279ra241.

193. Cooper ZA, Juneja VR, Sage PT, Frederick DT, Piris A, Mitra D, Lo JA, Hodi FS, Freeman GJ, Bosenberg MW, et al: **Response to BRAF inhibition in melanoma is enhanced when combined with immune checkpoint blockade.** *Cancer Immunol Res* 2014, **2:**643-654.

194. Ny L, Rizzo LY, Belgrano V, Karlsson J, Jespersen H, Carstam L, Bagge RO, Nilsson LM, Nilsson JA: **Supporting clinical decision making in advanced melanoma by preclinical testing in personalized immune-humanized xenograft mouse models.** *Annals of Oncology* 2020.

195. Ribas A, Lawrence D, Atkinson V, Agarwal S, Miller WH, Jr., Carlino MS, Fisher R, Long GV, Hodi FS, Tsoi J, et al: **Combined BRAF and MEK inhibition with PD-1 blockade immunotherapy in BRAF-mutant melanoma.** *Nat Med* 2019, **25:**936-940.

196. Sullivan RJ, Hamid O, Gonzalez R, Infante JR, Patel MR, Hodi FS, Lewis KD, Tawbi HA, Hernandez G, Wongchenko MJ, et al: **Atezolizumab plus cobimetinib and vemurafenib in BRAF-mutated melanoma patients.** *Nat Med* 2019, **25:**929-935.

197. Ascierto PA, Ferrucci PF, Fisher R, Del Vecchio M, Atkinson V, Schmidt H, Schachter J, Queirolo P, Long GV, Di Giacomo AM, et al: **Dabrafenib, trametinib and pembrolizumab or placebo in BRAF-mutant melanoma.** *Nat Med* 2019, **25:**941-946.

198. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, Schadendorf D, Dummer R, Smylie M, Rutkowski P, et al: **Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma.** *N Engl J Med* 2015, **373:**23-34.

199. Weber JS, Hodi FS, Wolchok JD, Topalian SL, Schadendorf D, Larkin J, Sznol M, Long GV, Li H, Waxman IM, et al: **Safety Profile of Nivolumab Monotherapy: A Pooled Analysis of Patients With Advanced Melanoma.** *J Clin Oncol* 2017, **35:**785-792.

200. Wang DY, Salem JE, Cohen JV, Chandra S, Menzer C, Ye F, Zhao S, Das S, Beckermann KE, Ha L, et al: **Fatal Toxic Effects Associated With Immune Checkpoint Inhibitors: A Systematic Review and Meta-analysis.** *JAMA Oncol* 2018, **4:**1721-1728.

201. Puzanov I, Diab A, Abdallah K, Bingham CO, 3rd, Brogdon C, Dadu R, Hamad L, Kim S, Lacouture ME, LeBoeuf NR, et al: **Managing toxicities associated with immune checkpoint inhibitors: consensus recommendations from the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group.** *J Immunother Cancer* 2017, **5:**95.

202. Haanen J, Carbonnel F, Robert C, Kerr KM, Peters S, Larkin J, Jordan K, Committee EG: **Management of toxicities from immunotherapy: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up.** *Ann Oncol* 2017, **28:**iv119-iv142.

203. Brahmer JR, Lacchetti C, Schneider BJ, Atkins MB, Brassil KJ, Caterino JM, Chau I, Ernstoff MS, Gardner JM, Ginex P, et al: **Management of Immune-Related Adverse Events in Patients Treated With Immune Checkpoint Inhibitor Therapy: American Society of Clinical Oncology Clinical Practice Guideline.** *J Clin Oncol* 2018, **36:**1714-1768.

204. Hsiehchen D, Watters MK, Lu R, Xie Y, Gerber DE: **Variation in the Assessment of Immune-Related Adverse Event Occurrence, Grade, and Timing in Patients Receiving Immune Checkpoint Inhibitors.** *JAMA Netw Open* 2019, **2:**e1911519.

205. Shoushtari AN, Friedman CF, Navid-Azarbaijani P, Postow MA, Callahan MK, Momtaz P, Panageas KS, Wolchok JD, Chapman PB: **Measuring Toxic Effects and Time to Treatment Failure for Nivolumab Plus Ipilimumab in Melanoma.** *JAMA Oncol* 2018, **4:**98-101.

206. Kostine M, Rouxel L, Barnetche T, Veillon R, Martin F, Dutriaux C, Dousset L, Pham-Ledard A, Prey S, Beylot-Barry M, et al: **Rheumatic disorders associated with immune checkpoint inhibitors in patients with cancer-clinical aspects and relationship with tumour response: a single-centre prospective cohort study.** *Ann Rheum Dis* 2018, **77:**393-398.

207. Tetzlaff MT, Nelson KC, Diab A, Staerkel GA, Nagarajan P, Torres-Cabala CA, Chasen BA, Wargo JA, Prieto VG, Amaria RN, Curry JL: **Granulomatous/sarcoid-like lesions associated with checkpoint inhibitors: a marker of therapy response in a subset of melanoma patients.** *J Immunother Cancer* 2018, **6:**14.

208. Tsimberidou AM, Levit LA, Schilsky RL, Averbuch SD, Chen D, Kirkwood JM, McShane LM, Sharon E, Mileham KF, Postow MA: **Trial Reporting in Immuno-Oncology (TRIO): an American society of clinical oncology-society for immunotherapy of cancer statement.** *J Immunother Cancer* 2018, **6:**108.

