Immunotherapy in advanced melanoma: crossing borders

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CHAPTER
1

General introduction and outline

Incidence and survival

Melanoma is a deadly form of cancer that originates from melanocytes. These neural crest cells control pigmentation and are present in various parts of the human body, including the skin and uvea. Their malignant counterparts result in cutaneous melanoma (CM) and uveal melanoma (UM), respectively.

The survival rate of patients with melanoma is dependent on the stage of the disease. The staging system as defined by the American Joint Committee on Cancer (AJCC) focusses on tumor thickness, mitotic rate, and the presence of ulceration, nodal metastases, and distant metastases. Most is known about CM as the incidence is much higher when compared to UM. Further research studying the survival, treatment options, and prognostic factors in melanoma is still ongoing.

In the Netherlands, approximately 7.500 patients were diagnosed with early stage CM in 2021, and in 2020 808 patients died due to the consequences of their melanoma. Globally, the number of patients diagnosed with CM is around 325.000, with a registered mortality of nearly 57.000 patients per year[1-3]. This difference in survival between patients diagnosed in the Netherlands versus melanoma patients world-wide could be due to the more accurate registration and screening of patients in the Netherlands.

Approximately one in five patients with melanoma will develop an advanced stage of the disease (inoperable stage III and stage IV with distant metastases). When the tumor is not operable due to size or location, patients can be treated with either local treatment with radiation or talimogene laherparepvec (T-VEC) intra-tumoral injections, or systemic treatment with either chemotherapy (dacarbazine), targeted therapy (BRAF- and MEK-inhibitors), or immune checkpoint inhibitors (anti-PD-1, anti-CTLA-4, or the combination of both).

Currently, multiple clinical trials are ongoing studying the combination of abovementioned standard of care treatment options. Tumor directed treatment for advanced melanoma is evolving quickly, and is dependent on clinical characteristics of the patient, and can differ between countries. In this introduction, I will further discuss treatments and a novel combination of adoptive cell therapy and conventional immunotherapy implemented in the Netherlands.

New treatments for advanced melanoma

In recent years, multiple new treatment options have become available for patients with advanced melanoma (Figure 1). First, targeted therapy with inhibitors of the

mitogen-activated protein kinase (MAPK) pathway was introduced around 2010. This pathway is crucial in cell proliferation, cell differentiation, and cell death.

Activating mutations in the BRAF gene are present in approximately 40 to 60 percent of advanced melanomas. In 80 to 90 percent of cases, this activating mutation consists of the substitution of glutamic acid for valine at amino acid 600 (V600E mutation), in approximately 10 percent valine is replaced by lysine at the same residue (V600K mutation)[4-6]. Treatment of patients with a BRAF V600E or BRAF V600K mutation with a BRAF-inhibitor improves both overall and progression free survival[7]. Although targeted therapy is initially very effective, the tumor usually acquires resistance to these drugs within a year after start of treatment[8].

In 2011, immune checkpoint inhibition was introduced. Melanoma is one of the most immunogenic cancer types, probably due to a high mutational load[9,10]. Strong anti-cancer immunity and better clinical outcome is seen in patients with a high infiltration of T lymphocytes, presence of specific subsets of dendritic cells and dendritic cell-like macrophages, and in patients with a high M1/M2 macrophage ratio[11,12]. Cancer immunity can be inhibited by co-inhibitory signals, expressed not only by tumor cells but also by myeloid cells, both in the tumor microenvironment and the tumor draining lymph nodes[13,14].

Multiple antibodies that stimulate anti-cancer immunity by blocking co-inhibitory signals have been developed. Most well-known immune checkpoint molecules to which blocking antibodies obtained regulatory approval are Cytotoxic T-Lymphocyte-Associated protein 4 (CTLA-4) and Programmed Death receptor 1 (PD-1) and its ligand (PD-L1).

