IMMUNOTHERAPY FOR LOCALLY ADVANCED AND METASTATIC MALIGNANT MELANOMA

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Ipilimumab,
Nivolumab,
Pembrolizumab.

Abstract

Metastatic malignant melanoma is a potentially fatal cancer with a survival rate between 10% to 20% at 5-years and a poor prognosis that can be in part due to limited efficacy of standard chemotherapy regimens. To improve these results different immunological interventions, including cytokine based therapies, anti-tumour vaccination and checkpoint inhibition, have been developed, in order to stimulate the proliferation and activation of T lymphocytes to destroy tumor cells. In this review we summarize the main immunotherapy approaches against metastatic melanoma.

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Introduction

Metastatic melanoma is a potentially fatal cancer which responds poorly to most standard chemotherapies, the 5-years survival rate being comprised between less than 10% (brain and liver metastases) and 17%-23% (lung, skin and lymph nodes metastases) (1, 2). The therapeutic options for unresectable Stage III and Metastatic (Stage IV) or Recurrent Melanoma are Intralesional therapy, Immunotherapy, Signal-transduction inhibitors, Chemotherapy or Palliative local therapy. Regarding Immunotherapy, there are several ways of acting on tumor cells such cytokine based therapies, anti-tumour vaccination and checkpoint inhibition (3).

A. Cytokines-based therapy in metastatic melanoma

A.1. Interleukin-2 (IL-2)

Interleukin-2 (IL-2), also known as T-cell growth factor (TCGF), is a potent multifunctional cytokine produced by T helper cells after antigen activation, and plays an important role in the control of proliferation and differentiation of immune system cells (4). Interleukin-2 induces the proliferation of Natural Killer (NK) cells and increases their cytolytic activity, promotes antibodies production, drives the development of Regulatory T Cells (Treg cells), and also plays a role in promoting the differentiation of T Helper cells (Th1 and Th2) (5-7).
**Subclinical studies**

The biological effects of IL-2 depend on the interaction between this cytokine and its specific receptor (IL-2R). In about 30% of murine melanoma cells, flow cytometry analysis revealed the presence of the Interleukin receptors (IL-2R alpha subunit). Moreover, the addition of IL-2 to the culture medium increases melanoma cell proliferation, suggesting a role for IL-2 in the tumoral activity of melanoma cells and its involvement in the metastatic progression of B16F10 melanoma.

Flow cytometric analysis reveals that human melanoma cell also expresses Interleukin receptors (both alpha-IL-2R alpha and beta-IL-2R beta chains), supporting the hypothesis that IL-2 acts directly on human melanoma cells, being able to contribute to tumor progression (9, 10). But at high doses, IL-2 stimulates the normal peripheral blood mononuclear cells resulting in an up regulation and endogenous production of IL-24, a tumor-suppressor cytokine that causes growth suppression and apoptosis in tumor cells (11). High doses of IL-2 can also up regulate IL-24 protein expression in some tumor cells, leading to melanoma tumor growth suppression (IL-24-mediated melanoma growth suppression) (12).

**Clinical studies and evidences**

*Phase II Studies, A systematic review*

Between 1985 and 1993, 270 patients with metastatic melanoma have been treated with intravenous bolus of 720,000 International Unit per Kilogram (IU/kg) IL-2 every eight hours for up to 14 consecutive doses per cycle (as clinically tolerated), cycle repeated every 6 to 12 weeks in stable or responding patients. This therapeutic scheme has produced 16% objective responses (6% complete responses and 10% partial responses), with a median response duration of 8.9 months for complete responding patients and 5.9 months for those who achieved a partial response. Forty-four percent of responders were long-term survivors beyond five years (range >70 months to >150 months) and none of the responding patients experienced disease progression after five years. Median survival rate for the entire group was 12 months and the toxicities, although severe (six patients=2% died from adverse events, all related to sepsis), were generally reversed rapidly after therapy was completed (13, 14).