209. Iwama S, De Remigis A, Callahan MK, Slovin SF, Wolchok JD, Caturegli P: **Pituitary expression of CTLA-4 mediates hypophysitis secondary to administration of CTLA-4 blocking antibody.** *Sci Transl Med* 2014, **6:**230ra245.
210. Menzies AM, Johnson DB, Ramanujam S, Atkinson VG, Wong ANM, Park JJ, McQuade JL, Shoushtari AN, Tsai KK, Eroglu Z, et al: **Anti-PD-1 therapy in patients with advanced melanoma and preexisting autoimmune disorders or major toxicity with ipilimumab.** *Ann Oncol* 2017, **28:**368-376.
211. Berner F, Bomze D, Diem S, Ali OH, Fassler M, Ring S, Niederer R, Ackermann CJ, Baumgaertner P, Pikor N, et al: **Association of Checkpoint Inhibitor-Induced Toxic Effects With Shared Cancer and Tissue Antigens in Non-Small Cell Lung Cancer.** *JAMA Oncol* 2019, **5:**1043-1047.
212. Johnson DB, Balko JM, Compton ML, Chalkias S, Gorham J, Xu Y, Hicks M, Puzanov I, Alexander MR, Bloomer TL, et al: **Fulminant Myocarditis with Combination Immune Checkpoint Blockade.** *N Engl J Med* 2016, **375:**1749-1755.
213. Haratani K, Hayashi H, Chiba Y, Kudo K, Yonesaka K, Kato R, Kaneda H, Hasegawa Y, Tanaka K, Takeda M, Nakagawa K: **Association of Immune-Related Adverse Events With Nivolumab Efficacy in Non-Small-Cell Lung Cancer.** *JAMA oncology* 2018, **4:**374-378.
214. Ricciuti B, Genova C, De Giglio A, Bassanelli M, Dal Bello MG, Metro G, Brambilla M, Baglivo S, Grossi F, Chiari R: **Impact of immune-related adverse events on survival in patients with advanced non-small cell lung cancer treated with nivolumab: long-term outcomes from a multi-institutional analysis.** *J Cancer Res Clin Oncol* 2019, **145:**479-485.
215. Indini A, Di Guardo L, Cimminiello C, Prisciandaro M, Randon G, De Braud F, Del Vecchio M: **Immune-related adverse events correlate with improved survival in patients undergoing anti-PD1 immunotherapy for metastatic melanoma.** *J Cancer Res Clin Oncol* 2019, **145:**511-521.
216. Maher VE, Fernandes LL, Weinstock C, Tang S, Agarwal S, Brave M, Ning YM, Singh H, Suzman D, Xu J, et al: **Analysis of the Association Between Adverse Events and Outcome in Patients Receiving a Programmed Death Protein 1 or Programmed Death Ligand 1 Antibody.** *J Clin Oncol* 2019, **37:**2730-2737.
217. Arbour KC, Mezquita L, Long N, Rizvi H, Auclin E, Ni A, Martinez-Bernal G, Ferrara R, Lai WV, Hendriks LEL, et al: **Impact of Baseline Steroids on Efficacy of Programmed Cell Death-1 and Programmed Death-Ligand 1 Blockade in Patients With Non-Small-Cell Lung Cancer.** *J Clin Oncol* 2018, **36:**2872-2878.
218. Ricciuti B, Dahlberg SE, Adeni A, Sholl LM, Nishino M, Awad MM: **Immune Checkpoint Inhibitor Outcomes for Patients With Non-Small-Cell Lung Cancer Receiving Baseline Corticosteroids for Palliative Versus Nonpalliative Indications.** *J Clin Oncol* 2019, **37:**1927-1934.
219. Faje AT, Lawrence D, Flaherty K, Freedman C, Fadden R, Rubin K, Cohen J, Sullivan RJ: **High-dose glucocorticoids for the treatment of ipilimumab-induced hypophysitis is associated with reduced survival in patients with melanoma.** *Cancer* 2018, **124:**3706-3714.
220. Yang JC: **Toxicities Associated With Adoptive T-Cell Transfer for Cancer.** *Cancer J* 2015, **21:**506-509.
221. Shimabukuro-Vornhagen A, Godel P, Subklewe M, Stemmler HJ, Schlosser HA, Schlaak M, Kochanek M, Boll B, von Bergwelt-Baildon MS: **Cytokine release syndrome.** *J Immunother Cancer* 2018, **6:**56.
222. Mort RL, Jackson IJ, Patton EE: **The melanocyte lineage in development and disease.** *Development* 2015, **142:**1387.
223. Aoki H, Yamada Y, Hara A, Kunisada T: **Two distinct types of mouse melanocyte: differential signaling requirement for the maintenance of non-cutaneous and dermal versus epidermal melanocytes.** *Development* 2009, **136:**2511-2521.
224. Chang SH, Worley LA, Onken MD, Harbour JW: **Prognostic biomarkers in uveal melanoma: evidence for a stem cell-like phenotype associated with metastasis.** *Melanoma Res* 2008, **18:**191-200.
225. Damato B: **Treatment of primary intraocular melanoma.** *Expert Rev Anticancer Ther* 2006, **6:**493-506.
226. Griewank KG, Westekemper H, Murali R, Mach M, Schilling B, Wiesner T, Schimming T, Livingstone E, Sucker A, Grabellus F, et al: **Conjunctival melanomas harbor BRAF and**

NRAS mutations and copy number changes similar to cutaneous and mucosal melanomas. *Clin Cancer Res* 2013, **19:**3143-3152.

227. Bergman L, Seregard S, Nilsson B, Ringborg U, Lundell G, Ragnarsson-Olding B: **Incidence of uveal melanoma in Sweden from 1960 to 1998.** *Invest Ophthalmol Vis Sci* 2002, **43:**2579-2583.

228. Nayman T, Bostan C, Logan P, Burnier MN, Jr.: **Uveal Melanoma Risk Factors: A Systematic Review of Meta-Analyses.** *Curr Eye Res* 2017, **42:**1085-1093.

229. Eskelin S, Kivelä T: **Mode of presentation and time to treatment of uveal melanoma in Finland.** *Br J Ophthalmol* 2002**:**333–338.

230. Virgili G, Gatta G, Ciccolallo L, Capocaccia R, Biggeri A, Crocetti E, Lutz J-MM, Paci E, Group E: **Incidence of uveal melanoma in Europe.** *Ophthalmology* 2007, **114:**2309-2315.

231. Moan J, Cicarma E, Setlow R, Porojnicu AC, Grant WB, Juzeniene A: **Time trends and latitude dependence of uveal and cutaneous malignant melanoma induced by solar radiation.** *Dermatoendocrinol* 2010, **2:**3-8.

232. Yu GP, Hu DN, McCormick SA: **Latitude and incidence of ocular melanoma.** *Photochem Photobiol* 2006, **82:**1621-1626.

233. Shah CP, Weis E, Lajous M, Shields JA, Shields CL: **Intermittent and chronic ultraviolet light exposure and uveal melanoma: a meta-analysis.** *Ophthalmology* 2005, **112:**1599-1607.

234. Royer-Bertrand B, Torsello M, Rimoldi D, El Zaoui I, Cisarova K, Pescini-Gobert R, Raynaud F, Zografos L, Schalenbourg A, Speiser D, et al: **Comprehensive Genetic Landscape of Uveal Melanoma by Whole-Genome Sequencing.** *The American Journal of Human Genetics* 2016.