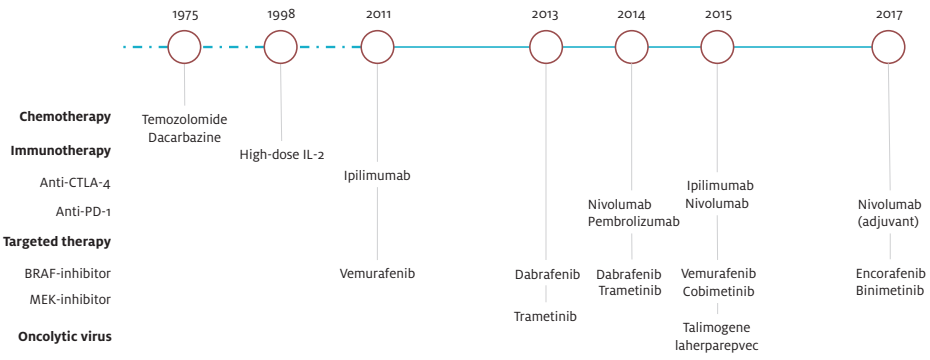

FIGURE 1 Introduction of new treatment options for patients with advanced melanoma. IL-2; interleukine-2, anti-CTLA-4; antibody against Cytotoxic T-Lymphocyte-Associated protein 4, anti-PD-1; antibody against Programmed Death receptor 1. Adapted from Van Zeijl et al. (Ned Tijdschr Geneeskd. 2018;162:D2420)

Before 2010, the median overall survival of patients with advanced melanoma was 6-9 months[15-17]. The recent 5-year follow-up data of a randomized controlled trial showed a median overall survival of 19.9 months after anti-CTLA-4, 36.9 months following anti-PD-1 and over 60 months for the group treated with the combination of anti-CTLA-4 and anti-PD-1[18]. A similar trend in prolonged annual survival rates since the introduction of these new treatments could also be observed on a nationwide scale in the Netherlands[19].

Although these results are promising, over half of the patients will not have a long-lasting response following treatment with immune checkpoint inhibition.

Furthermore, these antibodies can cause serious, and even life-threatening adverse events (AEs). In the previously mentioned trial 28%, 23% and 59% of the patients treated with anti-CTLA-4, anti-PD-1, or the combination of both experienced severe, life-threatening or disabling AEs. Most AEs are immune-related (irAE). These irAEs are thought to represent a bystander effect from activated immune cells[20,21].

Adjuvant treatment with anti-PD-1 is already approved as a standard treatment for patients with melanoma. At time of writing, trials investigating the safety and efficacy of neoadjuvant treatment with both anti-CTLA-4 and anti-PD-1 treatment are ongoing. So far, neoadjuvant treatment seems to lead to more expansion of tumor-resident T lymphocyte clones, a decrease in circulating myeloid-derived suppressor cells, and promising clinical responses. However, toxicity rates seem to be higher when compared to adjuvant therapy[22-24]. Recently, a randomized phase II trial identified a less toxic but equally effective dosing schedule for neoadjuvant ipilimumab and nivolumab in stage III melanoma[25]. An extension cohort showed that therapeutic lymph node dissection could be omitted in nearly all patients who achieved a complete or near-complete pathological response in the largest lymph node metastasis present[26].

Clinical trials and registry data

Whether a treatment gains market approval is based on data from large phase III randomized controlled trials. These large trials are considered to be the gold standard for determining the efficacy and safety of new treatments. However, these trials have strict inclusion and exclusion criteria. Overall, patients have to be in a (very) good clinical condition, with no active central nervous system metastases, and laboratory values within set parameters. The majority of the real-world advanced melanoma patients does not meet these criteria and is therefore not represented in the trials leading to market approval[27,28]. Thus, it is a matter of debate whether the results from these trials predict the response of the entire population of patients with advanced melanoma.

In July 2013 the Dutch Melanoma Treatment Registry (DMTR) was initiated. This first multipurpose nationwide registry for advanced melanoma patients registers all patients at time of diagnosis of advanced melanoma. The DMTR documents detailed information, including; tumor and patient characteristics, treatment patterns, AEs and clinical outcomes. In this thesis, I will show how databases like the DMTR make it possible to identify subsets of patients who have been excluded from large phase III trials, but can still benefit from immune checkpoint inhibition and targeted therapy[29].

Cancer immunity and new treatment options

In recent years, many clinical trials have been performed/initiated aiming to further improve the success rate of immunotherapy.

The efficacy of immunotherapy relies on a series of genetic and cellular alterations that provide the immune system of the patient with the means to generate a T cell response that recognizes and eradicates the cancer cells. This series of steps required for the final tumor eradication are part of the Cancer-Immunity Cycle, as was published by Chen and Mellman[30]. Additionally, the immune profile of an individual patient relies on an array of factors, including intrinsic tumor properties, extrinsic factors in the body, the presence of infection, and the exposure to sunlight and pharmacological agents[31].

