Revisão e Atualização dos Inibidores dos *Checkpoints* Imunológicos no Melanoma

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RESUMO – A incidência do melanoma está a aumentar de uma forma global e essa tendência manter-se-á muito provavelmente nas próximas duas décadas. Estes dados reforçam a necessidade de novos alvos terapêuticos, em alternativa à quimioterapia clássica para o tratamento do melanoma avançado. Com efeito, ao longo da última década, os novos conhecimentos relativos à biologia tumoral revolucionaram a terapêutica do melanoma, incluindo a imunoterapia com os inibidores dos "checkpoints" imunológicos, cujos alvos terapêuticos são as proteínas CTLA-4 (*cytotoxic T lymphocyte-associated protein* 4) e PD-1 (*programmed cell death protein* 1). A inibição destes alvos permite a modulação da resposta imune do hospedeiro contra o desenvolvimento tumoral, com respostas objetivas sustentadas no controlo da doença. Face a estes resultados, a imunoterapia tornou-se o tratamento de referência nos doentes com melanoma avançado (estadio III irressecável ou estadio IV, com metástases à distância), sem mutação BRAF identificada. O ipilimumab (anti CTLA-4) foi o primeiro "checkpoint" imunológico inibitório a demonstrar aumento na sobrevida global no tratamento do melanoma avançado. Mais tarde, o nivolumab e o pembrolizumab (ambos anti-PD-1) evidenciaram melhores resultados em termos de sobrevida global e tolerabilidade do que o ipilimumab. Estes resultados são expectáveis, na medida em que as vias de inibição dos "checkpoints" imunológicos são diferentes. Neste contexto, impõe-se a avaliação da eficácia da terapêutica combinada e a identificação de biomarcadores que possibilitem a previsão de resposta aos anti-CTLA-4 e anti-PD-1. Após um trabalho prévio em que foram sumariamente revistos os mecanismos de desenvolvimento tumoral e de ação dos "checkpoints" imunológicos inibitórios, propomo-nos efetuar uma revisão sobre os inibidores dos "checkpoints" imunológicos, atualmente disponíveis na prática clínica para o tratamento do melanoma avançado.
PALAVRAS-CHAVE – Anticorpos Monoclonais; Antígeno CTLA-4; Imunoterapia; Ipilimumab; Melanoma; Nivolumab; Pembrolizumab; Pontos de Controlo do Ciclo Celular; Receptor de Morte Celular Programada 1.

Immune Checkpoint Inhibitors in Melanoma: Review and Update

ABSTRACT – *The overall increasing incidence of melanoma will very probably be the trend over the next two decades. This data stresses the need for new therapeutic resources, other than classic chemotherapy. Nevertheless, the treatment of advanced melanoma has been changed in the last decade due to novel therapeutic strategies, including immunotherapy with immune checkpoint inhibitors targeting cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1). Inhibition of these targets enhances immune host response against cancer and results in durable objective responses, establishing immunotherapy as standard treatment for BRAF wild-type melanoma patients in advanced stages (III – unresectable and IV – metastases at distant sites). Anti-CTLA-4, ipilimumab, was the first–in-class immune checkpoint inhibitor to show improvement in overall survival in advanced melanoma. Latter, anti-PD-1 agents, nivolumab and pembrolizumab, have improved tumour response and tolerability in comparison with ipilimumab. Differences in outcome are expected considering the distinct target of checkpoint inhibition pathways. In this setting, it is of utmost importance the assessment of efficacy by combined therapy and the identification of biomarkers capable of predicting response to anti-CTLA-4 and anti-PD-1. After a previous review on cancer biology and mechanisms of action of immune checkpoint inhibitors we will focus on the main data on the immune checkpoint inhibitors for melanoma currently available in daily practice.*