Standard High-Dose Bolus of Interleukin-2 administration consists in the injection of 600,000 IU/kg to 720,000 IU/kg IL-2 (IV over 15 minutes) administered every eight hours for five consecutive days per cycle, for two cycles separated by a minimum of nine days, from days 1 to 5 for cycle 1 and from days 15 to 19 for cycle 2 (maximum of 14 doses/cycle and maximum of 28 doses per each two-cycle course, if well tolerated). Response evaluation is usually performed four weeks after the second cycle and, if tolerable and effective (evidence of anti-tumor response), the course may be repeated seven weeks after hospital discharge (15).

The major toxicities are infections (Bacterial sepsis requiring antibiotic prophylaxis), cardiovascular (hypotension, cardiac arrhythmias), renal (oliguria), flu like syndrome (fever, chills) and rash. Due to these risks of multi-organ complications, the administration of high dose HD-IL-2 is reserved to younger patients with excellent performance status and requires hospitalization with intensive monitoring (14, 15).

*Phase III trials*

No phase III randomized controlled trials comparing high-dose IL-2 to other treatments (standard therapy or placebo) have been conducted, eventually to provide an assessment of relative impact on overall survival.

**A.2. Interferon-alpha (IFN-α)**

Interferon-alpha (IFN-α), belonging to the Interferon type I class, is a multifunctional cytokine released mainly by plasmacytoid dendritic cells, with important role in potent innate immune response against both viral infection and cancers (16). The anticancer effects of Interferon-alpha are cell cycle inhibition (Go-G1 mitotic phase block), promotion of cell apoptosis (up regulation of pro-apoptotic proteins) and direct cytotoxic effect on some tumor cells (17-19).

In addition to the direct effects on tumor cells, Interferon-alpha (IFN-α) exerts immunomodulatory effects (activities on T cells and dendritic cells) that can play a central role in the overall antitumor response (20).

**Subclinical studies**

The treatment of human melanoma cell lines with recombinant IFN-α-2a doses from 7,000 to more than 50,000 IU/mL produces a 50% growth inhibition. The IFN alpha inhibits the formation of dendrites, decreases the proportion of differentiated cells (from 100% in untreated control group to 58%-74%), increases the melanin synthesis and modifies the cells surface markers (enhance HLA-I antigens) (21).

**Clinical studies and evidence**

*Phase I/II studies*

In phase I/II studies on patients with metastatic malignant melanoma, the Recombinant Interferon (rIFN-alpha) produces overall response rates in about 16% to 22% of patients, responses observed as late as six months after the initiation of therapy and persists (about four months) for up to one third of the responder patients. The toxic
A.3. Interleukin-2 (IL-2) and Interferon and chemotherapy combination

Clinical studies and evidences

A.3.1. Standard chemotherapy with Dacarbazine versus Interleukin-2 (IL-2) plus Interferon

In a multicenter randomized study, 241 patients with stage IV melanoma were randomized between standard chemotherapy (Dacarbazine 850 mg/m² every three weeks) and immunotherapy with Interleukin-2, 2.4 Million International Units (MIU/m² s.c., twice a day for five days) plus Interferon-alpha-2b (IFN-α, 3 MIU s.c., once daily for seven days) and Histamine Dihydrochloride (HDC, 1 mg, s.c., twice a day for five days). The immunotherapeutic regimen did not improve the response rate and overall survival compared with Dacarbazine (25).