The seven steps of the Cancer-Immunity Cycle guide our understanding of immunotherapy, treatment development over the past 50 years, and the rationale behind currently ongoing trials and new treatment combinations. For this purpose several representative treatments and trials of the many promising recent developments in the field of advanced melanoma were selected. The steps of the Cancer-Immunity Cycle are shown in Figure 2 and are described in the following text.

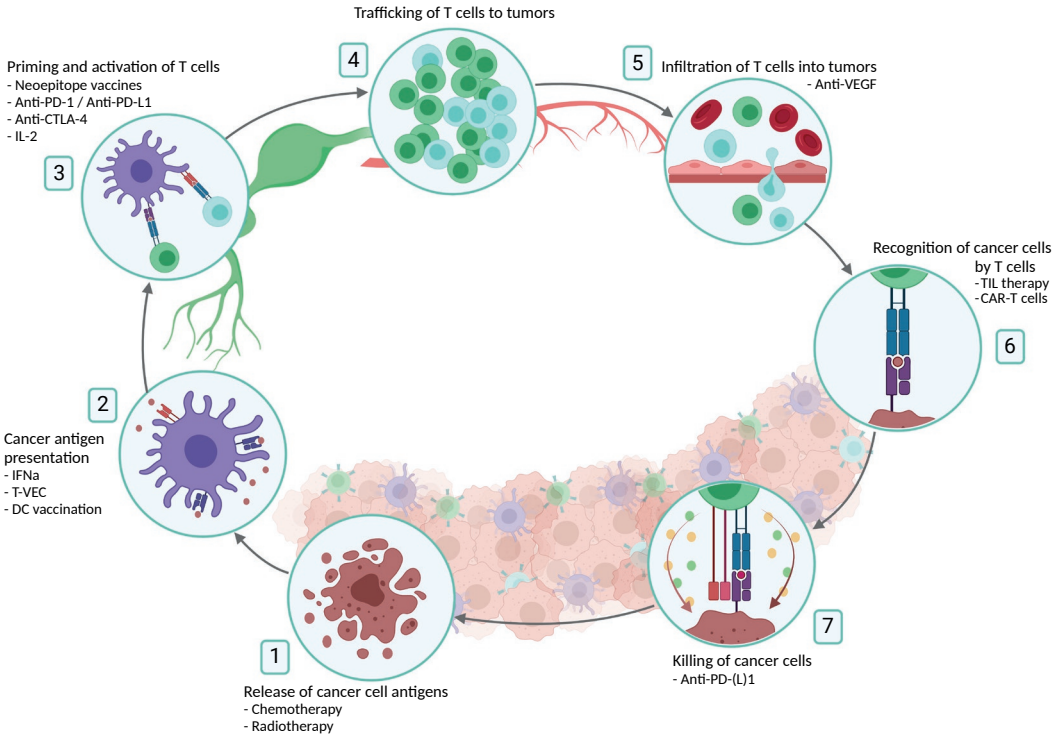

FIGURE 2 Cancer-immunity Cycle and anti-cancer treatment strategies. DC: dendritic cell, T-VEC: talimogene laherparepvec, IFNa: interferon-alpha, PD-(L)-1: programmed cell death (ligand)-1, CTLA-4: cytotoxic T-lymphocyte antigen 4, IL-2: interleukin 2, VEGF: vascular endothelial growth factor, CAR: chimeric antigen receptor, TIL: tumor infiltrating lymphocyte. This image was adapted from Chen and Mellman; Oncology Meets Immunology: The Cancer-Immunity Cycle, Immunity, Volume 39, Issue 1, 2013 (1-10), created with BioRender.com

1 Release of cancer cell antigens

In the first step tumor antigens, including neoantigens, are released after cell death, which are taken up by antigen presenting cells (APCs)[32]. These neoantigens are newly formed antigens that have not been previously recognized by the immune system. They arise from altered proteins formed as a result of mutations. As previously mentioned, melanoma has a high mutational load and therefore multiple neoantigens can be formed.

Treatment with chemotherapy and radiotherapy can lead to cell death. Currently, the only approved chemotherapy for metastatic melanoma is dacarbazine (DTIC). Since around 1970 patients have been treated with DTIC, leading to an objective response rate of approximately 20% with a median duration of 5-6 months[33]. Recently it was

shown that local treatments with radiotherapy can lead to regression of metastatic cancer at a distance. This so-called abscopal effect is mediated by activation of the immune system by the release of cancer cell antigens. Combining radiotherapy with immune checkpoint inhibitors could further enhance this effect[34].

