Immunotherapy of Melanoma

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Summary

Arising from melanocytes in skin, mucosal membranes, eye, and meninges, melanoma is a tumor that has been associated with poor prognosis in advanced disease stages. Given the poor response to chemotherapy and radiation therapy, new treatment approaches with targeted therapy, immunotherapy, and adoptive T-cell therapy have revolutionized the standard of care for patients with advanced melanoma. This review provides a short overview of past, present, and future immunotherapeutic approaches and their limitations, with a focus on new combination agents in early clinical trials.

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Especially in cutaneous melanoma (CM), there have been a number of case reports observing spontaneous tumor regression. This phenomenon occurs mostly in primary melanoma lesions, but when observed in metastases, it is often associated with a favorable outcome [4]. Spontaneous tumor regression has been linked to efficient immune response to melanoma, and tumor-infiltrating lymphocytes (TILs) and tumor antigens have been studied in this context. Thomas et al. [5] conducted a follow-up of 2,845 patients with primary melanoma and reviewed specimens for the amount of TIL infiltration (absent, nonbrisk, or brisk). A higher TIL infiltration grade in the primary melanoma was associated with a lower melanoma-specific mortality, independently of the tumor characteristics currently used for AJCC tumor staging.

Further clinical evidence for immunosurveillance in melanoma development is seen in immunosuppressed patients, e.g. transplant recipients. These patients have an increased risk for developing melanoma and for death as a result of melanoma [6].

Melanoma – a Model Tumor for Immunotherapy

In 2012, melanoma was among the 10 most common cancers in Europe, with an estimated 100,000 new cases diagnosed and more than 22,000 expected deaths due to the aggressive skin cancer [1]. When diagnosed and treated at an early stage according to the American Joint Committee on Cancer (AJCC) classification [2] based on the depth of invasion, ulceration, and mitotic rate, it is curable by surgical excision in more than 90% of cases. In contrast, advanced melanoma is associated with a poor prognosis [3].

Arising from melanocytes in skin, mucosal membranes, eye, and meninges, melanoma is a tumor with poor response to chemotherapy and radiation therapy; yet, it has developed into a model tumor for immunotherapy.

Current Therapeutic Options in Melanoma Therapy

Until 2011, dacarbazine (DTIC) and interleukin-2 (IL-2) were the only Food and Drug Administration (FDA)-approved systemic treatments for metastatic melanoma (MM); in Germany, DTIC, cisplatin and vindesine were approved. Hence, besides treatment in clinical studies, chemotherapy was one of the only options in the treatment of MM with no proven benefit on overall survival (OS) [7].

The discovery of genetic and epigenetic modifications leading to tumor transformation and melanoma progression has led to the advent of new treatment approaches.

One example of individualized, targeted therapy is BRAF inhibition (BRAFi) and, more recently, combined BRAF/MEK inhibition (BRAFi/MEKi). Mutations in the BRAF gene account for 40–60% of oncogenic driver mutations in melanoma [8, 9]. Most oncogenic BRAF mutations cause the valine-to-glutamic acid substitution at codon 600 (V600E), which constitutively activates the

mitogen-activated protein kinase (MAPK) pathway [8]. These agents have shown significant clinical activity in patients with BRAF mutation-positive advanced melanoma [10, 11]. Yet, melanoma progression on BRAFi treatment is a major obstacle for the treating physicians. Despite recent regulatory approval for BRAFi/MEKi, which prolongs progression-free survival (PFS) compared to BRAFi monotherapy, the emergence of resistance remains a clinical problem in melanoma treatment [12].

In the last 5 years, a total of 7 monotherapies and another 3 combination therapies were FDA-approved, more than tripling the number of available treatments and displaying the rapid development in dermato-oncology. Current therapeutic approaches for MM include targeted therapies for patients with BRAF-mutant MM, immunotherapy, oncolytic virus therapy, and chemotherapy.

Immunotherapeutic Agents in Melanoma Therapy

Cytokines

In the adjuvant setting, interferon alpha (IFN- α) is the only approved treatment for patients with melanoma at high risk of recurrence or melanoma with locoregional metastases after surgical resection of the primary lesion and/or local metastases.