A.3.2. Dacarbazine plus Interferon versus Dacarbazine plus Interferon and Interleukin

Two hundred ninety metastatic melanoma patients were randomized in a phase III multicentric trial conducted by the Dermatologic Cooperative Oncology Group (DeCOG) between Dacarbazine (DTIC, 850 mg/m² every 28 days) and Interferon α (IFN-α; 3 MIU/m², twice on day 1, once daily from days 2 to 5; 5 MIU/m² three times a week from week 2 to 4) only versus the same regimen plus Interleukin 2 (IL-2, 4.5 MIU/m² for three hours i.v. on day 3; 9.0 MIU/m² i.v. day 3/4; 4.5 MIU/m² s.c. days 4 to 7). No significant overall survival difference was noted (median overall survival 11.0 months each) neither for objective response (18.0 % for DTIC plus IFN-α versus 16.1% for DTIC, IFN-α and IL-2). The three drugs combination was more toxic (13.9% adverse reactions versus 5.6%) than DTIC-IFN-α (26).

A.3.3. Combination Dacarbazine-Cisplatin-Vinblastine chemotherapy versus same chemotherapy plus Interleukin-2 and Interferon

1) One hundred and ninety metastatic melanoma patients were included in a phase III randomized trial and treated with chemotherapy or biochemotherapy combination. The chemotherapy consisted of Dacarbazine (days 1 and 22) plus Cisplatin and Vinblastine (days 1 to 4 and 22 to 25), and the Bio-Chemotherapy involved the same chemotherapy (Vinblastine dose reduced by 25%) plus Interleukin-2 in a 24-hour continuous infusion (on days 5 to 8, 17 to 20, and 26 to 29) and Interferon alfa-2b in a subcutaneous injection (on days 5 to 9, 17 to 21, and 26 to 30). Response was assessed every six weeks.

The bio-chemotherapy combination almost doubled the response rate (48% versus 25%; p = .001) and the median time to progression (4.9 vs 2.4 months, p = .008) and produced a 3-month prolongation in median overall survival (from 9.2 to 11.9, p = .06). Biochemistry produced substantially more constitutional, hemodynamic, and myelosuppressive toxic effects (27).

2) The largest phase III randomized clinical trial comparing Dacarbazine-Cisplatin-Vinblastine chemotherapy with Bio-Chemotherapy was conducted by the Eastern Cooperative Oncology Group (E3695 trial) on 395 metastatic melanoma patients. Bio-Chemotherapy gets better results in terms of response rate (19.5% against 13.8%), median progression-free survival (4.8 against 2.9 months) and percentage of patient’s progression free at six months (38.9% against 18.9%). On the contrary, no treatment-related survival benefit was observed for any of the stratified categories, the median overall survival being 8.7 months for Chemotherapy and 9.0 months for Bio-Chemotherapy. The treatment of patients with metastatic melanoma with biotherapy, even in combination with chemotherapy, remains disappointing (28).

B. Anti-Tumor Vaccination

The principle of anti-tumor vaccination is to stimulate the immune system and develop specific immunity against tumor cells. The first step of
immune system activation is the presentation of tumor-specific antigens (TSAs) by professional antigen presenting cells (Dendritic cells DCs) to the naïve T cells (29). Consequently, the naïve T cells are activated and differentiated into T effector (Teff) cells, so activated CD8+ T cells will directly kill target tumor cells, activated CD4+ T cells can promote inflammation, cooperate in the induction of CD8+ T effector and memory cells, and provide help for B cells to produce destructive anti-tumor antibodies (30, 31).

Subclinical studies
Melanoma tumors can be induced in mice by subcutaneous inoculation to obtain superficial palpable tumors (a tumor that grows to a 1 cc in 14 to 21 days) or by intravenous tumor injection to produce pulmonary metastasis (50 and 250 pulmonary nodules visible on the lung surface) (32). Regarding Dendritic Cells (DC) vaccination, intravenous (i.v.) injected DC efficiently enter the spleen, whereas subcutaneously (s.c.) injected DC access peripheral lymph nodes LN draining the injection area (33, 34). The in vivo studies demonstrate that DC vaccination has a preventive immunization effect against melanoma cells, and also protected mice from tumor dissemination in a therapeutic model, the ability of DC to trigger an effective T cell response being dependent on their state of maturation and on cytokine production (35).

