Immunotherapy in advanced melanoma: crossing borders

Kooij, M.K. van der

CHAPTER
10

General discussion and future perspectives

In this thesis I have been crossing borders in the field of melanoma research, including uveal versus cutaneous melanoma, the use of real-world data to assess safety and efficacy of immune checkpoint inhibition treatment, and the use of adoptive cell therapy in cutaneous melanoma. Now it is time to focus on the horizon.

Part I: Systemic therapies for uveal melanoma

As **Chapter 1** already summarized, many new treatment options have become available for patients with cutaneous melanoma. Fortunately, several of these new therapies are also studied in patients with uveal melanoma, as the treatment options for this group of patients is still limited. **Chapter 2** of this thesis gave us an overview of some of the differences between cutaneous and uveal melanoma. One of the most striking differences in the metastatic setting is the lower mean somatic mutation rate in uveal melanoma, and therefore the potential lack of neoantigens to be recognized by the patient's immune system. This could be one of the reasons for the very limited effect of immune checkpoint inhibition treatment in these patients with either anti-CTLA-4 (**Chapter 3.1**), and anti-PD-1 (**Chapter 3.2**).

In a retrospective analysis 2 of the 6 uveal melanoma patients had a partial response to treatment with the combination of both anti-CTLA-4 and anti-PD-1. Both patients had received a liver metastases-directed therapy before the start of immune checkpoint inhibition[1]. One of these liver-directed therapies is isolated hepatic perfusion (IHP). The principle of IHP is to temporarily isolate the liver from the systemic circulation in a surgical procedure. Subsequently, the liver is flushed with high-dose melphalan (chemotherapy) for an hour. This leads to a local high dose intensity, which would be toxic and induce complications and serious adverse events when administered systemically. However, as the (surgical) procedure is associated with morbidity and even mortality, a new procedure was developed in which hepatic infusion with simultaneous chemofiltration can be performed percutaneously[2-9]. Percutaneous hepatic perfusion (PHP) is a relatively novel alternative to IHP that enables vascular isolation and perfusion of the liver by using endovascular techniques. Important advantages of PHP over IHP are the minimal invasiveness and the repeatability[10]. As metastatic uveal melanoma is associated with isolated diffuse hepatic disease (**Chapter 2** and **4**) PHP has gained popularity over the past two decades. Returning to the cancer-immunity cycle in Figure 2 of **Chapter 1**, we see that PHP treatment with high-dose melphalan could lead to the release of cancer cell antigens, which may be ingested and processed by antigen presenting cells for subsequent presentation to T cells. In 26 patients with advanced cutaneous melanoma, the combination of isolated limb infusion with melphalan followed by systemic administration of anti-CTLA-4 led to a response rate in 85% of patients[11]. Combining the locally administered melphalan by PHP with systemic treatment with immune checkpoint inhibition could as such

also induce a systemic effect by stimulating the endogenously activated T cells in uveal melanoma. A phase 1b/2 study combining hepatic percutaneous perfusion with anti-CTLA-4 and anti-PD-1 in advanced uveal melanoma is ongoing in the Leiden University Medical Center (NCT04283890)[12]. Similar trials are ongoing in other tumor types where responses to monotherapy with immune checkpoint inhibitors (ICI) are rare, including myxofibrosarcoma[13] (NCT04332874). The potential synergistic effects of these combinations will hopefully lead to new standard of care treatment options for patients with these (rare) tumor types.

It was previously shown that long-term survival and clinical benefit from adoptive cell therapy in cutaneous melanoma was determined by a four-parameter tumor immune signature; more CD8 T cells, a high M1/M2 macrophage ratio, more galectin-9 dendritic cells, and the expression of galectin-3 by tumor cells[14].