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1 - INTRODUCTION

Cutaneous melanoma represents a minority of all skin cancers; however, it is responsible for 80% of skin cancer deaths.[1] Diagnosis at earlier stages is associated with a better prognosis. The risk of metastasis correlates directly with primary tumour thickness, occurring in approximately 35% to 50% of patients with T4b primary melanoma (thickness > 4.0 mm, with ulceration).[2] The incidence of melanoma is increasing, and this trend will very probably continue throughout the next two decades.[3,4] This data stresses the need for new therapeutic resources other than conventional chemotherapy, which is largely ineffective in patients with unresectable or metastatic melanoma.[5] In the last decade, due to identification of activating mutations in melanoma[6] and the role of the immune system in cancer, multiple new therapeutic strategies have become available and radically transformed the care of melanoma patients, particularly those with advanced stage disease.[7,8] Thirty-five to 50% of patients with cutaneous melanoma harbour a *BRAF 600* mutation.[9] Therefore, treatment of these patients with molecular targeted therapy using BRAF inhibitors alone or in combination with MEK inhibitors is recommended, although the majority will develop secondary resistance.[10,11]

Immunotherapy using immune checkpoint inhibitors (ICI) against cytotoxic T lymphocyte antigen - 4 (CTLA-4) and programmed death - 1 (PD-1) are now recommended for BRAF wild-type melanoma patients in advance stages (III – unresectable and IV – metastases at distant site(s))2 (Fig. 1). After review cancer biology and mechanisms of action of immune checkpoint inhibitors,[8] we will focus on the use of immune checkpoint inhibitors for melanoma currently available in daily practice.

2 - IMMUNE CHECKPOINT INHIBITORS: MONOTHERAPY

2.1 – CTLA-4 inhibitors

2.1.1 – Ipilimumab

a) Mechanism of action

The identification of CTLA-4 as a key molecule in the downregulation of T-cell activation leads to the hypothesis that a CTLA-4 blockade could promote anti-tumour immunity mediated by effector CD4 and CD8 T cells.[12] Encouraging anti-tumour effects in animal models led to the development of human monoclonal antibodies which block CTLA-4.

Ipilimumab, a fully human monoclonal immunoglobulin (Ig)G1 antibody against CTLA-4, was the first drug introduced as immunotherapy for melanoma. Interaction of the monoclonal antibody with CTLA-4 blocks inhibitory signals generated through this receptor and enhances T cell activation, leading to increased anti-tumour responses.[8,13,14] (Fig. 1).

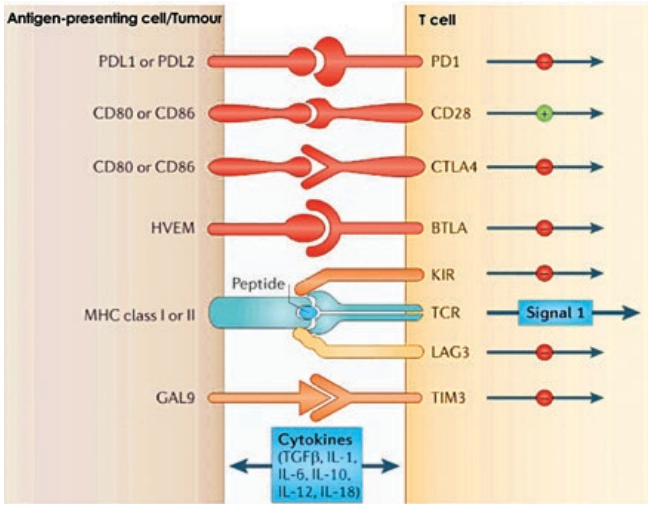

Figure 1 - (Adapted from Pardoll DM. Nat Rev Cancer. 2012;12:252–64[14]): Regulation of T-cell response: balancing activating and inhibitory signals. Inhibition receptors (CTLA-4 and PD-1) are well-established targets for immunotherapy. BTLA, LAG-3, TIM-3 are ongoing emerging targets for immunotherapy.