Adjuvant IFN- α therapy has been shown to significantly increase PFS. Meta-analyses also display a small benefit for OS, adding up to a 3–5% increase in the 5-year survival rate [13]. Data regarding treatment regimens (low- vs. intermediate- vs. high-dose IFN- α), duration of therapy, and the patient subgroup that will benefit most from adjuvant treatment is inconclusive. At present, the European Organisation for Research and Treatment of Cancer (EORTC) (NCT01502696) prospectively evaluates whether pegylated interferon alpha-2b (pegIFN) will selectively benefit patients with ulcerated node-negative melanoma.

IFN- α exerts antineoplastic activity via multiple mechanisms, leading to the inhibition of tumor cell proliferation and growth. Indirect immunomodulatory effects include upregulation of major histocompatibility complex (MHC)-dependent antigen expression (classes I and II), enabling immune cells to form an effective anti-tumor response and facilitating a decrease in regulatory T cells (Tregs) and a change in cytokine levels in the tumor microenvironment [14]. Directly affecting all phases of the cell cycle, IFN- α prolongs the cell cycle and can induce apoptosis of tumor cells. Furthermore, IFN- α reduces the expression of fibroblast growth factor 2 (FGF2) and the transcription of the vascular endothelial growth factor (VEGF) gene, thus inhibiting tumor angiogenesis [15].

In view of the above, IFN- α as the only approved agent for adjuvant treatment is an eligible option for patients with high-risk melanoma. Yet, efficacy and toxicity have to be discussed with the patient, especially with regard to comorbidities. With IFN- α therapy, the most common toxicities are fatigue, alteration of liver enzymes, pyrexia, headache, and depression [14].

Currently, numerous agents for the adjuvant therapy of MM are being tested inside clinical trials. These include approved treat-

ments for MM stage IV like vemurafenib, dabrafenib, trametinib, and cobimetinib, or antibodies (Abs) against cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and programmed death-1 (PD-1) (see below), as well as vaccines.

Results from EORTC 18071 (ipilimumab 10 mg/kg vs. placebo) in patients with AJCC stage III resected melanoma have shown significant impact for adjuvant ipilimumab on PFS, with data for OS expected next year. This treatment is FDA-approved and is already used in clinical routine in the USA. Another interesting trial evaluating the effect of adjuvant immunotherapy (NCT01274338) comparing ipilimumab 3 mg/kg versus ipilimumab 10 mg/kg versus high-dose IFN- α is still recruiting patients (high-risk stage III/IV melanoma that has been removed by surgery) and will show if ipilimumab has a role in the management of high-risk melanoma.

In Europe, KEYNOTE-054 (NCT02362594) is enrolling patients with completely resected high-risk melanoma (stages IIIA (> 1 mm metastasis), IIIB, and IIIC) to compare adjuvant pembrolizumab therapy to placebo, and will further assess the impact of programmed cell death ligand 1 (PD-L1) expression on the response rates to PD-1 Ab therapy.

In-transit metastases can be treated by intralesional injection of IL-2. IL-2 has been shown to deliver long-lasting remission in this patient subgroup. Intralesional IL-2 led to response rates of up to 80% [16, 17]. The rate of durable complete remissions (CRs) in treated metastases was also remarkable: 70% of the treated patients remained in CR for more than 6 months [17].

A new treatment option for unresected melanoma is intralesional therapy with the oncolytic virus talimogene laherparepvec (T-VEC).

Oncolytic Virus Therapy

First-in-class oncolytic virus T-VEC is a herpes simplex virus type 1-derived oncolytic immunotherapy. T-VEC has been designed to selectively replicate within tumors and produce granulocyte macrophage colony-stimulating factor (GM-CSF) to augment local and even distant immune responses [18]. The European Commission has approved the use of IMLYGIC™ (talimogene laherparepvec/T-VEC) for the treatment of adults with unresectable melanoma that is regionally or distantly metastatic (stages IIIB, IIIC, and IV M1a), with no bone, brain, lung, or other visceral disease. IMLYGIC is the first oncolytic immunotherapy to demonstrate therapeutic benefit for patients with MM in a phase 3 clinical trial. Here, T-VEC was compared with GM-CSF in patients with unresected stage IIIB/IV melanoma [18]. T-VEC is applied as intralesional or intranodal injection and has been shown to enhance the antitumor immune response especially in patients with early-stage disease, low tumor burden and activity, and limited visceral metastatic disease (M1a).

