

Immunotherapy of melanoma

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Melanoma is considered one of the immunogenic – if not the most immunogenic – malignancies. This is based on several observations.

1. Spontaneous remissions occur occasionally.
2. In about 5% of melanomas no primary tumour is found. The genetic aberrations of these tumours closely resemble those of cutaneous melanomas, and therefore are suggestive of spontaneous regressions of the primary tumours.
3. Both primary tumours and metastases often have brisk lymphocytic infiltrates, a phenomenon that is correlated with better outcome.
4. Studies of isolates of these tumour-infiltrating T lymphocytes have revealed that a proportion of these cells recognise melanoma antigens.
5. Melanomas respond to immunotherapy.

These observations have led to over 30 years of research on immunotherapy for melanoma; many of these efforts have failed, with only a few exceptions: interleukin-2 (IL-2) and to a lesser degree interferon- α (IFN- α). Recently, new developments in immunotherapy have revolutionised this treatment modality. Anti-CTLA4 has received approval from the Food and Drugs Administration (FDA) and the European Medicines Agency (EMA) for the treatment of stage IV melanomas based on the improvement in overall survival in phase III trials, and more recently blockade of PD1/PDL1 interactions has shown objective clinical responses in a stage IV melanoma in early-phase clinical trials. In addition, several independent single-institution phase I/II trials using adoptive cell therapy have shown a consistently high response rate, including durable complete remissions in a substantial percentage of treated patients.

Now, for the first time, immunotherapy has moved beyond the treatment of melanoma as both CTLA4 and PD1 blockade have been shown to induce objective responses in other tumour types as well.

This chapter will discuss the mechanism of action, clinical efficacy and side effects of IL-2, the novel treatments consisting of the immune checkpoint blockade drugs anti-CTLA4 and anti-PD1 and adoptive cell therapy.

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1. Introduction

Of all treatments for malignancies, immunotherapy has been the most extensively studied in metastatic melanoma. These often experimental, immunotherapeutic interventions can be divided into: (1) biologicals such as cytokines, including interleukin-2 (IL-2), interferons and granulocyte-monocyte colony-stimulating factor (GM-CSF); (2) vaccination strategies such as

peptide vaccines, whole-protein vaccines, virus-based vaccines, DNA vaccines and dendritic-cell-based vaccines; (3) adoptive cell therapy with lymphokine-activated killer cells (LAKs), tumour-infiltrating lymphocytes (TILs), peripheral-blood-derived melanoma-specific T cells and gene-modified T-lymphocytes and (4) immune checkpoint inhibitors, including anti-CTLA4, anti-PD1 and anti-PDL1 and immune co-stimulatory molecules, including anti-CD137. These

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experiments were initiated because of the observations that not only primary melanomas – especially primary superficial spreading skin melanomas, but also metastatic disease – can spontaneously regress [1]. In addition, about 5% of patients present with melanoma metastases, often lymph-node metastases, and sometimes also visceral metastases, without any sign of primary melanoma on dermatological inspection. Recently, it was shown that the genetic make-up (BRAF and NRAS mutations) of these unknown primary melanomas is very similar to that from non-chronic sun-damaged (non-CSD) skin melanomas, suggesting that the primary melanomas may have spontaneously regressed [2].

Little is known about the exact frequency of spontaneous regressions in melanoma, but it is considered to be low (around 3%), although some reviews have mentioned frequencies above 15%. In a review from 2009, describing 76 cases from 1866 and onwards, the proposed mechanisms for spontaneous regressions are thought to involve immune, endocrine, inflammatory and tumour environmental nutritional factors [3]. Although all of the above are probably involved, the focus of this review is on immune factors.