Clinical Studies and Evidences
Phase I/II studies
1) Thirty-three patients with stage IV melanoma, good performance status (ECOG status of 0) and low volume disease (clinically or radiologically measurable). Dendritic Cells vaccines were given by intra nodal (into inguinal lymph) injection under ultrasound control at weekly intervals for the first four injections then once two weeks later and twice at 4-week intervals. There were three responses (partial responses), stable disease (defined as no progression over a period of three months) was seen in nine patients and the treatment was not associated with significant side effects. There was no correlation between assays of IFN-gamma production and clinical responses (36).

2) In another phase I/II trial including 17 patients suffering from metastatic melanoma with measurable disease, 12 patients received a complete priming phase of six cycles of either 0.9 x 10^6 or 5 x 10^6 Dendritic Cells intradermal injection, at 2-weekly intervals, treatment continued where possible with the lower dose at 6-week intervals. A complete and durable response (average duration of more than 35 months) was obtained in three patients, three patients had partial responses, and the remaining six had progressive disease. The treatment had minimal side-effects and was well tolerated by all patients (37).

3) In a mono centric, open-label phase I/II DC vaccination trial, 53 stage IV melanoma patients HLA-A1- and/or HLA-A2.1-positive received DC vaccinations. No major (> grade 2) toxicity was observed in any patient, the most common side effects being transient mild rise in body temperature and vaccine injection site reactions (in 92% of patients). No complete or partial regression of all metastases was observed, but a slow regression and eventual complete disappearance of individual metastases was noticed. Regarding survival, after a minimum of a 12-years follow-up, 19% of patients suffering from metastatic melanoma with measurable disease were still alive. There was no significant correlation between long Overall Survival in metastatic patients and the strength of vaccine-specific immune response (38).

Phase III study: Standard chemotherapy with Dacarbazine versus vaccination with autologous peptide-pulsed dendritic cells (DC)
One hundred and eight stage IV melanoma patients were randomized between standard Dacarbazine chemotherapy (DTIC 850 mg/m^2 intravenously, in 4-week intervals) and Dendritic Cells vaccines (subcutaneously at 2-week intervals for the first five vaccinations and every four weeks thereafter). No significant differences in overall response (DTIC: 5.5%, DC: 3.8%) and overall survival was observed (39).

C. Checkpoint inhibitors
Immune checkpoints are regulatory signals that modulate T cell responses, and can be co-stimulatory signals that promote immunity against pathogens and co-inhibitory proteins that negatively regulate T-cell activation and prevent self-immunity in normal tissue. Therefore, the ultimate magnitude and quality of the response is determined by the summation of positive and negative signals delivered by cell surface immune checkpoint molecules (40). Destruction of cancer cells involves a T-cell activation, process that requires simultaneously recognition of antigenic peptides (Tumor-Associated Antigens) bound to the Major Histocompatibility Complex (MHC) by the T-cell receptor (TCR) and the presence of co-regulatory signals. These co-regulatory molecules ultimately determine the fate of the T-cell response; activation and differentiation into effector T-cells, deletion or anergy (41).
Most co-signalling molecules are members of the Immunoglobulin Superfamily (IgSF) and Tumor Necrosis Factor Receptor Superfamily (TNFRSF), and can be further subdivided into specific families on the basis of primary amino acid sequence, protein structure and function (42).
There are two checkpoint inhibitors classes actually used in advanced melanoma treatment:

1) Anti-PD-1 (Programmed Cell Death-1) and PD-L1 (Programmed Death Ligand 1).
2) Anti-CTLA-4 (Cytotoxic T-Lymphocyte Antigen-4)