In Europe, the phase 2 study TVEC-325 (NCT02366195) presently evaluates the safety and tolerability profile of this agent in unresected melanoma. Also, studies evaluating a combination therapy with other immune therapies like ipilimumab and pembrolizumab are currently enrolling patients (NCT01740297, NCT02263508).

In the last decades, vaccines for adjuvant treatment of high-risk melanoma have been extensively studied, without demonstrating significant clinical benefit.

Cancer vaccination approaches in melanoma include peptide and protein vaccines, recombinant vector-based vaccines, dendritic cell vaccines, and viruses, with the goal to stimulate the immune system and eradicate micrometastatic disease. The targets of these vaccines are tumor-associated antigens. Melanoma-associated antigens comprise differentiation antigens (Melan-A/MART-1, gp100, tyrosinase), cancer/testis antigens (MAGE-A3, NY-ESO-1), antigens with specific mutations (tumor cell-derived antigens), and viral antigens [7].

Results from the DERMA phase III trial, in which melanoma patients received a MAGE-A3 antigen-specific peptide vaccine in combination with an adjuvant, are expected in 2016. However, first results presented in September 2013 were that the coprimary endpoint disease-free survival (DFS) was not met in the overall study population. Current vaccine approaches use polyvalent vaccines targeting multiple tumor-associated antigens like MAGE-A3, NY-ESO-1, and tyrosinase, or dendritic cell vaccines, or investigate patient-specific approaches targeting the patient's individual neoantigen signature.

Immune Checkpoint Blockers

T and natural killer (NK) lymphocytes are considered the main effector cells in cancer immunity and an efficient antitumor immune response has been linked to TILs. In particular, ratios of different TIL subsets are of prognostic value [19].

T cell activation relies on 2 stimulating signals mediated via several surface receptors. The first signal is T cell receptor (TCR) recognition of antigens associated with MHC antigen-presenting molecules. A second costimulatory molecule such as CD28, CD27, GITR, OX40, or ICOS is needed for full activation [20]. Depending on the context of antigen presentation, there are also co-inhibitory receptors leading to downregulation of the T cell response and T cell anergy [21]. Co-inhibitory receptors are CTLA-4, PD-1, TIM-3, and LAG3 and belong to the category of so-called checkpoints.

The clinical impact of monoclonal Abs (mAbs) for checkpoint inhibition (or stimulation) has marked a breakthrough in cancer immunotherapy. Immunotherapeutic agents such as CTLA-4 Abs and PD-1 Abs now offer potent treatment options to melanoma patients. Approved mAbs mainly act as repressors of negative checkpoints, with agonists of costimulatory TCRs being tested in clinical trials.

Immune Checkpoints

CTLA-4

CTLA-4 is a transmembrane protein that emerges in the plasma membrane upon antigen recognition and it is a negative coregulator

of T cell activation. The CTLA-4 receptor acts via different inhibitory mechanisms. It outcompetes binding of CD28 to either CD80 or CD86 (costimulatory pathway), having a 10–100-fold higher affinity. CTLA-4 on Tregs directly leads to their activation and can co-opt CD80/CD86 by transcytosis, resulting in the ablation of costimulatory ligands from antigen-presenting cells (APCs) [21].

Ipilimumab, a mAb directed against CTLA-4, is the first approved immune checkpoint blocker and significantly improves OS, with a subset of patients reaching long-term survival [22]. The approval of the drug was based on a randomized (3:1:1) three-armed study comparing ipilimumab 3 mg/kg intravenous (i.v.) to gp100 peptide vaccine (gp100) to ipilimumab 3 mg/kg i.v. plus gp100. A total of 676 pretreated patients with unresectable melanoma or MM were included. The median OS was longer with ipilimumab and ipilimumab plus gp100 compared to gp100 alone (10.1 and 10.0 vs. 6.4 months, respectively) [22]. Despite reaching the primary endpoint of improved OS (ipilimumab 3 mg/kg i.v. plus gp100), an observation of higher interest was that, after a median follow-up of 17 months, 22% of the treated patients were still alive. Besides IL-2 treatment, this was the first time that durable responses in MM patients were seen.