Unfortunately, published information on the tumor and stromal composition of uveal melanoma metastases is limited. A recent article described the immune cell composition of 21 metastatic uveal melanomas, including both hepatic (n=17) and extra-hepatic (n=4) metastases, and correlated the outcome with patient response to various systemic and local treatments (immune checkpoint inhibition and/or chemoembolization with irinotecan charged microbeads), and survival. This led to the conclusion that the percentage of intratumoral granzyme B positive CD8 T cells (activated cytotoxic T lymphocytes) was a prognostic indicator. They also showed that the intra-tumoral density of CD163 positive tumor-associated macrophages (generally immunosuppressive M2-like) was higher in liver metastases when compared to extra-hepatic metastases[15]. Unfortunately, the number of extra-hepatic metastases was small (n=4) and there were no matched samples of both types of metastases from one patient. It would be interesting to validate these findings in a larger group of uveal melanoma patients, including multiple matched samples.

The most recent new treatment option for patients with irresectable uveal melanoma is systemic therapy with tebentafusp monotherapy. This immune-mobilizing monoclonal T cell receptor is a fusion of a soluble affinity-enhanced HLA-A*02:01-restricted T cell receptor for a glycoprotein 100 peptide (gp100) which is fused to an anti-CD3 single-chain variable fragment. The recently published open-label, phase 3 trial, included 378 patients with metastatic uveal melanoma. The overall survival at 1 year was 73% in the tebentafusp group versus 59% in the control group (hazard ratio for death 0.51, 95% confidence interval 0.37-0.71)[16]. This has led to the approval of this new treatment option by the FDA and EMA.

Another promising type of treatment involving T cell engagement, might be adoptive cell therapy. A first stage and ongoing expansion stage of a phase 2 trial with adoptive

cell therapy in uveal melanoma showed that seven of the 20 evaluable patients had an objective tumor regression (6 partial response, 1 complete response)[17]. There was a positive association between the frequency and absolute number of tumor-reactive tumor infiltrating lymphocytes (TIL) in the infusion product and response to treatment.

These specific TIL were determined by the sum of flow cytometric measurements of OX-40 positive CD4 T cells and CD137 positive CD8 T cells, following co-culture of the TIL with cryopreserved autologous tumor digests (when available).

Additionally, the absolute interferon-gamma production of these TIL following co-culture also seemed associated with response to treatment. No difference was observed in the number of non-synonymous mutations harboured by responding versus non-responding patients, in both groups the mutational burden was low[17]. Therefore, the question arises what is actually recognized by the tumor-reactive TIL. Are these, for example, neo-epitopes that have derived from the few somatic mutations present in the metastatic uveal melanoma? It will be interesting to further elucidate the specificity and identify the targets recognized by these reactive TIL in responding patients. At the same time it would be of importance to determine if TIL in non-responding patients are suppressed by the expression of immunomodulatory molecules that lead to T cell suppression, like Galectin-3, PD-L1, CTLA-4, Indoleamine 2.3-Dioxygenase-1, and Lymphocyte Activating 3, as was shown in a recent article in primary uveal melanoma[18].

Currently, two phase II trials are ongoing evaluating the efficacy of adoptive cell therapy in a larger cohort of amongst others uveal melanoma patients. The first trial will study 47 patients with metastatic uveal melanoma, who will be treated with a lymphocyte depleting preparative regimen followed by TIL and high-dose intravenous aldesleukin (NCT03467516). The second trial aims to determine whether the addition of dendritic cell vaccination to the combination of lymphodepleting chemotherapy, high-dose IL-2 and TIL leads to sustained persistence of the infused T cells when compared to lymphodepleting chemotherapy, high-dose IL-2 and TIL. This trial specifically includes patients with uveal melanoma, alongside patients with cutaneous melanoma (NCT00338377). In the first report on one of the different cohorts of the trial, the authors did not show a difference in the persistence of MART-1 TIL between the two groups. However, in the small group of 18 patients in total it seemed that there might be a better clinical response in the combination group (4/8 versus 3/10). Unfortunately, no uveal melanoma patients were included in this initial report[19]. The fact that more studies in metastatic melanoma include uveal melanoma in their inclusion criteria seems hopeful. This could potentially lead to new treatment combinations with adoptive cell therapy in this specific subgroup of melanoma patients.