### C.1. Anti-PD-1 and PD-L1 Checkpoint inhibitors

Programmed death 1 (PD-1) is an inhibitory receptor (type I transmembrane glycoprotein, composed of an immunoglobulin V-type extracellular domain) expressed on activated lymphocytes in order to regulates tolerance and autoimmunity (43). PD-1 has two ligands with distinct expression patterns: PD-Ligand 1 (PD-L1; B7-H1), expressed broadly on hematopoietic (resting T cells, B cells, DCs, and macrophages) and parenchymal cells (vascular endothelial cells and pancreatic islet cells) and PD-Ligand 2 (B7-DC), which is restricted to macrophages and dendritic cells (44). Some tumors can escape from the host immune system by expressing PD-L1 (B7-H1) on their surface, so the PD-L1/PD-1 blockade can be an effective treatment of primary PD-L1-expressing tumors, which are moderately immunogenic (45).

**Subclinical studies**

Melanoma cell lines B16 cells (1 × 10⁶) were injected to mice subcutaneously to obtain superficial tumors, into the spleen for hematogenous dissemination to the liver and intravenously for hematogenous dissemination to the lung. The study demonstrated that:

- a) the presence and the high level of PD-1 is correlated with faster growth of subcutaneous B16 tumors;
- b) the absence of PD-1 signals induced accumulation of cytotoxic T cells in tumor sites and strong cytotoxic activity against weakly immunogenic tumor cells;
- c) the PD-1 blockade immunotherapy could inhibit hematogenous cancer spread (tumor formation model in the liver after intrasplenic injection) of poorly immunogenic B16 melanoma (46).

**Clinical studies**

**Pembrolizumab (humanized IgG4 monoclonal antibody with high affinity for human PD-1)**

Phase I trial, KEYNOTE 001 trial, a multicenter, open-label, dose-comparative randomized phase IB trial, enrolled 173 patients with unresectable or metastatic melanoma who had progressed after previous treatment. The patients received Pembrolizumab, at either 2 mg/kg or 10 mg/kg intravenously (IV) every three weeks until disease progression or unacceptable toxicity. The Objective Response Rate (ORR) was similar in both arms (26%), with one complete response in the 2 mg/kg arm and no complete responses in the 10 mg/kg group. Treatment was well tolerated in both arms, the most common drug-related adverse events of any grade being fatigue (33% and 37%), pruritus (26% and 16 19%) and rash (18% vs 18%) (47).

Phase II trial, KEYNOTE 002 study, compares the two Pembrolizumab dosages (2 or 10 mg/kg every three weeks) with investigator-choice Chemotherapy (Paclitaxel-Carboplatin, Paclitaxel, Carboplatin, Dacarbazine, or oral Temozolomide) on 540 patients with Ipilimumab-refractory unresectable stage III or stage IV melanoma. Pembrolizumab 2 or 10 mg/kg every three weeks significantly improved Progression-Free Survival (primary endpoint) related to chemotherapy: from 16% to 34-38% at six months and from 8% to 24-29% at nine months for Chemotherapy and Pembrolizumab arms. Treatment-related grade 3-4 adverse events occurred in 11%-14% patients in the Pembrolizumab group (fatigue, oedema, myalgia) against 26% in the chemotherapy group (anaemia, fatigue, neutropenia-leucopenia) (48).

**Nivolumab (a fully human IgG4 monoclonal antibody against programmed death receptor-1)**

Phase I study. One hundred and seven patients with advanced melanoma and good performance status (ECOG PS ≤1) received at 12 cycles (four doses/cycle) of Nivolumab treatment, expanded at 0.1, 0.3, 1, 3, and 10 mg/kg. Drug-related adverse effects (any grade) occurred in 82% of patients with grade 3-4 toxicity in 21% of patients, including lymphopenia (3%), fatigue, and increased lipase (2%). Nivolumab at the 3 mg/kg, dose selected for phase III trials, produces a median overall survival of 20.3 months at the 3 mg/kg dose selected for phase III trials with 44% and 40% of patients alive at two and three years (49).