2 Cancer antigen presentation

Once the tumor antigens are released, they have to be taken up by dendritic cells (DCs). These cells can be attracted to the tumor site by proinflammatory cytokines and factors released by dying tumor cells. These cytokines include interferon-alpha (IFNa) and tumor necrosis factor alpha (TNFa).

Treatment of melanoma with high-dose IFNa was introduced around 1985[35,36]. Studies showed that IFNa promoted tumor immunogenicity and enhanced DC attraction to the tumor, their polarization and maturation, survival and the antigen cross presentation[37,38]. IFNa also has a role in T helper 1 lymphocytes traffic to the tumor[39-41]. Three large trials by the European Cooperative Oncology Group have led to the approval of IFNa as adjuvant therapy for high-risk surgically resected melanoma (stage IIB or III)[42-44]. Clinical tumor responses in patients with metastatic melanoma were modest, with a duration of response of approximately 4 months[35].

Another way of gaining tumor antigen specific DCs in the tumor is by injecting them by DC vaccination[45]. Many clinical trials have been conducted in metastatic melanoma patients showing a moderate objective response rate of 8.5%, as reported in a meta-analysis in over 1200 melanoma patients treated with DC vaccination[46]. Interestingly, a recent study showed that following DC vaccination there is a significant increase in CD8 tumor infiltrating lymphocytes (TIL). This *de novo* inducing of a T cell inflamed tumor microenvironment was however co-occurring with the up-regulation of the T cell inhibiting signal PD-L1[47].

In 2015, vaccination with T-VEC was approved for the treatment of advanced melanoma with metastases in the skin and/or lymph nodes, based on a phase III trial with an objective response rate of 26.4%[48]. This vaccine, which is based on an oncolytic virus that is directly injected in or near the tumor, has a dual function in step 1 and step 2 of the cancer-immunity cycle. First, the genetically engineered attenuated herpes simplex virus type 1 that is injected has a lytic function and destroys tumor cells directly. This source of antigens favors local recruitment of immune cells into the tumor microenvironment. Additionally, the attenuated virus holds the gene for the human pro-inflammatory granulocyte macrophage-colony stimulating factor (GM-CSF). As the virus replicates in the tumor cells, GM-CSF is produced[49]. This cytokine promotes the recruitment and maturation of DCs and macrophages into potent APCs[50].

3 Priming and activation of T cells

Once the APCs have migrated from the tumor to a lymph node, they can present their captured antigen on MHC class I and MHC class II molecules to T cells which results in the priming and activation of an effector T cell response against these antigens. Many checkpoints and cytokines play a role in this delicate balance between suppression and overactivation of the immune system.

In 1992 the first approved immunotherapy for stage IV melanoma patients was systemic treatment with high-dose IL-2. This is a nonspecific T cell growth factor that can lead to expansion of all T cell subsets. The overall response rate was between 16-18%, with a median survival of 9.6-12 months[51,52]. Widespread use of high-dose IL-2 has mainly been hampered by its toxicity profile; capillary leak syndrome (oliguria, generalized edema, hypotension), fever, nausea, sepsis and even death.

Other more recent studies have identified several key immune checkpoints that can hamper the activation of T cells, including CTLA-4 and PD-1. CTLA-4 is an inhibitory checkpoint that is expressed on activated T cells. Once T cells recognize an antigen as non-self, a regulatory interaction occurs between the CD28 surface marker on the T cell and the molecules of the B7 family (CD80 and CD86) on the APC. This results in a stimulatory signal for the T cell. However, upon this activation CTLA-4 expression is upregulated. This can bind to CD80/CD86 with a much higher affinity when compared to CD28. If this occurs, it leads to an inhibitory signal. Blocking CTLA-4 with anti-CTLA-4 results in a more unrestrained activation of the T cell and can therefore enhance anti-tumor activity.

It was initially believed that the main interaction between PD-1 and its ligand PD-L1 occurred at the tumor site. There, recognition of the antigen presented by MHC molecules on the surface of cancer cells leads to T cell activation. Upon activation T cells produce cytokines (interferon-gamma) that induce surface expression of PD-L1 on tumor cells. This increased expression of PD-L1 inhibits the initially activated T cells. The blockade of the PD-1/PD-L1 axis results in an enhanced cytotoxic T cell response[53]. To protect DCs from cytotoxicity of activated T cells after antigen-presentation they simultaneously upregulate PD-L1. This expression on tumor infiltrating DCs plays a critical role in limiting anti-tumor immune responses[54]. More recent research revealed that tumor draining lymph nodes (TDLN) also harbor significant proportions of tumor-specific PD-1 expressing T cells, which are co-localizing with PD-L1 expressing DCs. Selectively targeting the PD-L1 in the TDLN could lead to an effective anti-tumor immune response. Therefore, it is currently believed that blockade of the PD-1/PD-L1 axis both occurs at the tumor site and the lymph nodes[13].