The role of lymphocytic infiltrates in melanoma was first described by Clemente et al., showing that brisk infiltration by tumour-infiltrating lymphocytes (TILs) into primary melanomas was correlated with better survival [4,5]. Later this was also shown for TILs in metastatic lesions [6], suggesting a causal role for TILs in tumour control. In addition, in the past 20 years many tumour antigens have been discovered that we now know are recognised by TILs. T cells derived from TILs were shown to recognise melanocyte differentiation antigens gp100, tyrosinase and MART-1/Melan-A. Other genes were discovered in the 1990s, such as melanoma-associated genes (MAGE) and NY-eso-1 [7–16]. In contrast to proteins that belong to the melanocyte differentiation antigens, these gene products are derived from aberrantly expressed genes by tumours, which play a physiological role during foetal development, are silenced thereafter, but are still present mainly in the testis. Thus, these genes have been named cancer/testis genes. Very recently, it was demonstrated that TILs can also recognise mutated antigens (van Rooij et al., *J Clin Oncol*, in press). Melanoma has the highest frequency of mutations of all cancers [17,18]. The vast majority of these mutations carry a typical ultraviolet light signature. Using next-generation DNA sequencing and RNA sequencing of tumours from paired tumour and TIL samples, many mutations that potentially carried a new T-cell epitope were found. Using major histocompatibility complex (MHC) tetramers, TILs from these tumours were screened for the presence of T cells specific for these mutated or neo-antigens. Within four tumour-TIL pairs, four mutated antigen-specific T-cell populations could be detected, some at high frequencies.

On the basis of these studies and clinical responses observed in patients treated with immunotherapy, melanoma can be considered one of the immunogenic types of cancer – perhaps even the most immunogenic cancer.

In the past 30 years many trials focusing on immunotherapy have been performed: in the early days with cytokines, combinations of chemotherapy and cytokines, peptide vaccine trials, other vaccine trials (including DNA vaccines, viral vaccines, whole-protein vaccines, tumour-cell vaccines and

dendritic-cell vaccines) and adoptive cell therapy with LAK cells, melanoma-specific T-cell clones or peripheral-blood-derived melanoma-specific T cells. With the exception of high-dose IL-2, many trials failed, including the combination of chemotherapy and cytokines, the LAK cell therapy and many vaccine trials. Others showed responses in a minority of patients, some of which were very durable, but many strategies were not taken to phase III trial level because of lack of activity. In the past decade, immunotherapy has become much more successful, and ipilimumab is the first therapy to show an improvement in overall survival (OS). It is likely that also new developments such as anti-PD1/PDL1 will change the survival of patients. Adoptive cell therapy has become a potent therapy and will hopefully be investigated in randomised controlled trials (RCTs) as well. This review focuses on the therapies that have impacted on the lives of stage IV melanoma patients.

2. Clinical immunotherapy of metastatic melanoma

2.1. Immunotherapy by infusional high-dose IL-2 boluses

High-dose interleukin-2 was tested in murine models of sarcoma and melanoma and shown to lead to regression of established transplantable pulmonary metastases and subcutaneous tumours. The idea was that infusion of high-dose IL-2 led to the activation of lymphocytes, generating lymphokine-activated killer cells *in vivo*, since infusion of *in vitro* activated lymphocytes was highly active in murine tumour models. In 1985, based on the observations in mouse models, the first patients with metastatic cancer (mostly melanoma) were treated with purified IL-2, given as bolus infusions intravenously (i.v.) every 8 h. In some patients with melanoma objective partial clinical responses were seen [19]. Toxicity in these patients consisted of fever, chills and gastrointestinal tract symptoms such as nausea and diarrhoea, hypotension, severe weight gain, anaemia, leucocytopenia and thrombocytopenia [20]. In Europe, studies with continuous high-dose IL-2 i.v. infusion led to even more toxicity; many patients required admission to the intensive care unit (ICU) and some patients succumbed to this treatment. In many studies, especially in patients with either metastatic melanoma or metastatic renal cell carcinoma, lower doses of IL-2 have been tested. Although clinical responses were observed in a minority of patients, the durability of these responses has been short. On the basis of consistent achievement of durable complete remissions in 5–10% of patients with high-dose bolus IL-2 infusions in phase II trials, this treatment was Food and Drugs Administration (FDA)-approved for the treatment of metastatic melanoma in 1998, because of an unmet need in this patient population [21]. High-dose IL-2 still is one of the treatment options for stage IV melanoma (and for metastatic renal-cell carcinoma) in the United States (US). In particular, patients with good performance status and M1a or M1b disease may benefit from this treatment. In Europe, high-dose IL-2 for these indications has not been approved and is therefore hardly used.