Phase II study. In a single-arm, open-label, multicenter, phase II study 24 patients previously untreated with unresectable stage III/IV or recurrent malignant melanoma received i.v. Nivolumab 3 mg/kg every two weeks in each 6-week cycle until progressive disease (PD) or unacceptable toxicity. The overall response rate (primary endpoint) evaluated radiologically was 34.8% and the overall survival rate at 18 months was 56.5%. Grade 3 or 4 treatment-related adverse events occurred in three patients (12.5%) (50).

Phase III CheckMate 037 trial, randomized 272 patients with advanced melanoma previously treated, between Nivolumab 3 mg/kg intravenously every two weeks or investigator’s choice chemotherapy (Dacarbazine 1,000 mg/m² every three weeks or Carboplatin area under the curve 6 plus paclitaxel 175 mg/m² every three weeks). Nivolumab demonstrated higher (27% versus 10%) and more durable (32 months versus 13 months) responses, but no significant difference in overall survival (16 versus 14 months) compared with chemotherapy regimens (51).
Phase III CheckMate 066 trial, randomly assigned 418 previously untreated patients with metastatic melanoma without a BRAF mutation to receive Nivolumab (3 mg/kg every two weeks) and Dacarbazine-matched placebo every three weeks or Dacarbazine (at a dose of 1000 mg per square meter of body-surface area every three weeks and Nivolumab-matched placebo every two weeks). The study demonstrated that Nivolumab significantly improves overall survival (72.9% versus 42.1% at one year) and progression-free survival (5.1 against 2.2 months) compared with Dacarbazine. There were 7.6% complete responses (CR) with Nivolumab against only 1% in Dacarbazine-treated patients, and among the responding patients the median duration of response was not reached in the Nivolumab group, but it was six months in those treated with Dacarbazine. Common adverse events associated with Nivolumab included fatigue, pruritus, and nausea (52).

C.2. Anti-CTLA-4 (Cytotoxic T-Lymphocyte Antigen-4)

Naive T cells require two distinct signals to proliferate and differentiate into the armed effector cells, an antigen-specific signal and a co-stimulatory signal. Cytotoxic T lymphocyte-Associated Molecule-4 (CTLA-4), member of the immunoglobulin super family (contains a V domain, a trans-membranar domain, and a cytoplasmic tail) is a powerful negative regulator of T cell activation (inhibitory signal to T cells) and therefore plays a major role in regulating T cell tolerance or autoimmune immunity (53, 54).

Subclinical studies

Mice were inoculated with melanoma cells line B16 by subcutaneous route (subcutaneous injection of 104 cells) to develop superficial tumor or by hematogenic route (intravenously injection of 5 x 105 cells) to develop lung metastases. The same day or later, mice were subcutaneously inoculated with irradiated tumor cells engineered to secrete Granulocyte/Macrophage Colony-Stimulating Factor (GM-CSF producing cells). The mice were then sacrificed when the cutaneous tumors displayed severe ulceration or reached a size of 300 mm² or after 25 days for lung metastases group. An important detail is that B16 cells line is a very poorly immunogenic tumor, MHC class II being undetectable by flow cytometry in vitro and ex vivo. The study proves that CTLA-4 blockade plus GM-CSF-producing Cellular Vaccines causes rejection of established B16 tumors (overall cure rate of 80%), suppression of lung metastases and induction of long-term survival. Mice surviving of subcutaneous melanoma cells inoculation or lung metastases develop within 4-8 weeks after challenge skin and hair depigmentation, starting at the sites of vaccination (55).