b) Long-term efficacy and toxicity

Ipilimumab was investigated in several phase I and phase II clinical trials, which reported durable and consistent responses in patients with stage III unresectable or stage IV metastatic melanoma.[15-19] The first phase III clinical trial was a large prospective multicentre randomised study that included patients diagnosed with unresectable stage III or IV melanoma who had failed previous treatments. Patients were randomly assigned in a 3:1:1 ratio to receive ipilimumab 3 mg/kg body weight alone, in combination with gp100 (vaccine derived from melanossomal glycoprotein 100) or gp100 alone. The results showed no difference between the 2 ipilimumab groups, with a median overall survival (OS) of 10 months, better than 6.4 months observed in patients receiving gp100 alone.[17] Moreover, responses in the ipilimumab groups were sustained and durable, and gp100 vaccine was inactive. The most common adverse events related to the study drugs were immune-related events, which occurred in approximately 60% of the patients treated with ipilimumab and 32% of the patients treated with gp100. Grade 3 or 4 toxicities occurred in 10% to 15% of the patients in the ipilimumab groups and in 3.0% of those in the gp100-alone group. Seven of the 14 total deaths occurred due to autoimmune side effects. The gastrointestinal tract (diarrhoea/colitis) and the skin (injection site reactions and vitiligo) were the most affected organs. Endocrine immune-related adverse events (e.g., inflammation of the pituitary

Table 1 - Average outcomes, progression free survival (PFS) and overall survival (OS), of immune checkpoint inhibitors, based on selected trials (Adapted from[103])

Immunotherapy	PFS at 6 months (%)	OS at 12 months (%)	OS at 24 months (%)
FIRST LINE			
Anti-CTLA-4 (Ipilimumab)	39.3	50.4	28.6
Anti-PD-1 (Nivolumab or Pembrolizumab)	51.1	72.2	59.3
Combined therapy (Anti – CTLA-4 + Anti-PD-1)	63.8	73.1	62.9
SECOND LINE			
Anti-CTLA-4 (Ipilimumab)	21.8	48.6	29.1
Anti-PD-1 (Nivolumab or Pembrolizumab)	40.2	62.1	45.3
Combined therapy (Anti – CTLA-4 + Anti-PD-1)	73.2	86.9	n.a.

Legend: n.a. not available

gland) were also reported. Corticosteroids were critical for the management of immune-related adverse events.

The second phase III clinical trial was carried out in previously untreated patients.[20] Patients were randomly assigned in a 1:1 ratio to receive ipilimumab (10 mg/kg) plus dacarbazine (850 mg/m^2 of body-surface area) or dacarbazine (850 mg/m^2) plus placebo. In this trial, the OS was significantly longer in the group receiving ipilimumab plus dacarbazine than in the group receiving dacarbazine plus placebo (11.2 months *vs* 9.1 months) (hazard ratio – HR for death with ipilimumab-dacarbazine: 0.72; $p < 0.001$) and the 3-year OS was 20.8% *vs* 12.2%, respectively. The incidence of adverse effects, regardless of the cause, was higher in the ipilimumab plus dacarbazine group than in the dacarbazine group, and included elevation of serum levels of alanine aminotransferase (in 33.2% of patients *vs* 5.6%) and aspartate aminotransferase (29.1% *vs* 5.6%), diarrhoea (36.4% *vs* 24.7%), pruritus (29.6% *vs* 8.8%) and rash (24.7% *vs* 6.8%). Further, grade 3 or 4 adverse events occurred in 56.3% of patients receiving ipilimumab plus dacarbazine and in 27.5% of patients receiving placebo plus dacarbazine. Overall, immune-related adverse events were considerably higher in the group treated with ipilimumab plus dacarbazine (77.7% *vs* 38.2%), with elevated liver function values, including grade 3 or 4, as the most common immune-related adverse event.