High-dose bolus IL-2 is given in a dose of 600,000–720,000 IU/kg as an i.v. bolus (15 min infusion), every 8 h for no more than 15 boluses, followed by about 10 days of rest, followed by another 15 infusions. This is considered one course. Patients are followed every 2–4 months for prolonged periods of time.

The exact mechanism of action of high-dose IL-2, despite its presence in the clinic for over 20 years, remains elusive. IL-2, discovered as a T-cell growth factor in 1976 [22], is a 133-amino-acid protein which binds to the IL-2 receptor (IL-2R) present on T cells, B cells and NK cells. The IL-2R can consist of two or three chains, the IL-2R α , IL-2R β and IL-2R γ chains. The IL-2R α and β chains form the low-affinity IL-2R and all three chains form the high-affinity IL-2R. Both receptors can deliver signals upon binding IL-2. Since the IL-2R is widely expressed on cells from the adaptive immune system, the presence of IL-2R (on subpopulations of cells) is not a predictive biomarker for response to treatment. In fact, so far no biomarker of response has been found for high-dose IL-2 treatment. Recently, in a retrospective study, a non-statistically greater objective response rate was found for patients with melanomas harbouring an NRAS mutation (compared to BRAF mutation or wild-type tumours) [23]. Wang et al. studied the molecular patterns associated with response to treatment and observed that high-dose IL-2 has immense impact on gene profiles of peripheral-blood mononuclear cells (PBMCs), while the molecular changes within the tumours were small and differed between lesions [24]. Analyses of transcriptional profiles pre- and post-treatment with high-dose IL-2 in PBMCs did not reveal a statistically significant signature. Interestingly, within the same tumours analyses on pre- and post-fine-needle aspirates did not show important changes within genetic profiles; however, an immune response signature present pre-treatment was associated with better prognosis: complete remission (CR), partial remission (PR) and stable disease SD versus progressive disease PD. These results suggest that response to immune therapy with IL-2 is predetermined and can be measured by the presence of an immune response genetic signature within the tumour. However, the study was small, and validation in a larger study is warranted before gene profiling can be used to select patients for high-dose IL-2 treatment.

Clinical biomarkers that are associated with response result from pooled retrospective analyses of metastatic melanoma patients treated with high-dose IL-2 in several trials. Durable responses were almost only observed in patients with Eastern Cooperative Oncology Group (ECOG) 0–1 performance status and pulmonary, lymph-node and subcutaneous metastases (M1a and M1b) [25].

Despite the lack of knowledge on the mechanism of action of high-dose IL-2, this treatment remains one of few that gives rise to durable CRs. Probably a large proportion of these CRs are cured from melanoma [26].

In the past years, IL-2 has been combined with other therapies. These combined modalities consisted of IL-2 with or without gp100 peptide vaccination [27], IL-2 combined with stereotactic radiotherapy (RT) [28], IL-2 combined with anti-CTLA4 antibody ipilimumab [29] and IL-2 combined with infusion of ex vivo expanded tumour-infiltrating lymphocytes [30]. The only randomised controlled study was performed by Sch-

wartzentruber et al., which illustrated an improved response rate and progression-free survival for the combined modality arm consisting of gp100 peptide vaccine + high-dose IL-2 compared with high-dose IL-2 alone [27]. Combinations of stereotactic RT and IL-2 or ipilimumab and IL-2 were tested in small single-arm phase I/II studies [28,29]. Both combinations showed an unexpectedly high response rate, including complete remissions.