Unfortunately, the reported durability of the clinical responses following TIL therapy in uveal melanoma is relatively short compared to the responses seen in cutaneous melanoma. A possible explanation for this might be that the infused T cells are suppressed by the intra-tumoral M2-like macrophages. In order to support these infused T cells, combining this treatment with M2 targeting therapy might be necessary to overcome the immune suppressive environment in hepatic metastases of uveal melanoma. Several treatment options have been described that can induce a M2 to M1-phenotype macrophage repolarization, including local low-dose irradiation[20] and tumor vaccines formulated with GM-CSF[21,22]. Recently, targeting of M2-like tumor-associated macrophages with a hybrid peptide MEL-dKLA was used *in vivo* in a lung cancer model[23], and in a breast cancer model where it enhanced the PD-L1 mediated anti-tumor effect[24]. Multiple clinical trials are currently ongoing with macrophage targeting agents. For melanoma patients these include trials with CD40 agonists and CSF-1 receptor inhibitors[25].

Interestingly, a recent abstract on the NCT03123783 clinical trial with a CD40 agonist showed that 6 out of 33 patients with anti-PD-1 refractory metastatic cutaneous melanoma developed a partial response to the combination of anti-PD-1 and a CD40 agonist[26]. The same was seen in a mouse model of another immunologically desert tumor; pancreatic carcinoma. The authors conclude that the CD40 agonist leads to priming of both CD4 and CD8 T cell subsets, while anti-PD-1/anti-CLTA-4 treatment removes negative feedback signals for these newly primed T cells[27]. Multiple trials are ongoing in both immunologically hot and cold tumors to further study the effect of CD40 agonists in combination with immune checkpoint inhibition.

Another potentially promising adoptive T cell therapy for uveal melanoma, might be with Chimeric Antigen Receptor (CAR)-T cells. Hereby, the T cell receptors (TCR) of isolated peripheral T cells are further engineered to express extracellular antigen recognition domains targeting a tumor-specific cell surface protein[28]. So far, treatment with CAR-T cells has shown great promise in hematologic malignancies, including acute lymphoblastic leukemia, chronic lymphocytic leukemia, lymphoma, and multiple myeloma. In cutaneous melanoma multiple potential stable target antigens for CAR-T cells have been identified, including CD20, disialoganglioside GD2, CD171[29], chondroitin sulfate proteoglycan 4 (CSPG4)[30], and HER2[31]. Currently, there are no clinical trials ongoing for CAR-T cell therapy in uveal melanoma. Based on data from The Cancer Genome Atlas, HER2 mRNA is expressed at appreciable levels by both cutaneous and uveal melanoma. In the pre-clinical trial where it was shown that CAR-T cells directed against HER2 could kill cutaneous melanoma cells *in vitro* and in a humanized mouse model, also two uveal melanoma cell lines were included. These commercially available cell lines were sensitive to HER2 CAR-T cells[31].

A challenge for CAR-T cell therapy in solid tumors versus hematological malignancies, is that the tumors are poorly infiltrated by immune cells, that tumor microenvironment blocks the effect, that the infused cells become exhausted before they can eradicate the tumor, or that the targeted antigen is not uniformly expressed on the tumor cell surfaces or different metastases. In order to overcome these hurdles, recently the combination of an RNA vaccine and CAR-T cells targeting the same target (tight junction protein claudin 6) was studied in mice. This trial showed an enhanced efficacy of the infused CAR-T cells when combined with an RNA vaccine, designed for body-wide delivery of the CAR antigen[32]. A recent study showed that also an intracellular oncogenic transcription factor (WT1) could be targeted by CAR-T cells[33]. Another treatment option might be to target the malignant melanoma stem cells. Markers for this specific subgroup of melanoma cells include the previously named CD20[34] and CD133[35]. At writing, a phase 1 trial is ongoing that studies the safety of CD-targeting CAR-T cells in advanced melanoma patients (NCT03893019). It will be interesting to see the future developments in the treatment for metastatic uveal melanoma. When compared to cutaneous melanoma, it seems that more hurdles have to be overcome to reach lasting clinical responses.