Another phase III multicentric clinical trial ascertained two different ipilimumab doses.[21] Patients with unresectable stage III or IV melanoma, without previous therapy (BRAF inhibitors, CTLA-4 or PD-1/PDL-1 antagonists), were randomly assigned (1:1) to ipilimumab 10 mg/kg *vs* 3 mg/kg. The median OS was 15.7 months in the ipilimumab 10 mg/kg group and 11.5 months in the 3 mg/kg group (HR 0·84, 95% $p = 0·04$; 1-year OS was 54·3% in the 10 mg/kg group *vs* 47.6% in the 3 mg/kg group; 2-year OS was 38.5% *vs* 31.0% and 3-year OS was 31.2% *vs* 23.2%. The median progression-free survival (PFS) was 2.8 months in the 10 mg/kg group and 2.8 months in the 3 mg/kg group (Table 1). Globally, the incidence of adverse effects was higher in the 10 mg/kg group (79%) than in the 3 mg/kg group (63%). The most common grade 3 to 4 treatment-related adverse events were diarrhoea (10% in the 10 mg/kg group *vs* 6% in the 3 mg/kg group), colitis (5% *vs* 2%), increased alanine aminotransferase (3% *vs* 1%), and hypophysitis (3% *vs* 2%). Overall, immune-related adverse events occurred in 74% of patients in the 10 mg/kg group compared to 54% of patients in the 3 mg/kg group, most commonly diarrhoea, rash and pruritus. This trial demonstrated a significant improvement in OS with ipilimumab 10 mg/kg, however, these higher doses were associated with more adverse effects, including auto-immunity.

c) Dosage

Ipilimumab is currently approved for patients with unresectable or metastatic melanoma at a recommended dose of 3 mg/kg administered intravenously over 90 minutes every 3 weeks for a total of 4 doses.[22]

2.2 – PD-1 inhibitors

2.2.1 – Nivolumab

a) Mechanism of action:

Nivolumab, a fully human monoclonal IgG4 antibody, binds to PD-1 receptors on T cells and blocks the binding of its ligands, PD-L1 and PD-L2, thereby releasing PD-1-mediated inhibition of the immune response[8,14,22] (Fig. 1). *In vitro*, nivolumab exhibited high affinity and selective binding to PD-1 receptors, with no binding to other immunoglobulin super-family proteins, including CTLA-4 and CD28.[23]