Taken together, high-dose IL-2 has been the oldest approved form of immunotherapy for metastatic melanoma. Despite the development of new immunotherapies, high-dose IL-2 remains a valid treatment option, especially in the US.

2.2. Immunotherapy by immune checkpoint blockade

For T-cell activation a dual signalling step is required. The first essential step is binding of the T-cell receptor to its cognate antigen, the major histocompatibility complex (MHC) peptide complex presented by antigen-presenting cells (APCs). The second step is binding of the co-stimulatory molecule CD28 to CD80/CD86 (B7.1/B7.2) on the APC. The combined signalling leads to full T-cell activation, resulting in up-regulation of IL-2 and IL-2R gene expression and cell division. Next to CD28, T cells also express cytotoxic T-lymphocyte antigen-4 (CTLA4), a co-inhibitory molecule, which binds the same ligands as CD28 but with higher affinity [31,32]. Due to differences in both spatial and timely expression of CD28 and CTLA4, CTLA4 will appear at the cell surface later during the immune response and will then out-compete CD28 signalling [33]. Signalling through CTLA4 will stop IL-2 and IL-2R gene transcription and cell proliferation. Its key role as a regulator of immune responses was well established in CTLA4-deficient mice that, upon exposure to environmental antigens after birth, develop a severe and lethal lymphoproliferative disease due to uncontrolled and persistent T-cell activation, proliferation and infiltration in peripheral tissues [34]. Blockade of CTLA4 signalling by monoclonal antibodies has demonstrated anti-tumour activity in murine models. In the case of immunogenic tumours, single-agent CTLA4 blockade was enough to induce tumour shrinkage, whereas in other models anti-CTLA4 synergised with other treatment modalities to induce efficacious antitumour immune responses (reviewed in [35]). In the B16 melanoma model, the combination of vaccination with irradiated GM-CSF gene transduced tumour cells and CTLA4 blockade was successful in eradicating the tumour [36]. These animals developed autoimmune depigmentation or vitiligo, which was dependent on CD8 T cells, indicating breaking of immune tolerance in these animals. CTLA4 is expressed not only by CD8 T cells, but also by CD4 T cells and even high by CD4 FoxP3 regulatory T cells. Whether anti-CTLA4 works through the blockade of CTLA4 on CD4 and CD8 T cells, or through another mechanism involving regulatory T cells, has still not been revealed [37].

Two fully human monoclonal antibodies were developed for use in humans, ipilimumab (MDX-010) and tremelimumab (CP-675,206). Ipilimumab was the first monoclonal antibody to be tested in patients with metastatic melanoma [38]. In these early studies, which enrolled only a few patients, tumour regressions and autoimmune adverse events were observed.