235. Karlsson J, Nilsson LM, Forsberg EMV, Mitra S, Alsén S, Shelke G, Sah VR, Stierner U, All-Eriksson C, Einarsdottir BO, et al: **Molecular profiling of driver events and tumor-infiltrating lymphocytes in metastatic uveal melanoma.** Preprint: bioRxiv 742023; 2019.

236. Nasti TH, Timares L: **MC1R, eumelanin and pheomelanin: their role in determining the susceptibility to skin cancer.** *Photochem Photobiol* 2015, **91:**188-200.

237. Collaborative Ocular Melanoma Study G: **The COMS randomized trial of iodine 125 brachytherapy for choroidal melanoma: V. Twelve-year mortality rates and prognostic factors: COMS report No. 28.** *Arch Ophthalmol* 2006, **124:**1684-1693.

238. Kujala E, Makitie T, Kivela T: **Very long-term prognosis of patients with malignant uveal melanoma.** *Invest Ophthalmol Vis Sci* 2003, **44:**4651-4659.

239. **Factors Predictive of Growth and Treatment of Small Choroidal Melanoma: COMS Report No. 5.** *Archives of Ophthalmology* 1997, **115:**1537-1544.

240. Prescher G, Bornfeld N, Hirche H, Horsthemke B, Jockel KH, Becher R: **Prognostic implications of monosomy 3 in uveal melanoma.** *Lancet* 1996, **347:**1222-1225.

241. White VA, Chambers JD, Courtright PD, Chang WY, Horsman DE: **Correlation of cytogenetic abnormalities with the outcome of patients with uveal melanoma.** *Cancer* 1998, **83:**354-359.

242. Tschentscher F, Husing J, Holter T, Kruse E, Dresen IG, Jockel KH, Anastassiou G, Schilling H, Bornfeld N, Horsthemke B, et al: **Tumor classification based on gene expression profiling shows that uveal melanomas with and without monosomy 3 represent two distinct entities.** *Cancer Res* 2003, **63:**2578-2584.

243. Onken MD, Worley LA, Ehlers JP, Harbour JW: **Gene expression profiling in uveal melanoma reveals two molecular classes and predicts metastatic death.** *Cancer Res* 2004, **64:**7205-7209.

244. Onken MD, Worley LA, Tuscan MD, Harbour JW: **An accurate, clinically feasible multi-gene expression assay for predicting metastasis in uveal melanoma.** *J Mol Diagn* 2010, **12:**461-468.

245. Olofsson R, Cahlin C, All-Ericsson C, Hashimi F, Mattsson J, Rizell M, Lindner P: **Isolated hepatic perfusion for ocular melanoma metastasis: registry data suggests a survival benefit.** *Ann Surg Oncol* 2014, **21:**466-472.

246. Olofsson R, Ny L, Eilard M, Rizell M, Cahlin C, Stierner U, Lönn U, Hansson J, Ljuslinder I, Lundgren L, et al: **Isolated hepatic perfusion as a treatment for uveal melanoma liver metastases (the SCANDIUM trial): study protocol for a randomized controlled trial.** *Trials* 2014, **15:**1-6.

247. Diener-West M, Reynolds SM, Agugliaro DJ, Caldwell R, Cumming K, Earle JD, Hawkins BS, Hayman JA, Jaiyesimi I, Jampol LM: **Development of metastatic disease after enrollment**

in the COMS trials for treatment of choroidal melanoma: Collaborative Ocular Melanoma Study Group Report No. 26. *Archives of ophthalmology (Chicago, Ill: 1960)* 2005, **123:**1639-1643.

248. Khoja L, Atenafu EG, Suciu S, Leyvraz S, Sato T, Marshall E, Keilholz U, Zimmer L, Patel SP, Piperno-Neumann S, et al: **Meta-Analysis in Metastatic Uveal Melanoma to Determine Progression-Free and Overall Survival Benchmarks: an International Rare Cancers Initiative (IRCI) Ocular Melanoma study.** *Ann Oncol* 2019.

249. Van Raamsdonk CD, Griewank KG, Crosby MB, Garrido MC, Vemula S, Wiesner T, Obenauf AC, Wackernagel W, Green G, Bouvier N, et al: **Mutations in GNA11 in uveal melanoma.** *N Engl J Med* 2010, **363:**2191-2199.

250. Van Raamsdonk CD, Bezrookove V, Green G, Bauer J, Gaugler L, O'Brien JM, Simpson EM, Barsh GS, Bastian BC: **Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi.** *Nature* 2009, **457:**599-602.

251. Johansson P, Aoude LG, Wadt K, Glasson WJ, Warrier SK, Hewitt AW, Kiilgaard JF, Heegaard S, Isaacs T, Franchina M, et al: **Deep sequencing of uveal melanoma identifies a recurrent mutation in PLCB4.** *Oncotarget* 2016, **7:**4624-4631.

252. Moore AR, Ceraudo E, Sher JJ, Guan Y, Shoushtari AN, Chang MT, Zhang JQ, Walczak EG, Kazmi MA, Taylor BS, et al: **Recurrent activating mutations of G-protein-coupled receptor CYSLTR2 in uveal melanoma.** *Nat Genet* 2016, **48:**675-680.

253. Onken MD, Worley LA, Long MD, Duan S, Council ML, Bowcock AM, Harbour JW: **Oncogenic mutations in GNAQ occur early in uveal melanoma.** *Invest Ophthalmol Vis Sci* 2008, **49:**5230-5234.

254. Shain AH, Bagger MM, Yu R, Chang D, Liu S, Vemula S, Weier JF, Wadt K, Heegaard S, Bastian BC, Kiilgaard JF: **The genetic evolution of metastatic uveal melanoma.** *Nat Genet* 2019, **51:**1123-1130.

255. Ambrosini G, Pratilas CA, Qin LX, Tadi M, Surriga O, Carvajal RD, Schwartz GK: **Identification of unique MEK-dependent genes in GNAQ mutant uveal melanoma involved in cell growth, tumor cell invasion, and MEK resistance.** *Clin Cancer Res* 2012, **18:**3552-3561.

256. Carvajal RD, Sosman JA, Quevedo JF, Milhem MM, Joshua AM, Kudchadkar RR, Linette GP, Gajewski TF, Lutzky J, Lawson DH, et al: **Effect of selumetinib vs chemotherapy on progression-free survival in uveal melanoma: a randomized clinical trial.** *JAMA* 2014, **311:**2397-2405.

