

ADVANCES IN MELANOMA IMMUNOTHERAPY

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Summary

Melanoma is considered to be the most immunogenic malignant tumour. This fact is recognized for many years, and certain forms of immunotherapy have been used in melanoma therapy for a considerable time.

Treatment options for patients with metastatic melanoma have changed dramatically in the past 5 years, with the FDA approval of eight new therapeutic agents (immunotherapies and targeted therapies). During this period, melanoma immunotherapy has transitioned from cytokine-based treatment to antibody-mediated blockade of the cytotoxic T lymphocyte-associated antigen 4 (CTLA4) and, recently, the programmed cell-death protein 1 (PD1) immune checkpoints. These changes in the treatment options have dramatically improved patient outcomes, with the median overall survival of patients with metastatic melanoma increasing from approximately 9 months before 2011 to at least 2 years, and probably longer.

Various types of immunotherapy, like pembrolizumab, nivolumab, ipilimumab, combined therapy with nivolumab and ipilimumab, and T-VEC, have been established in recent years as the standard-of-care treatment for metastatic melanoma patients.

KEY WORDS: *immunotherapy, melanoma, checkpoint inhibitors, oncolytic vaccines*

NOVOSTI U IMUNOTERAPIJI MELANOMA

Sažetak

Melanomi se smatraju najimunogenijim zloćudnim tumorima. Ova je činjenica prepoznata već niz godina, te se neki oblici imunoterapije već dugo primjenjuju u liječenju melanoma.

Terapijske opcije za liječenje bolesnika s metastatskim melanomom dramatično su se promijenile u zadnjih pet godina, te je Američka agencija za lijekove (FDA) odobrila osam novih lijekova (imunoterapije i ciljane terapije). Tijekom ovog razdoblja, imunoterapija melanoma promijenila se iz terapije bazirane na citokinima u protutijelima posredovanu blokadu citotoksičnog T-limfocitnog antigena 4 (CTLA-4), a u zadnje vrijeme u protutijelima posredovanu inhibiciju imunoloških kontrolnih točaka, prvenstveno proteina programirane stanične smrti 1 (PD-1). Ove su promjene terapijskih opcija dramatično poboljšale ishod liječenja bolesnika, te se medijan ukupnog preživljenja kod bolesnika s metastatskim melanomom povisio s približno 9 mjeseci prije 2011. godine, na najmanje 2 godine, a vjerojatno i dulje. Različiti oblici imunoterapije, poput pembrolizumaba, nivolumaba, ipilimumaba, kombinirane terapije nivolumabom i ipilimumabom, te T-VEC, postali su zadnjih godina standardni oblici liječenja bolesnika s metastatskim melanomom.

KLJUČNE RIJEČI: *imunoterapija, melanom, inhibitori imunoloških kontrolnih točaka, onkolitičke vaccine*

INTRODUCTION

Melanoma is considered to be the most immunogenic malignant tumour with the highest

prevalence of somatic mutations (1). This fact is recognized for many years, and certain forms of immunotherapy have been used in melanoma therapy for a considerable amount of time.

Melanoma, particularly cutaneous melanoma, is amenable to immunotherapy for various reasons, including extensive tumour infiltration by T cells, a high mutational load, and crosstalk between oncogenic signalling pathways and immunobiology.

Treatment options for advanced-stage, unresectable or metastatic melanoma has changed dramatically over a short period of time. Before 2011, metastatic melanoma was considered a disease almost uniformly fatal within 18 months of diagnosis. Standard-of-care treatments during this time included limited chemotherapy options, sometimes in conjunction with interferon-alpha (IFN-alpha) but with no substantial added benefit, and in highly selected patients, immunotherapy with the cytokine interleukin-2 (IL-2) was applied (2).

The median overall survival of patients was roughly 9 months, and no treatment had been demonstrated to improve survival in a randomized phase 3 trial. Since then, due to advances in basic research and better understanding of genomic and immune responses against cancer, eight new melanoma therapies have been approved by FDA and EMA, from the field of immunotherapy and targeted therapy, rapidly changing the therapeutic landscape in metastatic melanoma patients. This transferred to, until now, unprecedented improvement in overall survival of metastatic melanoma patients, changing the therapeutic goals from palliative delay in disease progression in few patients, to durable clinical responses for a substantial patients' number, and effective disease control and palliation in the majority.

In addition, most patients who nowadays develop metastatic disease following a diagnosis of high-risk primary or regionally advanced melanoma have the possibility of surviving for years, owing to the availability of effective treatment options.

Immunotherapy has also achieved advances in adjuvant melanoma treatment, with the neoadjuvant treatment options currently under investigation.