PD-1

PD-1 is another co-inhibitory receptor expressed on T cells. Its ligands PD-L1 and PD-L2 have a broad tissue distribution and are expressed in peripheral tissues and cancers of hematopoietic and non-hematopoietic origin [23]. The normal function of PD-1, expressed on the cell surface of activated T cells, is to downmodulate unwanted or excessive immune responses, including autoimmune reactions [24]. PD-1 expression can be physiologically upregulated by interferon- γ (IFN- γ) and type I interferons and is often seen in exhausted T cells [21].

The PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and is a major pathway hijacked by tumors to suppress immune control [25, 26]. Binding of either PD-1 ligand to an anti-PD-1 Ab inhibits T cell activation triggered through the TCR. Blocking the interaction between PD-1 and its ligands PD-L1 and PD-L2 thereby results in augmentation of the immune response, including the antitumor response.

Pembrolizumab and nivolumab are mAbs directed against PD-1 and are currently approved to treat MM, metastatic non-small-cell lung cancer, and metastatic renal cell carcinoma (nivolumab).

In melanoma, PD-1 blockade was shown to prolong PFS and OS [27]. It is effective independently of the BRAF mutational status. Data from the KEYNOTE-006 study depicted improved response rates of 33.7% and 32.9%, respectively (pembrolizumab 10 mg/kg i.v. every 2 weeks or every 3 weeks, respectively), compared to CTLA-4 Ab ipilimumab 3 mg/kg (11.9%) and estimated 12-month survival rates of 74.1% and 68.4%, respectively [28].

The efficacy of first-line nivolumab monotherapy was shown in 418 previously untreated MM patients [29]. Study treatment was nivolumab at a dose of 3 mg/kg q2w or DTIC 1000 mg/m² body surface area q3w. OS after 12 months was 72.9% in the nivolumab group, as compared to 42.1% in the DTIC group. The median PFS

for nivolumab was 5.1 months, with an objective response rate of 40.0%. In MM, the approved dosing schedules are 2 mg/kg q3w for pembrolizumab and 3 mg/kg q2w for nivolumab.

Ipilimumab and nivolumab combination treatment raised the bar regarding higher objective response rates of 61% versus 11% with ipilimumab monotherapy, which led to FDA approval in late 2015 [30] and EMA approval just recently. This benefit comes at the cost of tolerability. Drug-related adverse events (AEs) of grade 3 or 4 were reported in 54% of the patients who received the combination therapy, and 38% had to discontinue treatment because of drug-related AEs. The median duration of response and the median PFS with the combination therapy were not reached at the time of publication.

Immune-Related AEs

Treatment with immune checkpoint inhibitors frequently leads to AEs [22, 27, 31]. The mechanism of action of these drugs is closely connected to the side effects: By removing CTLA-4- or PD-1-mediated protection from autoimmunity, autoimmune-inflammatory side effects are seen, classified as immune-related AEs (irAEs) [32].

The most common irAEs observed under ipilimumab therapy are diarrhea/colitis, dermatitis, hepatitis, and endocrinopathy [33]. These AEs can be severe to life-threatening (grades 3–5 according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) [34]) and have resulted in drug-related deaths [22]. Even though therapy can induce severe side effects, these are almost always manageable by treatment interruption, immunosuppressive therapy, or both [32]. When adhering to published algorithms on the management of side effects, most patients who experience irAEs return to baseline within weeks [33]. One exception is immune-related endocrinopathy, which is usually more persistent and can require ongoing hormone replacement therapy [35].

The occurrence of these irAEs is significantly lower in patients treated with anti-PD-1 Abs like pembrolizumab or nivolumab than with the anti-CTLA-4 Ab ipilimumab [28]. In clinical trials, Abs that block the PD1/PD-L1 axis display a greater therapeutic index mainly because their effect is more focused on the tumor microenvironment in which PD-L1 is overexpressed [36]. Safety data states that anti-PD-1 therapy is generally well tolerated, with fewer and less severe toxicities [28]. irAEs frequently affect the skin, the thyroid gland (leading to hypo- or hyperthyroidism), and the lungs. Patients previously treated with ipilimumab have a safety profile similar to that reported for ipilimumab-naïve patients [15, 27].

Biomarkers Corresponding with Response

In the treatment of MM, immunotherapy is widely used and applied as first- and second-line treatment. Yet, predictive and prog-

nostic biomarkers that have been prospectively validated in large patient cohorts are still missing.