b) Long-term efficacy and toxicity

A pilot study (phase I) with anti PD-1 immune checkpoint on solid tumours refractory to previous treatments demonstrated evidence of anti-tumour activity with a good safety profile.[24] Later, a larger phase I study corroborated the previous findings, showing a durable objective response with few adverse effects.[25] Data from phase I (II) trials encouraged further phase III studies, which supported the approval of nivolumab for unresectable/metastatic melanoma.[26,27] The Check-Mate-037[26] was a multicentric, open label phase III, clinical trial that investigated nivolumab versus the investigator's choice of chemotherapy (ICC) in patients with unresectable stage III C or IV metastatic melanoma that had progressed after ipilimumab, or ipilimumab and a BRAF inhibitor (for patients with melanoma harbouring a *BRAFV600E* mutation). Patients were randomly assigned in a 2:1 ratio to receive nivolumab 3 mg/kg every 2 weeks or ICC (dacarbazine 1000 mg/m^2 every 3 weeks or paclitaxel 175 mg/m^2 combined with carboplatin every 3 weeks). The median PFS was 4.7 months for the nivolumab group vs 4.2 months for the ICC group (HR 0.82). However, the median OS did not differ between the two treatment groups (16 months for patients treated with nivolumab *versus* 14 months for patients treated with chemotherapy).[28] Nevertheless, nivolumab demonstrated higher and more durable responses. Most patients developed adverse events related to treatment: 68% in the nivolumab group and 79% in the ICC group. The most frequent adverse events in the nivolumab group were fatigue, pruritus and diarrhoea and in the ICC group were nausea, fatigue, and alopecia. Grades 3 to 4 treatment-related adverse events occurred in 9% of the patients in the nivolumab group *vs* 31% of the patients in the ICC group. In the former group, the most common treatment-related grades 3 to 4 adverse events were increased serum lipase and alanine aminotransferase, fatigue and anaemia. Very few adverse events related to immunological causes requiring monitoring and potential intervention (immune suppression or endocrine treatment) were noted in the nivolumab group. In another multicentric phase III clinical trial, Check-Mate-066,[27] patients with BRAF wild-type metastatic melanoma were randomly assigned in a 1:1 ratio to receive 3 mg/kg of nivolumab every 2 weeks, or 1000 mg/m2 of dacarbazine every 3 weeks. Results showed that the 1 year OS rate 72.9% in the nivolumab group compared with 42.1% in the dacarbazine group (HR for death, 0.42; 99.79% confidence interval (CI), 0.25 to 0.73; $p < 0.001$). The median PFS was 5.1 months in the nivolumab group *vs* 2.2 months in the dacarbazine group (HR for death or progression of disease, 0.43; 95% CI, 0.34 to 0.56; $p < 0.001$). The survival benefit associated with nivolumab was noted regardless of PDL-1 expression, that is, even in those patients negative for PDL-1 the survival was improved in the nivolumab group in comparison with the ICC group. This trial reinforced the safety profile of nivolumab. The incidence of treatment-related adverse events of any grade was similar in the nivolumab group and the dacarbazine group (74.3% vs 75.6%). However, treatment-related grade 3-4 adverse events were reported less frequently in the nivolumab group (11.7% vs 17.6%) and included fatigue, pruritus and nausea. Immune-related adverse effects were uncommon with nivolumab and included diarrhoea (10%) and elevated alanine aminotransferase level (10%). Globally, this study demonstrated that nivolumab was associated with significant improvements in OS and PFS, as compared with dacarbazine.

c) Dosage and administration of nivolumab

Nivolumab is currently approved for patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if *BRAF V600* mutation positive, a BRAF inhibitor. The recommended dosage is 3 mg/kg once every 2 weeks, administered as a 60-minutes intravenous infusion.[29]

2.2.2 – *Pembrolizumab*

a) Mechanism of action:

Pembrolizumab is another highly selective humanised monoclonal antibody of the IgG4 isotype against PD-1. By binding to PD-1, pembrolizumab blocks its interaction with PD-L1 and PD-L2, thereby potentiating T-cell responses and reactivating anti-tumour immunity.[8,14,30] This drug improves the antigen responsiveness of immune cells *in vitro* (e.g., enhancing the production of several cytokines, including interleukin (IL)-2, IL-6, IL- 17, interferon gamma (IFN-γ) and tumour necrosis factor-a (TNF-α), by activated T cells.[31]

b) Long-term efficacy and toxicity

Key data supported the efficacy of pembrolizumab in the treatment of advanced melanoma. In Keynote 001,[32] a phase I study, pembrolizumab (formerly known as labrolizumab) was evaluated in patients with melanoma refractory to other immune therapy or BRAF inhibitors. Patients were treated with pembrolizumb at 10 mg/kg every 2 weeks, resulting in a high rate of sustained tumour regression with a reasonable safety profile.

Based on data from Keynote 001, in the phase II keynote 002 study two pembrolizumab dosages (2 or 10 mg/kg every 3 weeks) were compared with ICC in adults with ipilimumab-refractory advanced melanoma.[33] Results showed that the two pembrolizumab doses reduced the risk of disease progression or death compared with ICC and were well tolerated compared with chemotherapy, with fewer treatment-related adverse events, including grade 3 to 4. Potentially immune-mediated adverse events with pembrolizumab were infrequent, mostly grade 1 or 2, and generally manageable with immunosuppressive therapy. The benefit of pembrolizumab is further strengthened by more favourable health-related quality of life scores compared with chemotherapy. Those findings established pembrolizumab as a new standard of care for ipilimumab-refractory melanoma.