Immunotherapy in metastatic melanoma treatment

High dose interleukin-2 has shown to be the first efficient systemic immunotherapy in metastatic/advanced melanoma, and in USA has been used for many years in highly selective patients, leading to even long term survival, but at a cost of

extremely high, often life threatening toxicity (3). Other cytokines have been studied in advanced melanoma patients as well. Interferon-alpha therapy had limited efficiency in metastatic disease, but had been used combined with chemotherapy (so called biochemotherapy). However, interferon-alpha has a certain role in adjuvant treatment of high-risk melanomas (4). Different melanoma-specific vaccines can comprise of specific antigens, whole melanoma cells, or melanoma cells lysates, and have been investigated both in adjuvant therapy, and in treatment of metastatic disease.

CHECKPOINT INHIBITORS

CTLA-4 inhibitors

Turning point in melanoma immunotherapy has arrived in 2011 with **ipilimumab**, monoclonal antibody directed against cytotoxic T lymphocyte-associated antigen4 (CTLA4), as the first representative of modern immunotherapy options. Two randomized phase 3 studies have shown prolonged overall survival (OS) with ipilimumab in metastatic melanoma with *plateau* of survival curve over three years (5,6). However, new immunomodulatory drugs have emerged, more efficient and less toxic, directed against programmed cell-death protein 1 (PD1). Today, based on results of study CA184-002, ipilimumab monotherapy (where available) is considered valid second line immunotherapy option after failure of more efficient types of immunotherapy. The 3 mg/kg dose is considered the "standard" dose of ipilimumab, however, there has continued to be debate in the field about the most appropriate dose of ipilimumab, and clinical trials have continued to investigate the 10 mg/kg dose.

The use of ipilimumab has provided clinicians with experience regarding specific features of immunotherapy – longer period to therapeutic response (often several months after the beginning of therapy), duration of therapeutic response months after cessation of therapy with the possibility for long-term responses, even complete responses, the potential of partial responses to turn into complete responses with longer follow-up, immune-related side effects (endocrinopathies, pneumonitis, colitis, hepatitis, nephritis, dermatologic side effects...) demanding specific treatment, possible pseudoprogression i.e. transient worsening of the disease (progression of existing

or/and emerging of new metastatic lesions) with the need for different validation of tumour response to treatment using so called immune-related response criteria – irRC.

PD-1 inhibitors

Shortly after ipilimumab, *programmed cell-death protein 1 inhibitors, or anti-PD-1*, namely **pembrolizumab** and **nivolumab**, have appeared in the treatment of metastatic melanoma. These drugs have been shown to be more efficient than ipilimumab, with lower toxicity.

It is important to emphasize that immunotherapy can be used in metastatic melanoma patients regardless of BRAF V600 status (whereas BRAF V600 – positivity is the prerequisite for targeted therapies), and regardless of programmed-death ligand 1 (PD-L1) expression (unlike immunotherapy based on checkpoint inhibition in some other malignant diseases, i.e. lung cancer). Namely, although patients with higher expression levels of PD-L1 have higher response rates (RR) to immunotherapy, longer progression free survival (PFS), and longer overall survival, there is still sufficient benefit (sometimes with even long-term response) in the treatment of metastatic melanoma patients with low expression levels of PD-L1 to treat them with immunotherapy.

Pivotal clinical studies for **pembrolizumab** in metastatic melanoma treatment are Keynote (KN) 001 (7), KN-002 (8), and KN-006 (9, 10). In KN-001 study, overall response rate (ORR) was 33%, with median OS 24 months, OS rates of 73% after 12 months, 50% after 24 months, and 40% after 36 months of follow-up, respectively.

In registration study Keynote-006, pembrolizumab has shown to be more efficient than ipilimumab, with better objective RR (36% vs 13%), better PFS (in three-weekly regimen - 38% vs 19% after 12 months, 28% vs 14% after 24 months, HR=0.61), better OS (68% vs 59% after 12 months, 55% vs 43% after 24 months of follow-up, respectively, HR=0.68), and lower toxicity.

Pivotal clinical studies for **nivolumab** in metastatic melanoma treatment are CheckMate (CM) -037 (11) and -066 (12). In the phase 1 study with the longest follow-up of nivolumab treatment in metastatic melanoma, ORR was 32%, median overall survival was 17 months, and OS rates were 63% (after 1-year of follow-up), 48% (after 2 years),

42% (after 3 years), 35% (after 4 years), and 34% (after 5 years of follow-up), respectively.

In CheckMate-066, treatment naïve patients (i.e. previously untreated), have received either nivolumab or standard chemotherapy protocols with dacarbazine. Patients receiving nivolumab had significantly higher OS rates (73% vs 42%, HR for death 0.42), significantly higher median PFS (5.1 months vs 2.2 months), and significantly higher ORR (40% vs 14%).