Tremelimumab was tested in a classical dose-escalating phase I trial [39]. Comparable to ipilimumab, tremelimumab also leads to tumour regression, and also to uncommon toxicities, including dermatitis, colitis, hepatitis and hypophysitis, indicating that immunological tolerance was broken in some patients treated with CTLA4 blockade. Originally, an association between the incidence of immune-related adverse events and clinical response were thought to be present [40]; however, this could not be confirmed in the randomised controlled trials that have been performed with these agents. Ipilimumab was studied in two large randomised controlled trials [41,42]. The first trial was a second-line study in stage IV melanoma; 676 HLA-A*0201-positive patients were randomised in a 3:1:1 ratio between the combination of ipilimumab and gp100 vaccine, ipilimumab (+ placebo) and gp100 vaccine (and placebo). In this study ipilimumab was given in a dose of 3 mg/kg every 3 weeks four times. The primary endpoint of the study was overall survival. With a median follow-up of between 17 and 28 months, a statistically significant difference in median survival was observed in both ipilimumab arms (10.0 and 10.1 months) compared to gp100 vaccine alone (6.4 months). The objective response rate for ipilimumab plus vaccine was 5.7% and for ipilimumab alone 10.9% compared with 1.5% for the gp100 vaccine group. At 1 year 43.6% and 45.6% of patients in the ipilimumab arms and 25.3% in the vaccine arm were alive, also at 2 years 21.6%, 23.5% and 13.7% respectively. Grade 3-4 immune-related adverse events were experienced by 10-15% of the patients, and seven deaths (1%) were associated with immune-related side effects. Although preclinical data and an early clinical trial suggested synergy between gp100 vaccine and CTLA4 blockade [38], this could not be confirmed in this large RCT. Based on the statistically significant improvement in overall survival, ipilimumab was approved as first- (US) or second-line (European Union (EU)) treatment for patients with stage IV melanoma. In the second phase III trial ipilimumab combined with dacarbazine was compared with dacarbazine alone. Here ipilimumab was given in a dose of 10 mg/kg every 3 weeks four times, followed by maintenance every 3 months. Comparably to the second-line ipilimumab trial, this trial also found a statistically significant improvement in overall survival in the patients treated with ipilimumab plus DTIC (11.2 months) compared with DTIC plus placebo (9.1 months). At 3 years, 20.8% of patients in the ipilimumab arm were still alive compared with 12.2% in the DTIC alone arm. In 56.3% of patients grade 3-4 adverse events were observed. Whereas in the MDX-010-20 trial gastrointestinal adverse events were most frequent, only 36% of patients received in the second trial all four doses of ipilimumab treatment, mostly because of liver toxicity. This unexpected observation of hepatotoxicity was attributed to the combination of DTIC plus ipilimumab. Hence, the combination of DTIC plus ipilimumab is not recommended.

Tremelimumab was tested in a classical phase I design and the recommended dose for phase II studies was 15 mg/kg every 3 months [43]. Subsequently tremelimumab was studied in a randomised controlled phase III trial in stage IV melanoma patients as first-line therapy compared with dacarbazine [44]. Although a trend towards improved overall survival was seen in this study, this difference was not statis-

tically significant. In part this may have been due to the fact that patients with lactate dehydrogenase levels more than twice the upper limit of normal were excluded from the study, whereas these patients were included in the ipilimumab pivotal trials. Therefore, the survival in the control arm may have been better and the difference in overall survival (OS) between the two arms smaller. In addition, at least 16% of patients in the dacarbazine arm were treated with ipilimumab upon failure, which may also have contributed to a better OS in the control arm. Patients with an objective response to tremelimumab had a considerably longer duration of this response (35.8 months) compared with patients responding to dacarbazine (13.7 months).

Recently, in a series of 752 patients who were treated with ipilimumab for stage IV melanoma, 120 adverse events were described [45]. These adverse events ranged from drug reactions – sometimes severe and accompanied with eosinophilia and systemic symptoms (DRESS syndrome), small bowel perforation, ischaemic gastritis, hepatitis, pancreatitis, nephritis, hypophysitis, aseptic meningitis, alveolitis and even cardiac fibrosis. Others had already described rare conditions such as Guillain-Barré syndrome and sarcoidosis [46,47]. Algorithms have been developed to treat patients that develop adverse events. Most patients will require immediate corticosteroid therapy, and sometimes other immunosuppressive agents such as infliximab in the case of severe colitis that does not respond promptly to high-dose steroid therapy, and mycophenolate mofetil or even anti-thymocyte globulin (ATG) in the case of fulminant hepatitis.

In summary, CTLA4 blockade is an aspecific immunotherapeutic strategy which was the first therapy to show a statistical significant improvement in median overall survival in melanoma in two phase III trials. About 20-25% of patients will experience durable, mostly partial remissions, some even complete remissions. Ipilimumab is the only approved immunotherapeutic drug. Toxicity of ipilimumab occurs in about 50-70% of patients, with 10-20% being serious, mostly immune-related adverse events. Preferably, ipilimumab should be administered to patients by experienced clinicians. Ipilimumab has been approved for first- or second-line therapy in the US and as second-line treatment in Europe. Patients with absolute lymphocyte count $>1 \times 10^9/L$ or with an increase in ALC at the second infusion are more likely to benefit [48,49]. However, validated predictive biomarkers are still lacking.