Finally, Keynote 006,[34] a multicentric randomised, controlled, phase III study compared pembrolizumab with ipilimumab. In this study, 834 patients with advanced melanoma

were enrolled in a 1:1:1 ratio to receive pembrolizumab (10 mg/kg of body weight) every 2 or every 3 weeks, or four doses of ipilimumab (3 mg/kg) every 3 weeks. The results showed that the 6-month PFS rates were rates were 47.3% for pembrolizumab every 2 weeks, 46.4% for pembrolizumab every 3 weeks, and 26.5% for ipilimumab (HR for disease progression = 0.58; $p < 0.001$ for both pembrolizumab dosages versus ipilimumab; 95% CI: 0.46 to 0.72 and 0.47 to 0.72, respectively). The estimated 1-year OS rates were 74.1%, 68.4%, and 58.2%, respectively (HR for death for pembrolizumab every 2 weeks, 0.63; 95% CI: 0.47 to 0.83; $p = 0.0005$; HR for pembrolizumab every 3 weeks, 0.69; 95% CI: 0.52 to 0.90; $p = 0.0036$). Efficacy was similar in the two pembrolizumab groups. The most common treatment-related adverse events of any grade in the pembrolizumab groups were fatigue (20.9% in the 2-week group and 19.1% in the 3-week group), diarrhoea (16.9% and 14.4%, respectively), rash (14.7% and 13.4%, respectively) and pruritus (14.4% and 14.1%, respectively). Grades 3 to 4 adverse events occurred in less than 1% of patients, except for diarrhoea (2.5% and 1.1%, respectively). For ipilimumab, the most frequent adverse events were pruritus (25.4%), diarrhoea (22.7%), fatigue (15.2%) and rash (14.5%), with grades 3 to 5 severity in less than 1% of patients, except for diarrhoea (3.1%) and fatigue (1.2%). Adverse events of special interest on the basis of the likely autoimmune or immune-related mechanism observed most frequently with pembrolizumab were hypothyroidism (10.1% in the 2-week group and 8.7% in the 3-week group) and hyperthyroidism (6.5% and 3.2%, respectively). In the ipilimumab group, the most common adverse event of special interest was colitis (8.2%). Hypothyroidism and hyperthyroidism were more frequent in the pembrolizumab groups, whereas colitis and hypophysitis were more frequent in the ipilimumab group. The mean duration of exposure was 164 days among patients receiving pembrolizumab every 2 weeks, 151 days among those receiving pembrolizumab every 3 weeks and 50 days for those receiving ipilimumab. However, the rates of treatment-related adverse events of grades 3 to 5 severity were lower in the pembrolizumab groups (13.3% and 10.1%) than in the ipilimumab group (19.9%). This trial demonstrated that pembrolizumab, as compared with ipilimumab, significantly prolonged PFS and OS with fewer high-grade toxic events in patients with advanced melanoma.

A recent multicentric phase III study35 consolidated data from Keynote 006, showing that pembrolizumab continued to provide superior OS versus ipilimumab, with no differences between pembrolizumab dosing schedule.

c) Dosage and administration of pembrolizumab

Pembrolizumab is currently approved for patients with unresectable or metastatic melanoma. The recommended dosage is 2 mg/kg administered through a 30-minutes intravenous infusion every 3 weeks. Patients with the first signs of disease progression should continue to receive pembrolizumab until disease progression is confirmed.[36]

3 – IMMUNE CHECKPOINT INHIBITORS: COMBINED IMMUNOTHERAPY

3.1 – Principles

Targeting different checkpoint inhibition pathways triggers a different set of immune responses, based on a study on *ex-vivo* analyses of tumour tissue samples and blood-derived, purified human immune cells collected from patients treated with momotherapy with nivolumab, ipilimumab or combination therapy with nivolumab plus ipilimumab.[37] Pathway analysis revealed that CTLA-4 blockade induces a proliferative signature predominantly in a subset of transitional memory T cells, while PD-1 blockade instead leads to changes in genes implicated in cytolysis and natural killer cell function. Upregulation of genes was substantially higher in patients treated with combination therapy (442 genes upregulated) than in those treated with nivolumab or ipilimumab monotherapy (36 and 26 genes upregulated, respectively). Therefore, a combination blockade leads to non-overlapping changes in gene expression, including proliferation-associated and chemokine genes.