Clinical studies: Ipilimumab (a fully human immunoglobulin G1 monoclonal antibody designed to block CTLA-4)

Phase I/II study. Maximum-tolerated dose and dose-limiting toxicity

Eighty-eight patients with unresectable stage III or IV melanoma received single doses of Ipilimumab up to 20 mg/kg (group A, single dose), multiple doses up to 5 mg/kg (group A, multiple dose), and multiple doses up to 10 mg/kg (group B) in order to evaluate the safety and efficacy of single and multiple doses of Ipilimumab. The conclusion of the study is that Ipilimumab is well tolerated (group A and B without a maximum-tolerated dose and for group C dose-limiting toxicity in six of 23 patients) and has anti-tumor activity against metastatic melanoma (group B, disease control rate of 39% with one complete response more than 21 months and one partial response more than 23 months) (56).

Phase III clinical trial. Optimal Dose of Ipilimumab

In a randomised double-blind multicenter phase III study, 727 patients with unresectable stage III or IV melanoma (advanced melanoma) and no previous therapy were enrolled and randomly assigned to Ipilimumab 10 mg/kg or Ipilimumab 3 mg/kg. This trial demonstrated that Ipilimumab 10 mg/kg resulted in significantly longer overall survival than did Ipilimumab 3 mg/kg (15.7 against 11.5 months, p=0.04) but with increased treatment-related adverse events (diarrhoea 10% vs 6%, colitis 5% vs 2%, increased alanine aminotransferase 3% vs 1% and hypophysitis 3% vs 2%) (57).

Phase III clinical trial. Chemotherapy alone versus Chemotherapy plus Ipilimumab

Five hundred two patients with previously untreated metastatic melanoma were randomised to receive exclusive chemotherapy Dacarbazine (850 mg/m²) or combination therapy, Ipilimumab (10 mg/kg) plus Dacarbazine (850 mg/m²). The conclusion of the study is that adding Ipilimumab (10 mg/kg) to standard Dacarbazine chemotherapy improves the median overall survival (11.2 vs 9.1 months), the one year (47.3% vs 36.3%), two years (28.5% vs 17.9%) and three years survival (56.3% compared with 27.5%; p <0.001), but no drug-related deaths or gastrointestinal perforations occurred in the Ipilimumab-Dacarbazine group (58).

Phase III clinical trial. Ipilimumab versus antigen glycoprotein 100 (gp100) versus combination

In this randomized, double-blind, phase III study, 676 unresectable stage III or IV melanoma patients with progressive disease were randomly assigned to receive Ipilimumab (3 mg/kg) with or without gp100 every three weeks for up to four treat-
ments. In the vaccine groups, patients received two modified HLA-A*0201-restricted peptides, injected subcutaneously. The conclusion of the study is that Ipilimumab, with or without a gp100 peptide vaccine, as compared with gp100 alone, improved overall survival (10.0 against 6.4 months, \( p < 0.001 \)) in patients with previously treated metastatic melanoma. Adding gp100 to Ipilimumab does not improve overall survival. Regarding treatment tolerance, Grade 3 or 4 immune-related adverse events occurred in 10 to 15% of patients treated with Ipilimumab and in 3% treated with gp100 alone (59).

C.3. CTLA-4 inhibitors versus PD-1 inhibitors

**Phase III trial: Pembrolizumab versus Ipilimumab in Advanced Melanoma**

KEYNOTE 006 trial conducted between 2013 and 2014 randomized 834 patients with unresectable stage III or IV melanoma between Pembrolizumab (10 mg/kg every two or three weeks) and Ipilimumab (3 mg/kg q3w for up to four doses). The study proves that Pembrolizumab is superior to Ipilimumab in terms of response rate (33% versus 11.9%; \( p < 0.001 \)), progression-free survival (estimated 6-month progression-free survival 46.4-47.3% against 26.5%) and overall survival (estimated 12-month survival 74.1%-68.4% versus 58.2%, \( p=0.0036 \)). Grade 3 to 5 treatment-related adverse events were lower in the Pembrolizumab group (13.3% and 10.1%) than in the Ipilimumab group (19.9%) (60).