257. Carvajal RD, Piperno-Neumann S, Kapiteijn E, Chapman PB, Frank S, Joshua AM, Piulats JM, Wolter P, Cocquyt V, Chmielowski B, et al: **Selumetinib in Combination With Dacarbazine in Patients With Metastatic Uveal Melanoma: A Phase III, Multicenter, Randomized Trial (SUMIT).** *J Clin Oncol* 2018, **36:**1232-1239.

258. Piperno-Neumann S, Kapiteijn E, Larkin JMG, Carvajal RD, Luke JJ, Seifert H, Roozen I, Zoubir M, Yang L, Choudhury S, et al: **Phase I dose-escalation study of the protein kinase C (PKC) inhibitor AEB071 in patients with metastatic uveal melanoma.** *Journal of Clinical Oncology* 2014, **32:**9030-9030.

259. Kapiteijn E, Carlino M, Boni V, Loirat D, Speetjens F, Park J, Calvo E, Carvajal R, Nyakas M, Gonzalez-Maffe J, et al: **Abstract CT068: A Phase I trial of LXS196, a novel PKC inhibitor for metastatic uveal melanoma.** *Cancer Research* 2019, **79:**CT068-CT068.

260. Saraiva VS, Caissie AL, Segal L, Edelstein C, Burnier MN, Jr.: **Immunohistochemical expression of phospho-Akt in uveal melanoma.** *Melanoma Res* 2005, **15:**245-250.

261. Populo H, Soares P, Rocha AS, Silva P, Lopes JM: **Evaluation of the mTOR pathway in ocular (uvea and conjunctiva) melanoma.** *Melanoma Res* 2010, **20:**107-117.

262. Abdel-Rahman MH, Yang Y, Zhou XP, Craig EL, Davidorf FH, Eng C: **High frequency of submicroscopic hemizygous deletion is a major mechanism of loss of expression of PTEN in uveal melanoma.** *J Clin Oncol* 2006, **24:**288-295.

263. All-Ericsson C, Girnita L, Muller-Brunotte A, Brodin B, Seregard S, Ostman A, Larsson O: **c-Kit-dependent growth of uveal melanoma cells: a potential therapeutic target?** *Invest Ophthalmol Vis Sci* 2004, **45:**2075-2082.

264. Gardner FP, Serie DJ, Salomao DR, Wu KJ, Markovic SN, Pulido JS, Joseph RW: **c-MET expression in primary and liver metastases in uveal melanoma.** *Melanoma Res* 2014, **24:**617-620.

265. Yoshida M, Selvan S, McCue PA, DeAngelis T, Baserga R, Fujii A, Rui H, Mastrangelo MJ, Sato T: **Expression of insulin-like growth factor-1 receptor in metastatic uveal melanoma and implications for potential autocrine and paracrine tumor cell growth.** *Pigment Cell Melanoma Res* 2014, **27:**297-308.

266. Shoushtari AN, Ong LT, Schoder H, Singh-Kandah S, Abbate KT, Postow MA, Callahan MK, Wolchok J, Chapman PB, Panageas KS, et al: **A phase 2 trial of everolimus and pasireotide long-acting release in patients with metastatic uveal melanoma.** *Melanoma Res* 2016, **26:**272-277.

267. Khalili JS, Yu X, Wang J, Hayes BC, Davies MA, Lizee G, Esmaeli B, Woodman SE: **Combination small molecule MEK and PI3K inhibition enhances uveal melanoma cell death in a mutant GNAQ- and GNA11-dependent manner.** *Clin Cancer Res* 2012, **18:**4345-4355.

268. Shoushtari AN, Kudchadkar RR, Panageas K, Murthy RK, Jung M, Shah R, O'Donnell B, Khawaja TT, Shames Y, Prempeh-Keteku NA, et al: **A randomized phase 2 study of trametinib with or without GSK2141795 in patients with advanced uveal melanoma.** *Journal of Clinical Oncology* 2016, **34:**9511-9511.

269. Feng X, Degese MS, Iglesias-Bartolome R, Vaque JP, Molinolo AA, Rodrigues M, Zaidi MR, Ksander BR, Merlino G, Sodhi A, et al: **Hippo-independent activation of YAP by the GNAQ uveal melanoma oncogene through a trio-regulated rho GTPase signaling circuitry.** *Cancer Cell* 2014, **25:**831-845.

270. Feng X, Arang N, Rigiracciolo DC, Lee JS, Yeerna H, Wang Z, Lubrano S, Kishore A, Pachter JA, Konig GM, et al: **A Platform of Synthetic Lethal Gene Interaction Networks Reveals that the GNAQ Uveal Melanoma Oncogene Controls the Hippo Pathway through FAK.** *Cancer Cell* 2019, **35:**457-472 e455.

271. Flaherty KT, Robert C, Hersey P, Nathan P, Garbe C, Milhem M, Demidov LV, Hassel JC, Rutkowski P, Mohr P, et al: **Improved survival with MEK inhibition in BRAF-mutated melanoma.** *N Engl J Med* 2012, **367:**107-114.

272. Annala S, Feng X, Shridhar N, Eryilmaz F, Patt J, Yang J, Pfeil EM, Cervantes-Villagrana RD, Inoue A, Haberlein F, et al: **Direct targeting of Galphaq and Galpha11 oncoproteins in cancer cells.** *Sci Signal* 2019, **12**.

273. Lapadula D, Farias E, Randolph CE, Purwin TJ, McGrath D, Charpentier TH, Zhang L, Wu S, Terai M, Sato T, et al: **Effects of Oncogenic Galphaq and Galpha11 Inhibition by FR900359 in Uveal Melanoma.** *Mol Cancer Res* 2019, **17:**963-973.

274. Harbour JW, Onken MD, Roberson ED, Duan S, Cao L, Worley LA, Council ML, Matatall KA, Helms C, Bowcock AM: **Frequent mutation of BAP1 in metastasizing uveal melanomas.** *Science* 2010, **330:**1410-1413.

275. Robertson AG, Shih J, Yau C, Gibb EA, Oba J, Mungall KL, Hess JM, Uzunangelov V, Walter V, Danilova L, et al: **Integrative Analysis Identifies Four Molecular and Clinical Subsets in Uveal Melanoma.** *Cancer Cell* 2017, **32:**204-220 e215.