Next to CTLA4, programmed death-1 (PD1) protein is another cell-surface molecule that has inhibitory properties [48,49]. In contrast to CTLA4, PD1 expression is involved in inhibition of T cells in peripheral tissues during inflammation [37,50]. Upon activation, PD1 is expressed on CD4⁺ and CD8⁺ T cells and B cells, which results in the inhibition of e.g. T-cell-receptor- (TCR-)mediated signalling, probably through activation of phosphatase SHP2 [51]. The ligands of PD1 are PD-L1 (B7-H1) and PD-L2 (B7-CD) on APCs [52]. However, PD-L1 expression may also be induced on tumour cells [53,54]. Interaction between PD1-positive T cells and PD-L1-expressing tumour cells was therefore suggested to hamper proper T-cell function and appears to be one of the immunosuppressive mechanisms executed by tumours to escape an initially ongoing immune control [54,55]. Similarly to CTLA4 expression on

regulatory T cells, also PD1 is highly expressed on these FoxP3⁺ CD4 T cells. Therefore the blockade of PD1 by anti-PD1 antibodies may work through breaking the inhibitory interaction between PD1⁺ CD4 and CD8 T cells and PD-L1-expressing tumour cells, or by decreasing the number or function of regulatory T cells. Similarly, antibodies specific for PD-L1 can restore the function of tumour-specific PD1⁺ CD4 and CD8 T cells.

Both anti-PD1 and anti-PD-L1 antibodies are now in clinical trials. Nivolumab (MDX-1106; BMS 936558; ONO-4538) was the first anti-PD1 antibody to be tested in a phase I study ($n = 39$) as a single agent in several tumour types, including melanoma, renal cell carcinoma and non-small-cell lung cancer (NSCLC) [56]. Based on its mechanism of action, similar toxicity as had been seen in anti-CTLA4 treatment was expected; however, anti-PD1 – given in doses ranging from 0.3 to 1, 3 and 10 mg/kg – was quite safe. After one dose no dose-limiting toxicity was observed. Grade 3 toxicity consisted of CD4 lymphopaenia, fatigue and musculoskeletal problems. As far as immune-related adverse events were concerned, one patient developed colitis and one patient hypothyroidism. The first responded to corticosteroids and infliximab, the other was treated by thyroid hormone replacement. Since anti-PD1 treatment was well tolerated, the maximum tolerated dose (MTD) could not be determined from this study.

Recently, the results from the extension phase of this phase I study – involving 296 patients with either melanoma, renal-cell carcinoma (RCC) or NSCLC – were published [57]. Objective response rate in melanoma was 28%, and the majority of these responses were durable, lasting longer than 1 year. Interestingly, the authors found a strong correlation between cell-surface expression of PD-L1 on tumour cells and response to anti-PD1 treatment. So far an objective response was observed in none of the patients lacking tumour PD-L1 expression, indicating that PD-L1 expression may be an important predictive biomarker for treatment with anti-PD1.

Brahmer et al. published the results from a phase I study with anti-PD-L1 (MDX-1105) [58]. In total, 207 patients were treated, of whom 52 had metastatic melanoma; 17% of the melanoma patients developed an objective response. Only a minority of patients developed grade 3–4 toxicity (9%). Immune-related adverse events were also observed during this study, but were manageable. Also for anti-PD-L1 the MTD could not be defined. Apart from MDX-1105 and MDX-1106, several other antibodies are currently in development: CT-011 and MK-3475 are both anti-PD1 antibodies, RG7446 and MEDI4736 are anti-PD-L1 antibodies, while MP-224 is an Fc-fused PD-L2, a molecule that inhibits PD-1 signalling.