Instead of stimulating cancer cell death in order to increase the chances of (neo) epitopes being taken up and expressed by DCs, a recent development is treatment with patient-specific neoepitope vaccines[55]. Based on individual screening of both the tumor and healthy tissue a prediction on specific neoepitopes and their affinity can be made[56]. Multiple different vaccine formats are currently used in clinical studies, including synthetic long peptides, polyepitope DNA or polyepitope RNA[57,58]. Studies in melanoma patients with a peptide neoantigen vaccine and an intranodally administered mRNA vaccine, encoding for ten personalized neoantigens, showed that vaccination could induce a T cell response, stimulate T cell infiltration into the tumor microenvironment[59] and led to a remarkable vaccine-specific antitumor immune response[55].

Currently several clinical trials are exploring the efficacy of neoantigen vaccines in the form of peptides (NCT03639714, NCT03223103 and NCT02721043), mRNA (NCT04163094) and DNA (NCT04015700 and NCT04251117)[60] in combination with immune checkpoint inhibition.

4 Trafficking of T cells to tumors

In this step the activated T cells traffic to the tumor(s) via the bloodstream. After activation in the lymph node T cells undergo a shift in expression of surface markers and inflammation-specific receptors. By losing surface markers like CD62L and CCR7 these cells lose the ability to access lymph nodes. Instead they gain the expression of multiple homing molecules that enable them to migrate to diseased tissue. Chemokine receptors like CXCR3 bind inflammatory chemokines, including CXCL9, -10, -11 and CCL5, secreted by infected/tumor tissue[61,62].

5 Infiltration of T cells into tumor(s)

In order to be able to perform their tumor eradicating function, T cells have to migrate into the tumor microenvironment. From the bloodstream, they have to cross the endothelial lining and move through the tissue. Several proteins produced by the tumor can hamper this process. One of them is vascular endothelial growth factor (VEGF). This protein is known to drive tumor angiogenesis. Therefore, an inhibitor of VEGF was clinically studied for its proposed blood-vessel-formation control. The normalized vasculature resulted in increased tumor blood perfusion[63]. VEGF was also shown to hamper the expression of several adhesion molecules on the endothelial cells lining the tumor blood vessels[64,65]. By inhibiting VEGF there was not only better penetration of the tumor with blood vessels from which T cells could migrate into the tumor, but also the trans-endothelial cell migration and influx of these T cells was restored.

Unfortunately, no difference in overall survival was found in a randomized trial studying over 1300 patients with resected melanoma, who were treated with either adjuvant anti-VEGF or surveillance. Patients who received anti-VEGF did have a longer distant metastases free interval[66]. Interestingly, a phase I trial combining anti-CTLA-4 with anti-VEGF showed that this treatment combination was feasible and safe. Moreover, endothelial changes were present in the patients treated with this combination. Higher CD31 expression was observed in the intratumoral endothelial and interendothelial junctions. These changes were associated with extensive immune cell infiltration in the tumors, especially CD8 T cells and CD163 positive DCs[67]. At writing, the phase II follow-up trial with anti-CTLA-4 and anti-VEGF is still ongoing, as well as multiple trials combining anti-VEGF with anti-PD-1.

6 Recognition of cancer cells by T cells

Once the CD8 T cells have infiltrated the tumor, they can specifically recognize and bind to cancer cells through the interaction between its specific T cell receptor (TCR) and its cognate antigen bound to MHC class I on the surface of the cancer cell. In order to reduce the recognition by T cells, cancer cells can reduce their peptide MHC expression[68].

CD4 T cells on the other hand can exert their anticancer function in multiple ways. They can either provide signals to DCs to prime cytotoxic T lymphocytes[69], provide direct help to CD8 T cells, and in some cases they can directly recognize antigens presented by MHC class II on the surface of the cancer cell, followed by secretion of type I cytokines[70], or direct tumor killing[71].