In general, higher TIL infiltrate grades prior to immunotherapy were associated with an improved clinical outcome. In contrast, tolerogenic APCs, Tregs, and myeloid-derived suppressor cells (MDSC) that suppress adequate tumor immune response were correlated with poor treatment outcomes [37, 38]. Serum markers like lactate dehydrogenase (LDH), C-reactive protein, and VEGF have been associated with CTLA-4 Ab therapy outcome [39]. Especially for patients with a high serum LDH level, it was demonstrated that the benefit from ipilimumab therapy is limited. High levels of LDH can reflect a high tumor burden or high tumor metabolism, leading to lactate accumulation and a subsequent decrease in extracellular pH through anaerobic glycolysis. This in turn negatively affects the function of lymphocytes in the tumor microenvironment [39]. Retrospectively, increased ICOS expression on T cells correlated with improved survival in a number of CTLA-4 Ab trials in patients with advanced solid tumors [40, 41].

PD-L1 expression as a biomarker has been a topic of controversy. In MM, evidence from trials with PD-1 Abs showed that the expression of PD-L1 by melanoma cells largely correlates with response [29, 37]. Yet, there were a number of PD-L1-negative patients who also responded to anti-PD-1 therapies, questioning PD-L1 expression as a robust predictive marker. In a longitudinal, immunohistochemical study in 96 primary melanoma lesions and metastases (from 58 patients), Madore et al. [42] assessed PD-L1 expression. Patients were immunotherapy naïve and the median positive tumor cell count overall was low. PD-L1 expression was frequently discordant between intra-patient metastases. PD-L1 was associated with higher TIL grade but not with other known prognostic features.

Lastly, missing harmonization of PD-L1 as a biomarker among the different trials (cut-offs for PD-L1 expression, sensitivity and specificity of the used assays) makes comparisons difficult.

Due to the complexity and heterogeneity of the interaction of the host's immune system with the tumor, convenient and easily acquirable biomarkers are not likely to be found. In a consensus paper on biomarkers for personalized cancer immunotherapy, the Immune Biomarkers Task Force reviewed the latest evidence and technologies to give a recommendation for biomarkers in this field [43]. They suggest a division into immunologically ignorant and immunologically responsive tumors characterized by the mutation antigen profile, the gene signature and epigenetic modification of the tumor and immune cells, the breadth of Ab responses as well as the magnitude, homing capacity, cytotoxic function, and TCR repertoire of the T lymphocytes. Immunologically responsive tumors show a high mutational burden and an activating gene signature/pattern in the mutational analysis. Epigenetic modifications lead to a low Treg/CD3+ ratio and high numbers of CD3+ T cells, and protein microarrays show a robust general Ab response. B/T cell receptors are high in clonality, also showing a high CD3+ T cell count. In the tumor microenvironment, high counts of effector cells are found with a high ratio of effector-to-regulatory T cells (Teff/Treg). Finally, multicolor immunohistochemistry shows a

high count of effector cells accompanied by low counts of suppressor cells and high PD-L1 expression on tumor cells as well as TILs. In this tumor microenvironment, immunotherapy is likely to be efficient in boosting the antitumor immune response.

Co-Inhibitory TCRs Being Targeted in Clinical Trials

Successful treatment with CTLA-4 and PD-1 Abs has validated the principle that modulation of the immune response can produce objective antitumor responses. Currently, a range of mAbs targeting other co-inhibitory TCRs are on trial.

LAG-3 and TIM-3

Combined LAG-3 and PD-1 inhibition demonstrated efficacy in mice that were largely resistant to Ab monotherapy [44], and most trials with LAG-3 Abs indeed investigate the effect of LAG-3 Abs with or without PD-1 Ab combination (NCT01968109, NCT02658981, NCT02061761, NCT02676869, NCT02460224) in various malignancies. TIM-3 inhibition is another target receptor on trial. It is selectively expressed on IFN- γ -secreting T cells and upregulated by interferon, with broad expression among healthy and cancerous tissue. As with LAG-3, TIM-3 and PD-1 are uniquely co-expressed in TILs, and in vitro models of combined TIM-3 and PD-1 have led to increased cytokine production in melanoma-specific T cells [45]. Novartis is testing MBG453, a TIM-3 mAb, in a phase I-Ib/II trial as single agent and in combination with its PD-1 Ab PDR001 in advanced malignancies (NCT02608268).