In this discussion several promising treatment options were already described, including treatment with the combination of PHP and immune checkpoint inhibitors. The first promising results from an initial clinical trial of autologous T cell transfer in uveal melanoma were also described. In order to reduce the T cell suppression by intra-tumoral M2-like macrophages adoptive cell therapy might be combined with MEL-dKLA. In order to enhance the release of cancer cell antigens due to cell death, to increase MHC class I expression, and to trigger more intratumoral antigen-specific T cells, the harvesting of TIL might be preceded by melphalan treatment[36]. For example by combining TIL treatment with PHP.

Meanwhile, the search for an suitable target for CAR-T cell therapy continues in uveal melanoma. As was described earlier, the HER2 directed CAR-T cells might hold great promise. We will have to await further trials to verify the effect of these cells in uveal melanoma. And following these reports, combinations with RNA vaccines targeting the same target might be considered.

Part II: From bench to registry and back

The current evidence pyramid visually depicts the evidential strength of different research types. At the foundation of the pyramid usually animal and laboratory studies are depicted. This is followed by case reports/series, case control studies, cohort studies, and at the top of the pyramid randomized controlled trials are placed. These

studies are the ones that can lead to market approval and the widespread use of the different interventions.

However, these large phase III randomized controlled trials do not typically represent the entire population of patients that will receive the medicinal product. A recent study comparing systemically treated patients with advanced melanoma showed that 40% of the patients treated in the Netherlands would not have been eligible for inclusion in phase III trials[37]. The inclusion and exclusion criteria for these trials exclude a vast number of patients, based on for example: age, disease progression, brain or leptomeningeal metastasis, comorbidity, and use of (immune-modulating) medication.

Medical registries were initially mainly used for calculating valuable epidemiological data, like incidence, prevalence, and mortality. However, these registries have evolved, and can now include data on adverse events, quality of life, laboratory values, and medical history of the patient. The Dutch Melanoma Treatment Registry (DMTR) is a national registry databases that includes information on all advanced melanoma patients in the Netherlands. In **Chapter 5**, **6**, and **7** I used the data from the DMTR to study the safety and efficacy of systemic treatments for advanced melanoma patients in different subgroups.

Stepping away from the widely used evidence pyramid that depicts animal and laboratory studies at the bottom, I would like to argue that real-world registry data could also be used to create new fundamental research questions. In **Chapter 6** of this thesis we showed that there were distinct differences in primary tumor characteristics, and tumor mutations between patients 15-39 years of age (AYA) and older adults. We showed that the common BRAF mutation was even more prevalent in the AYA age group. I hypothesize that this may implicate that the prevalence of mutations in more melanoma driver genes will differ between AYA and older patients. In order to compare these mutational profiles it would be interesting to have access to whole-genome sequencing data (especially single-nucleotide variants, multiple-nucleotide variants, small insertions and deletions, structural variants, UV radiation related mutation signatures, and the median tumor mutational burden). Currently, the treatment regimen is roughly the same for every metastatic melanoma patient, except for BRAF treatment that is dependent on the presence of the BRAF V600 mutation. Based on the findings presented in **Chapter 6**, I hypothesize that early onset melanoma is a separate entity with a different prevalence of mutations in melanoma driver genes, when compared to older patients. Studying these differences could help identify potential targetable genomic differences between young and older patients with metastatic melanoma, which in turn could lead to age-specific mutational analysis in the future.

In the current era of medicine we are fortunate to have databases that collect these type of whole-genome sequencing data. In the Netherlands, this data is collected by the Hartwig Medical Foundation. Currently, their data is being analyzed to correlate the findings in our nationwide registry with more in-dept sequencing data. The aim is to understand the exact differences and identifying the potentially targetable genomic differences between young and older patients with metastatic melanoma.