Multiple trials have shown that both neoantigen-specific CD4 and CD8 TIL are seen in patients that respond to adoptive cell therapy (ACT)[72-75].

To increase the number of tumor infiltrating T cells, two different treatment strategies with genetically modified T cells are being implemented in the clinic. First, TCR-transgenic T cells i.e. T cells derived from peripheral blood mononuclear cells that are genetically modified by viral transduction of T cell receptors capable of recognizing specific tumor antigens[76]. Secondly, genetically modified T cells that express an artificial chimeric antigen receptor (CAR-T cell) with an antibody domain specific for recognition of a cell surface expressed tumor-specific/associated antigen and an intracellular signaling domain for activation of the T cell[77].

Since the 1980s the group of Rosenberg (NCI, USA) has been working on ACT. This process requires harvesting of TIL from the tumor, expanding them in the laboratory to large numbers and reinfusing them to the same patient. This treatment can induce

clinical responses in patients with metastatic melanoma, with the first report in 1988 describing a response rate of 50%[78,79].

In order to be successful ACT transfer requires the generation of sufficient numbers of cells with highly avid recognition of autologous tumor cells *in vitro*.

Subsequently, these activated T cells must be able to home to the tumor site in order to exert their effector function. Previous clinical trials employing the transfer of highly active antitumor T cell clones, have demonstrated that engraftment and persistence of the transferred cells required concomitant administration of high dose IL-2 to maintain cell proliferation and activation status. Rosenberg et al. reported that lymphodepletion prior to infusion of T cells can further improve the persistence and function of adoptively transferred cells. The AEs mentioned in their trial were mostly due to this high dose IL-2 that was given in combination with the ACT and included somnolence, coma, disorientation, neutropenia, thrombopenia, respiratory distress and hypotension. In later trials the group led by Rosenberg added toxic lymphodepleting chemotherapy and Total Body Irradiation (TBI) to this treatment schedule to induce a stronger lymphodepletion[80]. A more recent randomized controlled trial showed that adding TBI to lymphodepleting chemotherapy did not yield better clinical outcome. The TBI was responsible for significantly more treatment-related toxicities on top of the known toxicities from lymphodepleting chemotherapy, namely thrombotic microangiopathy, weight loss and more intesive care unit transfers and interventions[81].

The current globally used "Rosenberg-protocol" consists of; cyclophosphamide for 2 days, followed by fludarabine for 5 days. The infusion of TIL follows one day after the final dose of fludarabine. Patients subsequently receive high dose IL-2 intravenously every 8 hours up to 15 doses or until intolerance[79,82].

To date, ACT is still not part of the standard of care and is only given in clinical trials. Currently, a randomized phase III trial in the Netherlands and Denmark comparing TIL to standard anti-CTLA-4 treatment completed inclusion. The preliminary results are promising, showing that TIL treatment has a significantly longer progression free survival when compared to anti-CTLA-1 treatment. This trial is designed to open doors to lead to market approval for ACT treatment in metastatic melanoma (NCT02278887).

If ACT were to become an EMA/FDA approved treatment for metastatic melanoma, one of the important aspects curtailing the feasibility is the toxicity of the conditioning and support regimen, leading to long hospitalization and high patient burden. In the LUMC this regimen was replaced by cotreatment with low-dose IFNa.

In a phase I/II study the feasibility and safety of the adoptive transfer of tumor-reactive T cells and daily injections of IFNa in advanced-stage metastatic melanoma patients with progressive disease was tested[83]. Analysis of peripheral blood samples of the patient treated with PBMC-derived T cells with a complete clinical response revealed that circulating tumor-specific T cells persisted for at least 36 weeks after start of the infusion, sustaining the notion that T cell persistence can be achieved by daily IFNa injections instead of high dose IL-2. Additionally, treatment with IFNa induces a relatively mild leukopenia, neutropenia and lymphopenia and due to the favorable toxicity profile, this combination could be administered in the outpatient clinic.

7 Killing of cancer cells

In the final step of the cancer immunotherapy cycle, before re-entering and accelerating the whole cycle once more, T cells kill their target cancer cells. As was already described under "3. Priming and activation of T cells", one of the modes of action of anti-PD-(L)1 treatment is at the tumor site. Upon activation T cells produce cytokines that lead to the surface expression of PD-L1 in both the tumor and its micro-environment. This reactive expression of PD-L1 inhibits the initially activated T cells. The blockade of the PD-1/PD-L1 axis results in a cytotoxic T cell response[53].