In summary, blockade of PD1/PD-L1 interaction at the tumour site by inhibitory antibodies appears to be another promising immunotherapeutic strategy for the treatment of melanoma. These drugs seem less toxic than anti-CTLA4 and appear to induce a higher objective response rate, of which a large proportion appears to be durable. Large randomised controlled trials with these drugs are ongoing. So far, on the basis of extended phase I trial results, the toxicity profile of anti-PD1 appears to be better compared with ipilimumab. In addition, the response rate of anti-PD1 appears

to be higher in patients with metastatic melanoma. Randomised controlled trials comparing ipilimumab directly with anti-PD1 or the combination of ipilimumab and anti-PD1 are ongoing and should reveal which patients will benefit most from anti-PD1 treatment and when.

3. Immunotherapy by adoptive cell therapy

3.1. Tumour-infiltrating lymphocytes (TILs)

In the 1980s, adoptive cell therapy was tested in clinical trials based on the observation in murine models that infusion of *in vitro* IL-2-activated lymphocytes was highly effective in the eradication of tumours. The infusion of these so-called lymphokine-activated killer (LAK) cells in melanoma patients was compared with high-dose IL-2 alone and was not shown to be statistically significantly superior in response rate, progression-free survival or other outcomes of the trial [59]. It took until early 2000 before the infusion of T cells led to impressive response rates in substantial numbers of patients with metastatic melanoma. Dr. S. Rosenberg and colleagues showed that infusion of *in vitro* cultured TILs derived from a large melanoma metastasis was able to induce regression of bulky metastatic disease and even complete remissions in some patients [30,60]. From previous experiments and mouse models, it became apparent that prior depletion of host lymphocytes greatly improved the *in vivo* survival of the infused TILs, and a short *in vitro* culture time was important for survival, *in vivo* expansion and efficacy. Based on these observations, heavily pre-treated metastatic melanoma patients were treated with lympho-depleting chemotherapy, consisting of high-dose cyclophosphamide and fludarabine, followed by infusion of large numbers of TILs (around 1×10^{11} cells) followed by bolus infusion of high-dose IL-2 (up to 15 boluses) [30,60]. This conditioning regimen results in short but deep leukopaenia, including neutropenia and lymphopaenia, but is non-myeloablative and thus does not require peripheral haematopoietic stem cell (HSC) support. Mouse models showed that depletion of the lymphocytic compartment not only results in the creation of physical space for the infused TILs, but also results in much less competition from host lymphocytes for the homeostatic cytokines IL-7 and IL-15, giving a head start to the infused cells [61]. Secondly, lympho-depletion also diminishes the immunosuppressive cell populations, such as regulatory T cells from the circulation. Based on the results from the first trial, in later studies lympho-depleting regimens were intensified to combination of chemotherapy with total body irradiation (TBI) up to 12 Gy [62]. Naturally, this heavy conditioning regimen necessitated bone-marrow rescue by HSC support. In small phase I/II studies each with 25 patients, escalation of TBI combined with cyclophosphamide plus fludarabine resulted in further improvement in response rates to up to 72%, with 10–20% of patients acquiring a durable complete response. Not surprisingly, the treatment was harsher on the patients, resulting in more acute adverse events and prolonged organ dysfunction [62]. In the early studies cultured TILs were selected for reactivity against autologous melanoma cell lines, and if not present HLA-matched allogeneic melanoma cell lines. The

process of selection, however, required extra culture time, since the final dose of infused T cells was around 1×10^{11} cells. In subsequent studies, this selection step was deleted from the protocol, which simplified the production process substantially. Importantly, this did not harm the objective response rate, which remained around or above 50%, including the occurrence of complete responses [63]. The simplified protocol of non-selected TILs was subsequently adopted by several laboratories in the US and in Israel [64–66]. By now several studies using this protocol, but without the addition of TBI to the non-myeloablative chemotherapy regimen, have shown a highly consistent objective response rate of 40–50% of treated patients. In order to widely distribute TIL treatment over Europe, a randomised controlled trial is required.