Investigating patient data on a national, or even international scale, will not only be beneficial for patients with cutaneous melanoma. Data-registries and collaborations will have an even greater benefit for patients with rare cancers. Approximately 200 malignancies are defined as rare cancers (6 or less cases per 100.000). In Europe, rare cancers account for 24% of all malignancies[38,39]. As Nathan et al. showed in their phase 3 trial with tebentafusp for patients with metastatic uveal melanoma, randomized studies are possible for rare cancer types albeit requiring large international consortia[16]. A potential way of reducing the number of patients with rare cancers that have to be included in these trials, is the use of "historical cohorts". In this thesis, we included nation-wide data on uveal melanoma that can be used as such (**Chapter 4**). I would encourage registries with rare cancer types to join forces on an international level. Combining survival data on such a large scale will make it possible to provide "historical cohorts" for researchers, leading to less patients being treated with "standard of care" therapies and possibly more trials for patients with rare malignancies.

Another benefit of joining forces on an international level could be to compare treatment strategies and stage-specific survival of patients with melanoma in, for example, Europe. Over the past decades treatment options have changed for patients with melanoma. However, not all countries in Europe added these treatments to their standard of care at the same point in time. It would therefore be interesting to see if survival changed since the introduction on these new treatment options. In addition "country" could be used as an instrumental variable in comparing neighboring countries to identify an association between treatment strategy and survival.

One of the key questions in medical oncology was whether patients with a preexisting autoimmune disease could be treated with ICI. Treating oncologists worldwide feared potential flares in patients with an already overactive immune system. Therefore, patients with this type of comorbidity were excluded from the phase III trials that led to market approval of both anti-CTLA-4 and anti-PD-1 treatment. However, using the DMTR database it was possible to publish data on this specific group of patients and to compare both treatment outcome and overall survival with a large group without an autoimmune disease (**Chapter 5**). Showing that ICI can be prescribed to patients with common autoimmune diseases of endocrine and rheumatologic origin, has had

a major clinical impact worldwide. This was evidenced by the interest for the subject on multiple (international) conferences and the reports from multiple clinicians that they indeed are now less hesitant to treat patients with common autoimmune diseases with anti-CLTA-4 or anti-PD-1.

Another important aspect for which these large registries can be used is validation of scoring systems or models that were based on (smaller) trials. One of the first examples is shown in **Chapter 7.2**. By using data from the DMTR, we found that a previously published prediction model for response to anti-PD-1 could not be validated. I can't emphasize enough how important these kinds of validation attempts are. Many researchers try to create an appealing and easy scoring system for response to drugs. However, as we have learned from amongst others the Cancer Immunity Cycle, tumor regression is (unfortunately) not so easily reached nor defined.

One of the variables used in the prediction model was gender. For many years it has been known that women have a survival advantage over men with melanoma. Many possible explanations have been studied, including; behavioral differences leading to earlier detection in women, possible differences in mitotic rate, and BRAF mutation rate. Interestingly, previous studies already showed that the survival advantage for women became smaller in patients with more advanced disease. A recently published theory states that women are prone to stronger immunoediting in early tumor development. This strong initial immune response leads to the fact that when tumors have grown and metastasized the effectively-presented driver mutations are already significantly depleted. This renders advanced melanomas in women less visible to the immune system and therefore more difficult to treat with ICI[40]. In line with this hypothesis, it was found that in (mostly) metastatic melanoma patients the tumor mutational burden was lower in women when compared to men[41-43]. Using gene expression pathway analysis, a recent report on mostly stage IIIB and IIIC melanoma patients showed that tumors from women were enriched in immune related pathways when compared to tumors from men. Apart from CD8 and CD4 T cell pathways, this also included the regulatory T cell pathway. However, when peripheral blood was analysed, it was shown that women had a higher percentage of CD3 positive cells, while men had higher percentages of monocytes and trends towards higher percentages of regulatory T cells[44].