The presence of high numbers of activated T cells is a requirement for a good response of PD-1 blocking therapy[84], consequently patients with low levels or absence of activated tumor-specific T cells may benefit from ACT treatment. To provide tumor-reactive TIL, alleviate immune checkpoint inhibition, reduce toxicity of ACT treatment and minimalize hospitalization and patient burden we combined ACT, with anti-PD-1 and low-dose IFNa in a new clinical trial (ACTME trial - NCT03638375).

Over the course of this PhD the clinical protocol was written, approved and the trial was initiated. The proposed mechanism of action of the treatment given in the ACTME trial is shown in Figure 3.

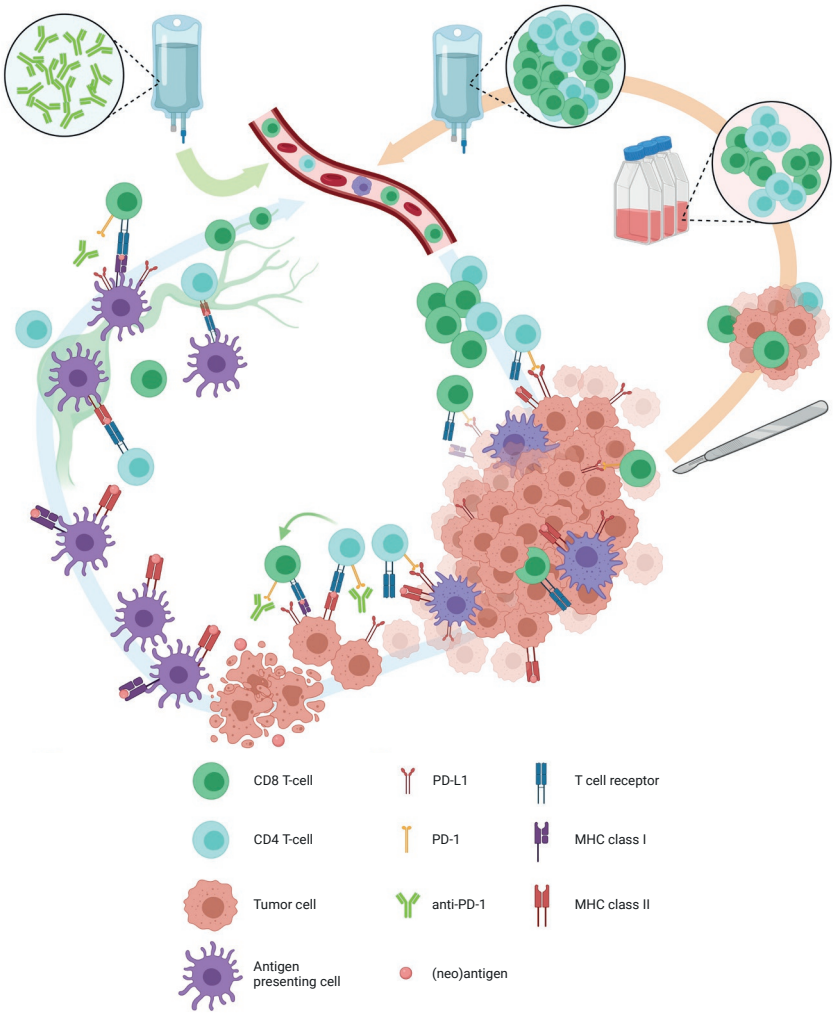

FIGURE 3 ACTME trial mode of action.

The **blue arrow** shows T cell activation. As these cells are programmed to be specific for one particular tumor antigen, they become activated after they recognize their cognate antigen in the context of an HLA molecule at the surface of an antigen presenting cell (APC). The T cell receptor (TCR) of the CD4 or CD8 T cell binds to the antigen that is presented in the MHC complex on the surface of the APC[85-89]. Upon activation a subset of helper CD4 T cells can provide critical signals to induce an adequate CD8 T cell response. Furthermore, inhibitory signals via PD-L1/PD-1 axis can inhibit the anti-tumor response of activated T cells. By using anti-PD-1 immune checkpoint inhibition it is possible to overcome this, resulting in cancer cell destruction by the patients' own T cells. Possible (neo)antigens that are released by the degrading tumor cells can be picked up by APCs to sustain the ongoing local response, or transported back to the lymphoid tissue to initiate new responses[10]. The **orange arrow** indicates the process of ACT by harvesting, culturing and reinfusing tumor-infiltrating lymphocytes (TIL) to the patient. The **green arrow** indicates the previously described systemic treatment with immune checkpoint inhibition (anti-PD-1). This figure was created with BioRender.com