276. Field MG, Kuznetsov JN, Bussies PL, Cai LZ, Alawa KA, Decatur CL, Kurtenbach S, Harbour JW: **BAP1 Loss Is Associated with DNA Methylomic Repatterning in Highly Aggressive Class 2 Uveal Melanomas.** *Clinical Cancer Research* 2019, **25:**5663-5673.

277. Harbour JW, Roberson ED, Anbunathan H, Onken MD, Worley LA, Bowcock AM: **Recurrent mutations at codon 625 of the splicing factor SF3B1 in uveal melanoma.** *Nat Genet* 2013, **45:**133-135.

278. Martin M, Masshofer L, Temming P, Rahmann S, Metz C, Bornfeld N, van de Nes J, Klein-Hitpass L, Hinnebusch AG, Horsthemke B, et al: **Exome sequencing identifies recurrent somatic mutations in EIF1AX and SF3B1 in uveal melanoma with disomy 3.** *Nat Genet* 2013, **45:**933-936.

279. Niederkorn JY: **Ocular immune privilege and ocular melanoma: parallel universes or immunological plagiarism?** *Front Immunol* 2012, **3:**148.

280. Ericsson C, Seregard S, Bartolazzi A, Levitskaya E, Ferrone S, Kiessling R, Larsson O: **Association of HLA class I and class II antigen expression and mortality in uveal melanoma.** *Invest Ophthalmol Vis Sci* 2001, **42:**2153-2156.

281. Whelchel JC, Farah SE, McLean IW, Burnier MN: **Immunohistochemistry of infiltrating lymphocytes in uveal malignant melanoma.** *Invest Ophthalmol Vis Sci* 1993, **34:**2603-2606.

282. Wu K, Kryczek I, Chen L, Zou W, Welling TH: **Kupffer cell suppression of CD8+ T cells in human hepatocellular carcinoma is mediated by B7-H1/programmed death-1 interactions.** *Cancer Res* 2009, **69:**8067-8075.
283. Vetter CS, Lieb W, Brocker EB, Becker JC: **Loss of nonclassical MHC molecules MIC-A/B expression during progression of uveal melanoma.** *Br J Cancer* 2004, **91:**1495-1499.
284. Chandran SS, Somerville RPT, Yang JC, Sherry RM, Klebanoff CA, Goff SL, Wunderlich JR, Danforth DN, Zlott D, Paria BC, et al: **Treatment of metastatic uveal melanoma with adoptive transfer of tumour-infiltrating lymphocytes: a single-centre, two-stage, single-arm, phase 2 study.** *Lancet Oncol* 2017, **18:**792-802.
285. Javed A, Arguello D, Johnston C, Gatalica Z, Terai M, Weight RM, Orloff M, Mastrangelo MJ, Sato T: **PD-L1 expression in tumor metastasis is different between uveal melanoma and cutaneous melanoma.** *Immunotherapy* 2017, **9:**1323-1330.
286. Algazi AP, Tsai KK, Shoushtari AN, Munhoz RR, Eroglu Z, Piulats JM, Ott PA, Johnson DB, Hwang J, Daud AI, et al: **Clinical outcomes in metastatic uveal melanoma treated with PD-1 and PD-1L antibodies.** *Cancer* 2016, **122:**3344-3353.
287. Heppt MV, Heinzerling L, Kahler KC, Forschner A, Kirchberger MC, Loquai C, Meissner M, Meier F, Terheyden P, Schell B, et al: **Prognostic factors and outcomes in metastatic uveal melanoma treated with programmed cell death-1 or combined PD-1/cytotoxic T-lymphocyte antigen-4 inhibition.** *Eur J Cancer* 2017, **82:**56-65.
288. Mignard C, Deschamps Huvier A, Gillibert A, Duval Modeste AB, Dutriaux C, Khammari A, Avril MF, Kramkimel N, Mortier L, Marcant P, et al: **Efficacy of Immunotherapy in Patients with Metastatic Mucosal or Uveal Melanoma.** *J Oncol* 2018, **2018:**1908065.
289. Piulats Rodriguez JM, De La Cruz Merino L, Espinosa E, Alonso Carrión L, Martin Algarra S, López-Castro R, Curiel García MT, Rodriguez Abreu D, Rullan Iriarte AJ, Berrocal Jaime A: **Phase II multicenter, single arm, open label study of nivolumab in combination with ipilimumab in untreated patients with metastatic uveal melanoma (GEM1402.NCT02626962).** In *ESMO 2018*: Annals of Oncology, Volume 29, Issue suppl_8; 2018.
290. Holliday R: **The inheritance of epigenetic defects.** *Science* 1987, **238:**163-170.
291. Anastasiadou E, Jacob LS, Slack FJ: **Non-coding RNA networks in cancer.** *Nat Rev Cancer* 2018, **18:**5-18.
292. Reddy KL, Feinberg AP: **Higher order chromatin organization in cancer.** *Semin Cancer Biol* 2013, **23:**109-115.
293. Greenberg MVC, Bourc'his D: **The diverse roles of DNA methylation in mammalian development and disease.** *Nat Rev Mol Cell Biol* 2019, **20:**590-607.
294. Feinberg AP: **The Key Role of Epigenetics in Human Disease Prevention and Mitigation.** *N Engl J Med* 2018, **378:**1323-1334.
295. Seto E, Yoshida M: **Erasers of histone acetylation: the histone deacetylase enzymes.** *Cold Spring Harb Perspect Biol* 2014, **6:**a018713.
296. Filippakopoulos P, Picaud S, Mangos M, Keates T, Lambert JP, Barsyte-Lovejoy D, Felletar I, Volkmer R, Muller S, Pawson T, et al: **Histone recognition and large-scale structural analysis of the human bromodomain family.** *Cell* 2012, **149:**214-231.
297. Xu Y, Vakoc CR: **Targeting Cancer Cells with BET Bromodomain Inhibitors.** *Cold Spring Harb Perspect Med* 2017, **7**.
298. Feinberg AP, Koldobskiy MA, Genetics G-A: **Epigenetic modulators, modifiers and mediators in cancer aetiology and progression.** *Nature Reviews Genetics* 2016.
299. Mack SC, Witt H, Piro RM, Gu L, Zuyderduyn S, Stutz AM, Wang X, Gallo M, Garzia L, Zayne K, et al: **Epigenomic alterations define lethal CIMP-positive ependymomas of infancy.** *Nature* 2014, **506:**445-450.
300. Pugh TJ, Weeraratne SD, Archer TC, Pomeranz Krummel DA, Auclair D, Bochicchio J, Carneiro MO, Carter SL, Cibulskis K, Erlich RL, et al: **Medulloblastoma exome sequencing uncovers subtype-specific somatic mutations.** *Nature* 2012, **488:**106-110.
301. Michalak EM, Burr ML, Bannister AJ, Dawson MA: **The roles of DNA, RNA and histone methylation in ageing and cancer.** *Nat Rev Mol Cell Biol* 2019, **20:**573-589.
302. Silverman LR, Demakos EP, Peterson BL, Kornblith AB, Holland JC, Odchimar-Reissig R, Stone RM, Nelson D, Powell BL, DeCastro CM, et al: **Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the cancer and leukemia group B.** *J Clin Oncol* 2002, **20:**2429-2440.