This could be a possible explanation for some findings presented in **Chapter 7.1**. The reported overall survival advantage of 10% for women when compared to men, was no longer present when only patients treated with ICI for advanced melanoma were analyzed. The primary melanomas of women were thinner when compared to men, and female patients had a longer time gap between primary disease and the development of advanced disease. Is this longer time gap explained by the fact that the

primary tumors were earlier detected, and therefore thinner, in women. Or does early strong immuno-editing play a role? If the theory about early immunoediting is true, we would expect to see a difference in response between men and women when ICI are given at an earlier stage.

Recently, the Checkmate-238 and EORTC 1325/Keynote-054 trials led to registration and approval of anti-PD-1 as adjuvant systemic treatment in resected stage III and IV melanoma. Interestingly, in 2021 De Meza et al. published the first data on adjuvant anti-PD-1 treatment in patients with melanoma using data from the DMTR. In their univariate Cox regression model women had a better recurrence-free survival (HR 0.64, 95% CI 0.48-0.87). Factors that were associated with recurrence-free survival in univariate Cox were included; sex, tumor stage, ulceration present in primary melanoma, Breslow thickness, and BRAF-V600 mutation status. These factors were included in a multivariate Cox in the supplemental material. Women recurrence-free survival advantage remained (HR 0.69, 95% CI 0.48-0.97)[45]. A comparable result was seen in the earlier mentioned trial that showed that women with a stage IIIB an IIIC had a higher infiltration with immune cells compared to men. When these women were treated with adjuvant anti-CTLA-4 they showed both a longer overall survival and relapse free survival[44]. Although these data cannot directly be compared with our data in **Chapter 7**, as age and patient performance score were not included, these results strengthen the theory that women might benefit more from early treatment with ICI, possibly due to the strong immune response early in disease development.

Neoadjuvant treatment in melanoma is not (yet) a registered treatment for melanoma. Therefore, we turn to the data from the recently published phase II OpACIN-neo and OpACIN neoadjuvant ICI trials[46,47]. The currently published data from these trials mainly focusses on the pathologic response rate following three different ICI treatment regimens. In the percentage of pathologic responses the OpACIN-neo did not show a significant response difference in response rate between women (62%; 95% confidence interval 45-78) and men (84%; 95% confidence interval 70-93)[47]. In coming years it would be very interesting to analyze the neoadjuvant data on a national scale, in order to really make a head to head comparison in the survival advantage of women versus men following neoadjuvant, adjuvant and regular ICI treatment.

Part III: Time for TIL

In this thesis I presented the data from our phase I/II clinical trial with adoptive T cell transfer in combination with low dose interferon-alpha (**Chapter 8**). It was shown that this combination was safe and could lead to clinical results, even in patients who already had progression of their melanoma under immuno- and targeted therapy. Interestingly, we found that a large portion of infused T cells expressed PD-1 on their surface[48].

These findings formed the basis for our currently ongoing trial, where we combine anti-PD-1, interferon-alpha and adoptive T cell transfer (**Chapter 9**)[49]. In this thesis the first preliminary data is published on both safety and efficacy of this novel treatment combination. We conclude that this combination can safely be prescribed to patients with melanoma who have already progressed on all standard of care treatment options. Additionally, several heavily pre-treated patients still show a clinical response.

Currently, the first phase III trial comparing TIL with ipilimumab has completed inclusion. The preliminary results show that the progression free survival of patients receiving TIL was significantly longer when compared to patients who were treated with ipilimumab. This could pave the way for TIL treatment to become part of the standard of care treatment options for patients with melanoma.