C.4. CTLA-4 inhibitors plus PD-1 inhibitors

**Phase III trial: Nivolumab versus Ipilimumab versus Combination**

In this double-blind phase III study, 945 previously untreated patients with unresectable stage III or IV melanoma are randomised to receive Nivolumab alone (3 mg/kg every two weeks), Ipilimumab alone (3 mg/kg every three weeks for four doses) or combination Nivolumab (1 mg/kg every three weeks) plus Ipilimumab (3 mg/kg every three weeks for four doses). The Nivolumab-Ipilimumab combination induces a higher rate of objective response (57.6%) and progression-free survival (11.5 months) than Nivolumab alone (40% and 6.9 months) or Ipilimumab alone (33% and 2.9 months). The prediction factor for the response to Nivolumab treatment is the presence of the PD-1 ligand (PD-L1); the median progression-free survival for the patients treated with Nivolumab was 14.0 months in positive patients versus 5.3 months for PD-L1-negative tumors. The combination treatment is more toxic than monotherapies with 55.0% grade 3 or 4 adverse events against 16.3% for Nivolumab alone and 27.3% for Ipilimumab group (61).

**Conclusions and recommendations**

No studies have compared IL-2 to the current Dacarbazine (DTIC) chemotherapy, High-dose IL-2 is considered a reasonable treatment option for a selected group of patients with metastatic melanoma (good performance status, up to three organs involved and no evidence of central nervous system metastases), patients to whom IL-2 treatment can produce durable complete remissions. Recombinant Interferon (rIFN-alpha) monotherapy has limited efficacy and significant toxicity for metastatic melanoma patients, so its clinical benefit is limited in the treatment of stage IV melanoma. The bio-chemotherapy combination with Interleukin-2 (IL-2) and Interferon has proved a progression-free survival benefit but without overall survival improvement. This combination can be used as second line therapy for metastatic patients.

Dendritic cells vaccines alone or in combination with standard Dacarbazine chemotherapy do not improve the overall response or overall survival, and for instance are not included in the therapeutic strategy.

On the contrary, anti-PD-1/PD-L1 Pembrolizumab and Nivolumab alone or in combination with anti-CTLA-4 Ipilimumab represent today the first line therapy for melanoma metastatic patients (Figures 1 and 2).

**Figure 1.** Therapeutic regimens

<table>
<thead>
<tr>
<th>High-dose Interleukin-2</th>
<th>Interferon alpha-2B induction phase</th>
<th>Interferon alpha-2B maintenance phase</th>
<th>Pembrolizumab</th>
<th>Nivolumab</th>
<th>Ipilimumab</th>
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<tr>
<td>600,000 IU/kg per dose</td>
<td>15 million IU/m2</td>
<td>10 million IU/ml</td>
<td>2 mg/kg</td>
<td>3 mg/kg</td>
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<td>1x over 15 minutes</td>
<td>i.v. over 20 minutes</td>
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<td>i.v. over 30 min</td>
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<td>every 8 hours</td>
<td>3 times per week</td>
<td>every 3 weeks</td>
<td>every 2 weeks</td>
<td>every 3 weeks</td>
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<td>up to 14 doses</td>
<td>days 1, 5, 8-12, and 15-19</td>
<td>48 weeks.</td>
<td>until disease progression max 2 years</td>
<td>until disease progression unacceptable toxicity</td>
<td>4 cycles Repeat at progression</td>
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Figure 2. Comparative treatment table

<table>
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<th>Cytokine-based therapy</th>
<th>Anti-Tumor Vaccination</th>
<th>Checkpoint inhibitors</th>
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<td><strong>Anti-CTLA-4</strong></td>
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<td><strong>No significant differences</strong></td>
<td>26%</td>
<td><strong>Improved:</strong></td>
</tr>
<tr>
<td><strong>5.5% vs 3.8%</strong></td>
<td><strong>27% vs 10%</strong></td>
<td><strong>10.9% vs 1.5%</strong></td>
</tr>
</tbody>
</table>

**Bibliography**