303. Hoglund A, Nilsson LM, Forshell LP, Maclean KH, Nilsson JA: **Myc sensitizes p53-deficient cancer cells to the DNA-damaging effects of the DNA methyltransferase inhibitor decitabine.** *Blood* 2009, **113:**4281-4288.
304. Eden A, Gaudet F, Waghmare A, Jaenisch R: **Chromosomal instability and tumors promoted by DNA hypomethylation.** *Science* 2003, **300:**455.
305. Gryder BE, Pomella S, Sayers C, Wu XS, Song Y, Chiarella AM, Bagchi S, Chou H-C, Sinniah RS, Walton A, et al: **Histone hyperacetylation disrupts core gene regulatory architecture in rhabdomyosarcoma.** *Nature Genetics* 2019, **51:**1714-1722.
306. Tomasi TB, Magner WJ, Khan NAH: **Epigenetic regulation of immune escape genes in cancer.** *Cancer Immunology, Immunotherapy* 2006, **55:**1159-1184.
307. West AC, Johnstone RW: **New and emerging HDAC inhibitors for cancer treatment.** *The Journal of clinical investigation* 2014, **124:**30-39.
308. Li Y, Seto E: **HDACs and HDAC Inhibitors in Cancer Development and Therapy.** *Cold Spring Harb Perspect Med* 2016, **6**.
309. Landreville S, Agapova OA, Matatall KA, Kneass ZT, Onken MD, Lee RS, Bowcock AM, Harbour WJ: **Histone Deacetylase Inhibitors Induce Growth Arrest and Differentiation in Uveal Melanoma.** *Clinical Cancer Research* 2012, **18:**408-416.
310. Haas NB, Quirt I, Hotte S, McWhirter E, Polintan R, Litwin S, Adams PD, McBryan T, Wang L, Martin LP, et al: **Phase II trial of vorinostat in advanced melanoma.** *Invest New Drugs* 2014, **32:**526-534.
311. Weintraub K: **Take two: Combining immunotherapy with epigenetic drugs to tackle cancer.** *Nature Medicine* 2016, **22**.
312. Busslinger M, Tarakhovsky A: **Epigenetic control of immunity.** *Cold Spring Harb Perspect Biol* 2014, **6**.
313. Philip M, Fairchild L, Sun L, Horste EL, Camara S, Shakiba M, Scott AC, Viale A, Lauer P, Merghoub T, et al: **Chromatin states define tumour-specific T cell dysfunction and reprogramming.** *Nature* 2017, **545:**452-456.
314. Adcock IM: **HDAC inhibitors as anti-inflammatory agents.** *Br J Pharmacol* 2007, **150:**829-831.
315. Kelly-Sell MJ, Kim YH, Straus S, Benoit B, Harrison C, Sutherland K, Armstrong R, Weng WK, Showe LC, Wysocka M, Rook AH: **The histone deacetylase inhibitor, romidepsin, suppresses cellular immune functions of cutaneous T-cell lymphoma patients.** *Am J Hematol* 2012, **87:**354-360.
316. Wang D, Iclozan C, Liu C, Xia C, Anasetti C, Yu XZ: **LBH589 enhances T cell activation in vivo and accelerates graft-versus-host disease in mice.** *Biol Blood Marrow Transplant* 2012, **18:**1182-1190 e1181.
317. Bridle BW, Chen L, Lemay CG, Diallo JS, Pol J, Nguyen A, Capretta A, He R, Bramson JL, Bell JC, et al: **HDAC inhibition suppresses primary immune responses, enhances secondary immune responses, and abrogates autoimmunity during tumor immunotherapy.** *Mol Ther* 2013, **21:**887-894.
318. Kim K, Skora AD, Li Z, Liu Q, Tam AJ, Blosser RL, Diaz LA, Jr., Papadopoulos N, Kinzler KW, Vogelstein B, Zhou S: **Eradication of metastatic mouse cancers resistant to immune checkpoint blockade by suppression of myeloid-derived cells.** *Proc Natl Acad Sci U S A* 2014, **111:**11774-11779.
319. Shen L, Pili R: **Class I histone deacetylase inhibition is a novel mechanism to target regulatory T cells in immunotherapy.** *Oncoimmunology* 2012, **1:**948-950.
320. Zhu S, Denman CJ, Cobanoglu ZS, Kiany S, Lau CC, Gottschalk SM, Hughes DP, Kleinerman ES, Lee DA: **The narrow-spectrum HDAC inhibitor entinostat enhances NKG2D expression without NK cell toxicity, leading to enhanced recognition of cancer cells.** *Pharm Res* 2015, **32:**779-792.
321. Peng D, Kryczek I, Nagarsheth N, Zhao L, Wei S, Wang W, Sun Y, Zhao E, Vatan L, Szeliga W, et al: **Epigenetic silencing of TH1-type chemokines shapes tumour immunity and immunotherapy.** *Nature* 2015, **527:**249-253.
322. Kassiotis G, Stoye JP: **Immune responses to endogenous retroelements: taking the bad with the good.** *Nat Rev Immunol* 2016, **16:**207-219.
323. Ritter C, Fan K, Paschen A, Reker Hardrup S, Ferrone S, Nghiem P, Ugurel S, Schrama D, Becker JC: **Epigenetic priming restores the HLA class-I antigen processing machinery expression in Merkel cell carcinoma.** *Sci Rep* 2017, **7:**2290.

324. Chiappinelli KB, Strissel PL, Desrichard A, Li H, Henke C, Akman B, Hein A, Rote NS, Cope LM, Snyder A, et al: **Inhibiting DNA Methylation Causes an Interferon Response in Cancer via dsRNA Including Endogenous Retroviruses.** *Cell* 2015, **162:**974-986.