Indoleamine-2,3-dioxygenase

A different approach is targeting indoleamine-2,3-dioxygenase (IDO), a negative immune modulator expressed by APCs in cancerous tissue. IDO is thought to be involved in tumor immune evasion, creating an environment that favors immune suppression and tolerance. It is an enzyme that catalyzes tryptophan degradation. Increased activity leads to a tryptophan deficit in the tumor microenvironment, starving cytotoxic T cells and triggering Tregs [46, 47]. In MM, a phase I/II trial combining IDO and ipilimumab is currently accruing patients (NCT02073123).

Costimulatory TCRs Being Targeted in Clinical Trials

While the focus is on mAbs targeting and thus inhibiting co-inhibitory TCRs, costimulatory TCRs like CD27, CD28, CD137, GITR, OX40, and ICOS can also have the ability to induce profound antitumor responses. Preclinical data suggest that the greatest potential of agents targeting costimulatory TCRs is achieved in combined treatment regimens.

There are costimulatory molecules that are constitutively expressed on resting antigen-naïve T cells such as CD28 and CD27, whereas expression of CD137, GITR, OX40, and ICOS is induced upon previous antigen priming [20]. Ligation with agonist Abs leads to T cell activation.

CD137

CD137 belongs to the tumor necrosis factor (TNF) receptor family and is expressed in several immune cells like CD4+ and CD8+ T cells, Tregs, dendritic cells, and NK cells. Agonistic CD137 mAbs stimulate CD4+ and CD8+ T cells with respect to cytokine production and upregulation of cell survival genes, and prevent their activation-induced cell death. Ligation to APCs results in activation and can depress Treg function [48].

CD137 Abs have shown efficacy in several tumor models, with 2 agents (urelumab and PF-05082566) being tested in early clinical trials. They are agonistic mAbs that bind to the extracellular domain of human CD137. Preclinical data showed increased hepatic toxicity for urelumab monotherapy and combination therapy [49], and 3 studies were withdrawn due to severe hepatotoxicity (NCT00351325, NCT00461110, and NCT00803374). Nonetheless, trials evaluating combination treatment with urelumab at lower doses and nivolumab or pembrolizumab for patients with advanced solid tumors or advanced B cell non-Hodgkin's lymphoma are recruiting patients, with pending safety results.

Combination of PF-05082566 and pembrolizumab is being evaluated in a phase IB study (NCT02179918) [50].

OX40

OX40 is a costimulatory receptor that also belongs to the TNF receptor superfamily and can potentiate TCR signaling, leading to T cell activation by a specifically recognized antigen. Agonistic mAbs targeting OX40 increase antitumor immunity and improve tumor-free survival in patients with advanced cancer [51]. Patients treated with OX40 mAbs showed an acceptable toxicity profile, but treatment failed to deliver strong efficacy, with no objective responses according to the Response Evaluation Criteria in Solid Tumors (RECIST) [52]. Currently, a number of trials evaluating combination regimens with radiation, chemotherapy, and immunotherapy (NCT02205333) have been undertaken. Of note, 5 different agents targeting OX40 are in use in clinical trials, with one of them being an OX40 ligand-Fc fusion protein (OX40L-Fc) with improved efficacy compared to the OX40 mAbs. Different to the Ab approach, OX40L-Fc also led to stimulation of dendritic cells and the tumor vasculature, facilitating the activation, expansion, and recruitment of T cells into established tumors [53].

CD27 and GITR Abs have recently entered clinical trials and phase I/IB studies have been initiated with these agents (NCT01239134, NCT01460134, and others).

Adoptive T-Cell Therapy

Another promising immunotherapeutic approach for MM involves adoptive T cell therapy (ACT), in particular, autologous in vitro expanded TIL therapy. While therapy of solid tumors with genetically engineered T cells (TCR and chimeric antigen receptor (CAR) gene therapy) is still in an early stage of development, TIL therapy has had the longest clinical history, with multiple clinical trials consistently demonstrating durable clinical response rates

near 50% or more [54–59]. An important advantage of TIL therapy is the broad nature of the T cell recognition against both defined and undefined tumor antigens against all possible MHCs, rather than the single specificity and MHC coverage of the newer TCRs and CAR transduction technologies [60]. Recent findings suggest that a crucial component of the therapeutic T cell response targets patient- and tumor-specific neoantigens resulting from somatic mutations and that only a minority of TILs respond to defined melanoma-associated antigens like differentiation antigens (Melan-A/MART-1, gp100, tyrosinase) or cancer/testis antigens (MAGE-A3, NY-ESO-1) [61–68]. This also explains why striking antitumor efficacy has been observed in the absence of significant autoimmune pathology.