Combined immunotherapy (CTLA-4 inhibitors and anti-PD-1 inhibitors)

Combined use of anti-CTLA-4 antibody **ipilimumab** and anti-PD-1 antibody **nivolumab** has higher efficacy in metastatic melanoma treatment compared to monotherapy with either drug, but at the cost of high(er) toxicity.

The largest study exploring this combination in metastatic melanoma treatment was double blind, placebo controlled phase 3 study – CheckMate-067 (13,14) with 945 therapy naïve patients randomized to receive either nivolumab and ipilimumab for three cycles, followed by nivolumab only, or nivolumab monotherapy, or ipilimumab monotherapy (latter for only 4 cycles). Objective response rates were 58% for combination therapy (with 12.1% of complete responses /CR/), 44% for nivolumab monotherapy (with 9.8% CR), and 19% for ipilimumab monotherapy (with 2.2% CR). Median PFS in combination therapy was 11.5 months, compared to 6.9 months using nivolumab monotherapy (HR=0.55), and 2.9 months using ipilimumab monotherapy (HR=0.42). PFS rates after 18 months of therapy were 46% for combination, 39% for nivolumab, and 14% for ipilimumab. Recent results showed the first OS data for CM-067. With a minimum follow-up of 28 months, the median OS had not yet been reached in either of the two nivolumab treatment groups and was 20 months for the ipilimumab monotherapy group. Nivolumab in combination with ipilimumab and as a monotherapy reduced the risk of death 45% (HR 0.55; P<0.0001) and 37% (HR 0.63; P<0.0001), respectively, compared with ipilimumab alone. The two-year OS rates were 64% for the nivolumab plus *ipilimumab* combination, 59% for nivolumab alone and 45% for ipilimumab alone. Severe toxicity and the need for discontinuation of treatment were more frequent in combination arm compared to both monotherapy arms, with 55% of adverse

effects of grade 3 and 4 in combination therapy vs 16% in nivolumab group, and 27% in ipilimumab group.

Despite expectations, sequential use of nivolumab and ipilimumab showed similar toxicity (as well as similar efficacy), as concomitant i.e. combined use of both drugs. Consequently, it does not provide any benefit over combined use.

Just recently, results from two clinical trials presented at ASCO 2017, CheckMate-204 trial (15), and Anti-PD-1 Brain Collaboration (ABC) trial (16), showed that combination checkpoint inhibitors (nivolumab and ipilimumab) resulted in significant PFS benefit for intracranial disease, especially as first line treatment and in asymptomatic CNS melanoma metastases. Therefore, systemic therapy as initial treatment for patients with asymptomatic CNS melanoma metastases is an important new option for the patients and is even considered practice changing.

Unfortunately, mucosal, and even more so, uveal melanoma, represent different entities, with limited efficacy of immunotherapy in mucosal melanoma (17), and basically no effect in uveal melanoma.

ONCOLYTIC VACCINES

Oncolytic vaccine, so called **talimogen – laherparepvec (T-VEC)** has been approved in local, intralesional treatment of patients with unresectable cutaneous, subcutaneous and/or palpable or ultrasonographically detectable lymph node metastases. It is an attenuated herpes-symplex virus type I, programmed to be replicated within tumor and to produce GM-CSF. Durable response rates (DRR), were significantly higher in patients receiving T-VEC compared to GM-CSF, as was overall response rate, with 10.8% of complete responses, and with median of response of 8.2 months. Median OS was not significantly different, but at the borderline of statistical significance (23.3 vs 18.9 months, HR 0.79, P=0.051). With the use of this type of immunotherapy, certain so called abscopal effect was noticed, i.e. antitumor effect in non-injected metastatic lesions and in visceral metastatic sites (18).

T-VEC is currently investigated in clinical trials with various systemic therapies in metastatic melanoma patients.

ADOPTIVE IMMUNOTHERAPY

Various types of adoptive immunotherapy in highly selected patients have, according to some studies, yielded considerable and long-term therapeutic responses, but additional randomized clinical studies conducted on larger number of patients are needed.

OTHER CYTOKINES AND VACCINES

In metastatic melanoma treatment, other cytokines, as well as tumour vaccines have been investigated.

Immunotherapy in adjuvant melanoma treatment

Clinical studies ECOG 1684 (19) and Intergroup E1694 (20), as well as meta – analysis of various studies (21), have shown efficacy of high dose **interferon – alpha (IFN-alpha)** in adjuvant treatment of high risk melanoma patients.