325. Campoli M, Ferrone S: **HLA antigen changes in malignant cells: epigenetic mechanisms and biologic significance.** *Oncogene* 2008, **27:**5869-5885.

326. Lee JH, Choy ML, Ngo L, Foster SS, Marks PA: **Histone deacetylase inhibitor induces DNA damage, which normal but not transformed cells can repair.** *Proceedings of the National Academy of Sciences of the United States of America* 2010, **107:**14639-14644.

327. Landreville S, Agapova OA, Matatall KA, Kneass ZT, Onken MD, Lee RS, Bowcock AM, Harbour WJ: **Histone Deacetylase Inhibitors Induce Growth Arrest and Differentiation in Uveal Melanoma.** *Clin Cancer Res* 2012, **18:**408-416.

328. Gray JE, Saltos A, Tanvetyanon T, Haura EB, Creelan B, Antonia SJ, Shafique M, Zheng H, Dai W, Saller JJ, et al: **Phase I/Ib Study of Pembrolizumab Plus Vorinostat in Advanced/Metastatic Non-Small Cell Lung Cancer.** *Clin Cancer Res* 2019, **25:**6623-6632.

329. Bhadury J, Nilsson LM, Muralidharan SV, Green LC, Li Z, Gesner EM, Hansen HC, Keller UB, McLure KG, Nilsson JA: **BET and HDAC inhibitors induce similar genes and biological effects and synergize to kill in Myc-induced murine lymphoma.** *Proc Natl Acad Sci U S A* 2014, **111:**E2721-2730.

330. Adeegbe DO, Liu S, Hattersley MM, Bowden M, Zhou CW, Li S, Vlahos R, Grondine M, Dolgalev I, Ivanova EV, et al: **BET Bromodomain Inhibition Cooperates with PD-1 Blockade to Facilitate Antitumor Response in Kras-Mutant Non-Small Cell Lung Cancer.** *Cancer Immunol Res* 2018, **6:**1234-1245.

331. Erkes DA, Field CO, Capparelli C, Tiago M, Purwin TJ, Chervoneva I, Berger AC, Hartsough EJ, Villanueva J, Aplin AE: **The next-generation BET inhibitor, PLX51107, delays melanoma growth in a CD8-mediated manner.** *Pigment Cell Melanoma Res* 2019, **32:**687-696.

332. Kersten K, de Visser KE, van Miltenburg MH, Jonkers J: **Genetically engineered mouse models in oncology research and cancer medicine.** *EMBO Mol Med* 2017, **9:**137-153.

333. Bhadury J, Einarsdottir BO, Podraza A, Bagge RO, Stierner U, Ny L, Davila Lopez M, Nilsson JA: **Hypoxia-regulated gene expression explains differences between melanoma cell line-derived xenografts and patient-derived xenografts.** *Oncotarget* 2016, **7:**23801-23811.

334. Ito M, Hiramatsu H, Kobayashi K, Suzue K, Kawahata M, Hioki K, Ueyama Y, Koyanagi Y, Sugamura K, Tsuji K, et al: **NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for engraftment of human cells.** *Blood* 2002, **100:**3175-3182.

335. Hidalgo M, Amant F, Biankin AV, Budinska E, Byrne AT, Caldas C, Clarke RB, de Jong S, Jonkers J, Maelandsmo GM, et al: **Patient-derived xenograft models: an emerging platform for translational cancer research.** *Cancer Discov* 2014, **4:**998-1013.

336. Quintana E, Shackleton M, Sabel MS, Fullen DR, Johnson TM, Morrison SJ: **Efficient tumour formation by single human melanoma cells.** *Nature* 2008, **456:**593-598.

337. Einarsdottir BO, Bagge RO, Bhadury J, Jespersen H, Mattsson J, Nilsson LM, Truve K, Lopez MD, Naredi P, Nilsson O, et al: **Melanoma patient-derived xenografts accurately model the disease and develop fast enough to guide treatment decisions.** *Oncotarget* 2014, **5:**9609-9618.

338. Girotti MR, Gremel G, Lee R, Galvani E, Rothwell D, Viros A, Mandal AK, Lim KH, Saturno G, Furney SJ, et al: **Application of Sequencing, Liquid Biopsies, and Patient-Derived Xenografts for Personalized Medicine in Melanoma.** *Cancer Discov* 2016, **6:**286-299.

339. Ben-David U, Ha G, Tseng YY, Greenwald NF, Oh C, Shih J, McFarland JM, Wong B, Boehm JS, Beroukhim R, Golub TR: **Patient-derived xenografts undergo mouse-specific tumor evolution.** *Nat Genet* 2017, **49:**1567-1575.

340. Hylander BL, Punt N, Tang H, Hillman J, Vaughan M, Bshara W, Pitoniak R, Repasky EA: **Origin of the vasculature supporting growth of primary patient tumor xenografts.** *J Transl Med* 2013, **11:**110.

341. Blomme A, Van Simaeys G, Doumont G, Costanza B, Bellier J, Otaka Y, Sherer F, Lovinfosse P, Boutry S, Palacios AP, et al: **Murine stroma adopts a human-like metabolic phenotype in the PDX model of colorectal cancer and liver metastases.** *Oncogene* 2018, **37:**1237-1250.

342. Choi Y, Lee S, Kim K, Kim SH, Chung YJ, Lee C: **Studying cancer immunotherapy using patient-derived xenografts (PDXs) in humanized mice.** *Exp Mol Med* 2018, **50:**99.

343. Allen TM, Brehm MA, Bridges S, Ferguson S, Kumar P, Mirochnitchenko O, Palucka K, Pelanda R, Sanders-Beer B, Shultz LD, et al: **Humanized immune system mouse models: progress, challenges and opportunities.** *Nat Immunol* 2019, **20:**770-774.

344. Huang JL, Urtatiz O, Van Raamsdonk CD: **Oncogenic G Protein GNAQ Induces Uveal Melanoma and Intravasation in Mice.** *Cancer Res* 2015, **75:**3384-3397.

345. Moore AR, Ran L, Guan Y, Sher JJ, Hitchman TD, Zhang JQ, Hwang C, Walzak EG, Shoushtari AN, Monette S, et al: **GNA11 Q209L Mouse Model Reveals RasGRP3 as an Essential Signaling Node in Uveal Melanoma.** *Cell Rep* 2018, **22:**2455-2468.