Outline of the thesis

This thesis gives an overview of different treatment aspects for patients and patient subgroups with advanced melanoma and consists of three main parts. In the first part the differences between UM and CM are discussed. The second part focusses on the use of real-world data to move beyond the previously described phase III clinical trials. In this part the safety and efficacy of immune checkpoint inhibition and targeted therapy is investigated in different patient subgroups with advanced melanoma. In the third part new treatment combinations for patients with advanced melanoma are reported and discussed, including preliminary results from our ongoing trial for advanced CM patients who progressed on standard of care treatment options.

Part I

In **chapter 2** the differences in genetic alterations, metastatic routes, tumor biology, and tumor-host interactions between UM and CM is the focus. The role of the adaptive immune system differs between CM and UM. Even if immune cells succeed in infiltrating metastatic UM lesions, these cells do not seem to be activated. The described differences in CM and UM form the basis for understanding the low clinical response rate following anti-CLTA-4 (**chapter 3.1**) and anti-PD-1 (**chapter 3.2**) treatment in UM patients.

This first part of the thesis is concluded with an overview of patient characteristics, treatment options, and survival rates of advanced UM in the Netherlands in **chapter 4**. As UM is a very rare type of cancer, large trials and even data describing the current state of treatment are scarce. Using unique nationwide data, we are able to give a broad overview of all patients in the Netherlands with an advanced UM. All patients, regardless of their treatment strategy are included. The initial treatments prescribed, the corresponding overall survival, and the influence of risk factors are shown.

Part II

The second part of this thesis focusses on the use of the nationwide data from the DMTR. In **chapter 5** the treatment of advanced CM patients with and without a preexisting autoimmune disease is compared. This particular group of patients was excluded from the large trials leading to market approval of immune checkpoint inhibition, because of concerns about unleashing their underlying autoimmunity.

However, based on our findings oncologists are encouraged not to withhold immune checkpoint inhibition from patients with the more common autoimmune diseases of rheumatologic or endocrine origin. In addition, it is advised to follow-up patients

with inflammatory bowel disease closely, as severe colitis and toxicity requiring early discontinuation of treatment were higher in this group following immune checkpoint inhibition.

In **chapter 6** the focus is on adolescents and young adults (AYAs, 15-39 years of age), a group that was underrepresented in the large phase III trials with a median age of 53-62 years. We show distinct differences in primary tumor characteristics, tumor mutations, and first-line treatments initiated between AYAs and older adults. Although immune checkpoint inhibition and targeted therapy led to similar tumor responses, no AYAs experienced grade 3-4 colitis following anti-CTLA-4 treatment, while 17% of the older adults did.

In **chapter 7** potential differences in responses between male and female patients with advanced melanoma are addressed. Over the years multiple studies have been published showing conflicting results on survival and treatment response in male and female patients with (advanced) melanoma. Therefore, the question arose whether both groups can be treated with the same regimens. An overall female survival advantage of 10% was observed (**chapter 7.1**), but sex was not clearly associated with prolonged survival following immune checkpoint inhibition.

In the second part (**chapter 7.2**) the validity of an existing prediction score, claiming that female patients had a lower response to anti-PD-1 immune checkpoint inhibition when compared to male patients, was tested. This result was not validated using our extensive database, showing the importance of external validation of prediction scores.

Part III

In the final part of this thesis I discuss the results of our phase I/II clinical trial using adoptive T cell transfer in combination with low dose IFNa as treatment for stage IV cutaneous melanoma. Data on clinical results, immunological parameters and possible prognostic factors is presented in **chapter 8**. An important finding from this trial was that even patients who had previously progressed on immune checkpoint inhibition and/or targeted therapy could still respond to treatment with TIL. Furthermore, we observed that a large portion of the infused TIL expressed activation marker PD-1, which could make them more prone to inhibition via the previously described PD-1/PD-L1 axis.

These findings formed the basis for a new clinical trial that we initiated in 2018, where we combine TIL with pegylated IFNa and anti-PD-1 treatment. The rationale behind this treatment combination is described in more detail in **chapter 9.1**. The first preliminary (clinical) results from the phase I part are included in **chapter 9.2**.

General discussion

In **chapter 10** the results obtained in this thesis are discussed and implications for further research are presented.

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