A possible way to further improve the clinical outcome of adoptive T cell therapy lies in the selection of the metastatic site to culture these cells from. Currently, this selection process is solely made on the basis of which metastases has the best access for surgical removal. However, we know that the presence of large numbers of infiltrating lymphocytes in the primary tumor, metastatic lesion, stroma, and (draining) lymph node has been shown to hold predictive value with respect to the natural history of melanoma[50-56]. It was already shown that the presence of higher concentrations of CD8+ lymphocytes in the (single) tumor from which TIL for adoptive T cell therapy were harvested, was correlated with a better survival[14]. As TIL play a central role in the response, an effective method to select patients and predict responses is crucial. Therefore, over the past years multiple mouse-studies and the first phase-I (human) clinical studies have been published using immune-PET/CT with zirconium-89 (^{89}Zr) labeled CD8+ antibodies to quantify tumor infiltration *in vivo*. This has the advantage that the technique is non-invasive and does not suffer from sampling error due to heterogeneity: the whole tumor burden can be quantitatively assessed. A recent study showed that a ^{89}Zr-labeled human CD8-specific minibody could detect CD8+ lymphocyte infiltration by small animal immuno-PET imaging in a xenograft mouse model[57]. It was shown that the radiopharmaceutical distribution not only spatially matched immunohistochemistry for CD8+, but also quantitatively. The first in human imaging study with this anti-CD8 minibody showed the procedure to be safe and confirmed a correlation between high radiopharmaceutical uptake determined by immuno-PET/CT and CD8 staining using immunohistochemistry[58]. In order to take adoptive T cell therapy a step further, I believe it would be promising to use radiolabeled CD8 antibodies as a selection tool for the lesion to culture T cells from to be used in adoptive cell therapy.

In order to further improve the effect of TIL therapy, it would also be beneficial to select TIL that respond to neo-antigens[48,59]. Detection of these neo-antigens can

be performed using genome and RNA sequencing data from the treated patients in comparison to healthy tissue. Using algorithms for amongst others HLA-binding, stability, and epitope foreignness the most potent neo-epitopes can be selected. Selecting and expanding only those TIL that respond to these neo-epitopes would yield better clinical results[60].

As the process of neo-epitope selection is both time-consuming and costly, one would ideally select TIL based on (a combination of) activation-induced surface markers. Over the years many surface markers have been studied[61]. CD137 is upregulated on CD8 and CD4 T cells following antigen-specific stimulation[62,63]. It was shown that the expanded CD137 positive fraction of TIL had been enriched for neoantigen-specific T cells[64]. Other markers that were suggested and exhibited antitumor activity were; PD-1, CD39, and CD103. Particularly, the combination of the latter two was shown to identify tumor-reactive CD8 T cells[65]. A recent comparative study on surface markers in human high-grade serous ovarian tumor samples showed that the antitumor abilities of PD-1, CD103 and CD39 positive T cells was mainly derived from a subset of CD137 expressing TIL[66].

Currently, there is a trial ongoing in the Erasmus Medical Center studying adoptive T cell therapy with autologous T cells, gene-engineered to express the MAGE-C2 antigen (NCT04729543). This is a tumor specific target in 40% of melanomas and 20% of head and neck squamous cell carcinomas[67-69]. As MAGE-C2 is not expressed in healthy tissues, except for the gonads, it will be interesting to see whether this treatment protocol indeed shows less toxicity when compared to previous trials with differentiation antigens, including MART-1, gp100, CAE and p53[70-72].

The past decade in medicine belonged to ICI with anti-CTLA-4 and anti-PD-1. Their development and clinical implementation has made a great impact on our understanding of cancer pathogenesis, and has importantly improved survival of patients with many different tumor types. However, we are now at the beginning of a new era, where we will face the challenges of immunotherapy-resistance.

Discussed here were some promising new developments for patients with uveal and cutaneous melanoma. Where cutaneous melanoma treatment will mostly have to battle secondary immunotherapy resistance, uveal melanoma treatments will have to overcome primary immunotherapy resistance. In order to offer TIL therapy to both groups of patients, immunologists, oncologists, pathologists, pharmacists, radiologists, and epidemiologists will have to join forces to determine the best treatment add-on to TIL therapy for these two very different types of melanoma.

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