For TIL therapy, patients undergo surgical tumor excision, from which TILs are derived followed by ex vivo expansion of these T cells and reinfusion after lymphoablative preconditioning (cyclophosphamide and fludarabine chemotherapy). This treatment schedule is accompanied by significant AEs, mostly related to the high levels of inflammatory cytokines produced by the infused T cells and the high-dose IL-2 that is given concomitantly with T cell infusion. But given the fact that most patients enrolled in TIL studies have advanced disease and have failed multiple prior treatments, the clinical results with overall response rates of approximately 50% and rates of durable CR of 20% show striking clinical activity [56, 57, 59, 69, 70].

Before TIL therapy can become standard of care in MM, a number of issues have to be addressed. First, phase II or III trials with consistent protocols and sufficient patient numbers are needed for regulatory approval of TIL treatment. Second, protocols for TIL expansion have to be improved concerning time in culture and TIL quality, to assure longer T cell persistence and enhanced antitumor activity in vivo. Third, as with immune checkpoint inhibitors, there is a critical need to identify surrogate and predictive biomarkers to better select suitable patients for TIL therapy [60].

At the Heidelberg University Hospital, we are currently planning a phase II trial of TIL therapy in MM patients who have progressed after initial clinical benefit from checkpoint inhibitor treatment, to establish TIL treatment in Germany.

Conclusion and Future Perspectives

The advent of immunotherapy has had a great impact on melanoma treatment and immunotherapy is or will be widely used in the treatment of other solid tumors. A significant fraction of patients responds to these therapies, but as with targeted therapies, the need for complementary strategies in immunotherapy is evident.

Ipilimumab/nivolumab combination therapy has shown that combined therapy approaches can boost response rates, but toxicity has to be carefully evaluated. New agents for combination treatment targeting co-inhibitory and costimulatory TCRs are currently being tested in early clinical trials with pending results.

Over the last years, the idea of a tailored, patient-specific anti-neoplastic treatment approach based on whole-genome sequencing data has evolved and treatments that target tumor-specific growth/survival pathways have shown significant clinical impact in MM (BRAFi, MEKi).

In immunotherapy as well, individual tumors should be assessed for predictive biomarkers such as a pre-existing antitumor immune response prior to initiation of therapy.

Technologies needed to subdivide tumors into immunologically ignorant or immunologically responsive ones are currently not broadly available and expensive. Above all, these potential biomarkers need to be validated in future clinical studies [43].

Efforts to further characterize cellular and humoral components in the tumor microenvironment, their relationship and impact on immunotherapeutic approaches need to be made to understand mechanisms of cancer immunoediting and cancer immune escape. This will provide a scientific basis to design better treatment schedules and combinations for MM patients.

In the near future, we await results for adjuvant treatment of patients with high-risk melanoma. Results from EORTC 18071 (ipilimumab 10 mg/kg vs. placebo) in patients with AJCC stage III resected melanoma have shown a significant impact for adjuvant ipilimumab on PFS, with data for OS expected next year. KEYNOTE-054 (NCT02362594) is currently recruiting melanoma patients for adjuvant pembrolizumab therapy compared to placebo and will deliver data on adjuvant PD-1 Ab treatment.

A growing number of medical centers (USA, Europe, and Israel) are pursuing TIL therapy for melanoma, with great outcomes concerning response rates and response durability. To date, TIL therapy is limited by the rigorous treatment schedule and the time-consuming expansion period. Lastly, in TIL treatment, there is also the need for predictive biomarkers to better select suitable patients.

To finish, new approaches could target other effector cells in the tumor microenvironment like NK cells, APCs, or Tregs and inhibitory T cell populations. So far clinical trials using dendritic cells and NK cells have had only modest clinical success in cancer patients [71, 72]. Further targetable molecules arising from basic science on the function of the immune system and the interaction between the tumor and its microenvironment can be expected and have the potential to increase the efficacy of treatment against MM even further.

Disclosure Statement

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