Interferon – alpha is the most efficient in high doses, that are also the most toxic ones, although in some countries, intermediate, even low IFN – alpha doses are used (latter particularly in Germany). **Pegilated interferon** has, in EORTC 18991 study (22) shown benefit in adjuvant treatment in two subgroups of patients, those with lymph node micrometastases (but not in lymph node macrometastases), and those with ulcerated melanomas (unlike non – ulcerated).

In USA, based of results of EORTC 18071 study (23) high doses of ipilimumab have been recently registered in adjuvant treatment of high risk melanoma patients with stage III disease (10 mg/kg every three weeks for four applications, then additional applications every 12 weeks until three years of therapy in total). In this phase III study, patients (N=951) have been randomized to receive either ipilimumab or placebo. Patients receiving ipilimumab had significantly longer relaps-free survival (RFS) – 5-year - RFS 40.8% vs 30.3%, HR 0.76, with significantly longer distant metastasis – free survival (DMFS); 5-year – DMFS 48.3% vs 38.9%, HR 0.76, and significantly longer OS; 5-year OS 65.4% vs 54.4%, HR 0.72, P=0.001. However, this benefit has been achieved at the cost of high toxicity (98.7% of patients receiving ipilimumab had some sort of toxicity, and even 54.1% had adverse events of grade 3 or 4, that, in five patients resulted in treat-

ment-related death), and reduced life quality, that, however, according to study results, did not reach statistical significance.

CheckMate-238, ongoing phase 3 study, presented at ESMO 2017 (24), showed that patients with stage IIIb/IIIc or stage IV melanoma at high risk of recurrence following complete surgical resection had greater recurrence-free survival (RFS) with adjuvant nivolumab compared to adjuvant high-dose ipilimumab. Stage IIIb, IIIc, and IV disease was reported for 34%, 47%, and 19% of patients, respectively. Thirty-two percent of patients had ulcerated primary disease, 48% had macroscopic lymph node involvement, and 42% of patients were positive for the BRAF mutation. RFS was significantly improved with nivolumab over ipilimumab at a median follow-up of 18.5 months; the 18-month RFS rates were 66.4% versus 52.7%, respectively.

CONCLUSIONS

Various types of immunotherapy have been present for a long time in melanoma treatment, and contemporary types of immunotherapy, like pembrolizumab, nivolumab, ipilimumab, combined therapy with nivolumab and ipilimumab, and T-VEC, have been established in recent years as the standard-of-care treatment for advanced-stage melanoma.

Immunotherapy of metastatic melanoma also makes headway in research of new and promising types of immunotherapy due to high immunogeneity of these tumours.

Several types of clinical studies in melanoma immunotherapy are currently ongoing – sequential studies that should, in BRAF V600-positive patients, answer the question regarding sequencing of targeted and immunotherapy, and concomitant studies that are combining immunotherapy and targeted therapy, or intralesional with systemic immunotherapy. Phase 1 clinical studies show promising antitumour activity of triplet regimens (immunotherapy with combined BRAF-inhibition and MEK-inhibition) in patients with BRAF-mutant melanoma. Studies are also investigating new types of immunotherapy in metastatic melanoma treatment, e.g. anti-PD-L1 antibodies and other checkpoint inhibitors, either separately or in different combinations. For instance, combinations

of anti-PD-1 with IDO-1 inhibitors (25), or with anti-LAG-3 (26), seem to be well tolerated and showed promising clinical activity.

However, in this ongoing enthusiasm, greater scrutiny of the results of clinical trials exploring combination therapies is required, in order to ensure that synergistic therapeutic interactions are achieved without synergistic toxicity.

Also, studies of adjuvant use of PD-1 inhibitors, either versus placebo, ipilimumab, or high dose IFN-alpha, are on the way, as well as study comparing adjuvant use of high dose ipilimumab, low dose ipilimumab, or high dose IFN-alpha.

Resistance mechanisms are being elucidated; effectiveness of therapy might be limited by loss of tumour antigen presentation and Tcell trafficking. Biomarkers of response should be further explored.

Substantial heterogeneity exists in the natural history of metastatic melanoma, including differences in the pace of disease progression and the sites of metastatic lesions, as well as heterogeneity in patients' characteristics; this should also be taken into account in further clinical studies.

Some interesting investigations have emerged recently, e.g. how diversity and composition of the gut microbiome influence response to anti-PD-1 immune checkpoint therapy in patients with metastatic melanoma (27). Also, clinicians treating melanoma patients are nowadays paying more attention to patients who would normally be excluded from clinical trials, such as elderly patients, or patients with autoimmune diseases, besides patients with CNS metastases, who have already been studied more closely, as mentioned earlier.

We anticipate that immunotherapy in melanoma treatment has not reached its peak, either as independent therapeutic option, or combined with other therapies, in metastatic, as well as in adjuvant, or neoadjuvant setting.

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