346. Nemati F, Sastre-Garau X, Laurent C, Couturier J, Mariani P, Desjardins L, Piperno-Neumann S, Lantz O, Asselain B, Plancher C, et al: **Establishment and characterization of a panel of human uveal melanoma xenografts derived from primary and/or metastatic tumors.** *Clin Cancer Res* 2010, **16:**2352-2362.

347. Besser MJ, Shapira-Frommer R, Treves AJ, Zippel D, Itzhaki O, Schallmach E, Kubi A, Shalmon B, Hardan I, Catane R, et al: **Minimally cultured or selected autologous tumor-infiltrating lymphocytes after a lympho-depleting chemotherapy regimen in metastatic melanoma patients.** *J Immunother* 2009, **32:**415-423.

348. Donia M, Junker N, Ellebaek E, Andersen MH, Straten PT, Svane IM: **Characterization and comparison of 'standard' and 'young' tumour-infiltrating lymphocytes for adoptive cell therapy at a Danish translational research institution.** *Scand J Immunol* 2012, **75:**157-167.

349. Tran KQ, Zhou J, Durflinger KH, Langhan MM, Shelton TE, Wunderlich JR, Robbins PF, Rosenberg SA, Dudley ME: **Minimally cultured tumor-infiltrating lymphocytes display optimal characteristics for adoptive cell therapy.** *J Immunother* 2008, **31:**742-751.

350. Jespersen H, Bagge RO, Ullenhag G, Carneiro A, Helgadottir H, Ljuslinder I, Levin M, All-Eriksson C, Andersson B, Stierner U, et al: **Concomitant use of pembrolizumab and entinostat in adult patients with metastatic uveal melanoma (PEMDAC study): protocol for a multicenter phase II open label study.** *BMC Cancer* 2019, **19:**415.

351. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, et al: **New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1).** *Eur J Cancer* 2009, **45:**228-247.

352. Simon R: **Optimal two-stage designs for phase II clinical trials.** *Controlled clin trials* 1989, **10:**1-10.

353. Levin AM, Bates DL, Ring AM, Krieg C, Lin JT, Su L, Moraga I, Raeber ME, Bowman GR, Novick P, et al: **Exploiting a natural conformational switch to engineer an interleukin-2 'superkine'.** *Nature* 2012, **484:**529-533.

354. Salmon H, Remark R, Gnjatic S, Merad M: **Host tissue determinants of tumour immunity.** *Nat Rev Cancer* 2019, **19:**215-227.

355. Carter L, Fouser LA, Jussif J, Fitz L, Deng B, Wood CR, Collins M, Honjo T, Freeman GJ, Carreno BM: **PD-1:PD-L inhibitory pathway affects both CD4(+) and CD8(+) T cells and is overcome by IL-2.** *Eur J Immunol* 2002, **32:**634-643.

356. Dijkstra KK, Cattaneo CM, Weeber F, Chalabi M, van de Haar J, Fanchi LF, Slagter M, van der Velden DL, Kaing S, Kelderman S, et al: **Generation of Tumor-Reactive T Cells by Co-culture of Peripheral Blood Lymphocytes and Tumor Organoids.** *Cell* 2018, **174**.

357. Neal JT, Li X, Zhu J, Giangarra V, Grzeskowiak CL, Ju J, Liu IH, Chiou S-H, Salahudeen AA, Smith AR, et al: **Organoid Modeling of the Tumor Immune Microenvironment.** *Cell* 2018, **175:**1972.

358. Votanopoulos KI, Forsythe S, Sivakumar H, Mazzocchi A, Aleman J, Miller L, Levine E, Triozzi P, Skardal A: **Model of Patient-Specific Immune-Enhanced Organoids for Immunotherapy Screening: Feasibility Study.** *Annals of Surgical Oncology* 2019.

359. Olson B, Li Y, Lin Y, Liu ET, Patnaik A: **Mouse Models for Cancer Immunotherapy Research.** *Cancer Discov* 2018, **8:**1358-1365.

360. Gottesdiener LS, O'Connor S, Busam KJ, Won H, Solit DB, Hyman DM, Shoushtari AN: **Rates of ERBB2 Alterations across Melanoma Subtypes and a Complete Response to Trastuzumab Emtansine in an ERBB2-Amplified Acral Melanoma.** *Clin Cancer Res* 2018, **24:**5815-5819.

361. Navai SA, Derenzo C, Joseph S, Sanber K, Byrd T, Zhang H, Mata M, Gerken C, Shree A, Mathew PR, et al: **Abstract LB-147: Administration of HER2-CAR T cells after lymphodepletion safely improves T cell expansion and induces clinical responses in patients with advanced sarcomas.** *Cancer Research* 2019, **79:**LB-147-LB-147.

362. Sullivan RJ, Moschos SJ, Johnson ML, Opyrchal M, Ordentlich P, Brouwer S, Sankoh S, Meyers ML, Agarwala SS: **Abstract CT072: Efficacy and safety of entinostat (ENT) and pembrolizumab (PEMBRO) in patients with melanoma previously treated with anti-PD1 therapy.** *Cancer Res* 2019, **(79)**.
363. Ritchie G, Gasper H, Man J, Lord S, Marschner I, Friedlander M, Lee CK: **Defining the Most Appropriate Primary End Point in Phase 2 Trials of Immune Checkpoint Inhibitors for Advanced Solid Cancers: A Systematic Review and Meta-analysis.** *JAMA oncol* 2018, **4:**522-528.
364. Nishino M, Giobbie-Hurder A, Gargano M, Suda M, Ramaiya NH, Hodi FS: **Developing a common language for tumor response to immunotherapy: immune-related response criteria using unidimensional measurements.** *Clin Cancer Res* 2013, **19:**3936-3943.
365. Seymour L, Bogaerts J, Perrone A, Ford R, Schwartz LH, Mandrekar S, Lin NU, Litiere S, Dancey J, Chen A, et al: **iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics.** *Lancet Oncol* 2017, **18:**e143-e152.
366. Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbe C, Maio M, Binder M, Bohnsack O, Nichol G, et al: **Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria.** *Clin Cancer Res* 2009, **15:**7412-7420.
367. Einarsdottir BO, Karlsson J, Söderberg EMV, Lindberg MF, Funck-Brentano E, Jespersen H, Brynjolfsson SF, Bagge R, Carstam L, Scobie M, et al: **A patient-derived xenograft pre-clinical trial reveals treatment responses and a resistance mechanism to karonudib in metastatic melanoma.** *Cell Death & Disease* 2018, **9:**810.

Appendix