3.2. Gene-modified T cells

Because TIL therapy is not always feasible, and because of the complexity of the treatment, alternatives for adoptive cell therapy with TILs have been developed. One of the most promising new strategies is the use of tumour-specific antigen receptors [67]. These tumour-specific receptors can be derived from a tumour-specific T-cell clone with a high-affinity T-cell receptor (TCR) that recognises a human MHC/tumour-derived peptide complex, or from a high-affinity antibody-specific for a cell-surface tumour antigen. These antibody-based receptors, called chimeric antigen receptors (CARs), are genetically fused to proteins of the T-cell receptor signalling machinery (CD3 ζ , CD28 and others), so that T lymphocytes genetically changed to express these receptors upon antigen binding will be properly activated [68].

For the genetic transfer of TCR or CAR genes to T cells several options exist; however, the most widely used is transfer by retroviral infection. These retroviruses and lentiviruses are genetically modified to contain the genes for a specific CAR/TCR. In addition, these viruses have been crippled to prevent replication. Upon infection of the T lymphocytes these viruses place the genes encoding the CAR/TCR more or less randomly into the host-cell genome. Thus, these receptors can be genetically transferred into peripheral-blood T lymphocytes, thereby creating an army of tumour killer cells.

So far tumour-specific T-cell receptors have been used only for diseases other than melanoma; for example, a high-affinity CD19 binding antibody has been used successfully in B-cell lymphomas/leukaemia [69–71].

For melanoma, patients have been treated with TCR-transduced T cells specific for MART-1, NY-eso-1 and more recently also for MAGE-A3 [72–75]. In all three trials, objective responses have been observed. So far, gene therapy with the NY-eso-1-specific TCR was most effective and safest. In the trials with the MART-1-specific TCR, a substantial portion of patients developed on-target toxicity due to T-cell attack on MART-1-positive cells in the body, leading to severe dermatitis and vitiligo, uveitis and hearing loss (Kogt–Koyanagi–Harada disease). In most instances these side effects were transient and responsive to topical corticosteroids.

Apart from on-target toxicity – which is more likely to occur for TCRs specific for over-expressed gene products or differentiation antigens, as normal tissues often also express

these antigens – off-target toxicity is another potential danger of this treatment. The most important reason for this type of toxicity is cross-reactivity of the introduced TCR with an unknown antigen. Since TCRs recognise MHC peptide complexes, and since most individuals have six different MHC class I alleles, the chance of a TCR having affinity for one of these plus an unknown peptide is not just theoretical. Choice of target and knowledge about tissue expression of the antigen for which the TCR is specific is going to be crucial.

Taken together, adoptive cell therapy is still at the level of early clinical trials; however, the efficacy of this treatment is promising, with establishment of durable remissions in some patients. With more centres performing these trials, the experience with this complex therapy is rapidly increasing. Therefore, this treatment should be taken to the next level: randomised controlled trials.

4. Conclusion

In conclusion, immune therapy of melanoma is by 2013 an established and expanding strategy that can induce durable responses in advanced-stage melanoma patients, some of whom may be cured for life. Immunotherapy, however, comes with a price. New and unexpected toxicities may develop during the course of the treatment or after cessation of treatment and requires experience in order to properly manage these therapies. In addition, these therapies are costly and sometimes highly complex (TIL and TCR/CAR gene therapy). Therefore research focused on finding biomarkers that predict response to treatment, such as PDL1 tumour expression for response to anti-PD1, will be one of the most important challenges for the coming years.

Apart from immunotherapy as a novel treatment option for patients with stage IV melanoma, targeted therapy has also recently changed the outcome of these patients. Drugs such as vemurafenib, dabrafenib and trametinib have been shown to prolong survival of metastatic melanoma patients harbouring the common BRAF V600 mutation. With all these new therapies now available, studies on selecting the best patient population for each therapy, and on the optimal sequence of treatments, will be key to most effectively prolonging the lives of metastatic melanoma patients, also on the use of these therapies in the most cost-effective manner.

Conflict of interest statement

None declared.

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