Likewise, oncolytic virus may have an attractive synergy interaction with immunotherapy as they directly lyse tumor cells, leading to the release of soluble antigens, danger signals and type I interferons, which drive anti-tumour immunity, among other immunologic functions.[38] Cytokines, well known as potent immune modulating agents, might also concur in this cytolysis activity.[39]

3.2 - Ipilimumab plus nivolumab

Some studies have shown that nivolumab and ipilimumab have complementary activity in metastatic melanoma.[40,41] These findings were confirmed in a randomised, double-blind, phase III study, in which nivolumab alone or nivolumab plus ipilimumab were compared with ipilimumab alone in patients with metastatic melanoma.[42] In this study, 945 previously untreated patients with unresectable stage III or IV melanoma were enrolled in a 1:1:1 ratio to receive nivolumab alone, nivolumab plus ipilimumab, or ipilimumab alone. The results showed that the median PFS was 11.5 months (95% CI: 8.9 to 16.7) for combined therapy, as compared with 2.9 months (95% CI: 2.8 to 3.4) for ipilimumab (HR for death or disease progression, 0.42; 99.5% CI: 0.31 to 0.57; $p < 0.001$) and 6.9 months (95% CI: 4.3 to 9.5) for nivolumab (HR for the comparison with ipilimumab, 0.57; 99.5% CI: 0.43 to 0.76; $p < 0.001$). The incidence of adverse events was lowest in the nivolumab group and highest in the combination group. Grade 3-4 treatment-related adverse events occurred in 16.3% of the patients in the nivolumab group, 55.0% in the nivolumab+ipilimumab group and 27.3% in the ipilimumab group. This study demonstrated that nivolumab alone or combined with ipilimumab resulted in significantly longer PFS than ipilimumab alone.

The 3-year OS outcomes of the previous trial were reported later.[43] This study showed that at a minimum follow-up of 36 months, the median OS had not been reached in the nivolumab-plus-ipilimumab group and was 37.6 months in

the nivolumab group, as compared with 19.9 months in the ipilimumab group (HR for death with nivolumab plus ipilimumab *vs* ipilimumab, 0.55 $p < 0.001$; HR for death with nivolumab *vs* ipilimumab, 0.65 [$p < 0.001$]). The OS rate at 2 years was 64% in the nivolumab-plus-ipilimumab group and 59% in the nivolumab group, as compared with 45% in the ipilimumab group. The 3-year OS rate was 58% in the nivolumab-plus-ipilimumab group and 52% in the nivolumab group, as compared with 34% in the ipilimumab group. This study corroborated that among patients with advanced melanoma, significantly longer OS occurred with combination therapy with nivolumab plus ipilimumab or with nivolumab alone than with ipilimumab alone.

On the basis of this data, the FDA approved combined use of ipilimumab and nivolumab for the treatment of stage IV melanoma.

3.3 - Pembrolizumab plus ipilimumab

Pembrolizumab in combination with ipilimumab was evaluated in the KEYNOTE-029, a multicentric open-label, phase Ib trial.[44] This study showed that standard-dose pembrolizumab (2 mg/kg) given in combination with four doses of low-dose ipilimumab (1 mg/kg) followed by standard-dose pembrolizumab has a manageable toxicity profile and provides robust anti-tumour activity in patients with advanced melanoma. These data suggest that standard-dose pembrolizumab plus reduced dose ipilimumab might be a tolerable and efficacious treatment option for patients with advanced melanoma. However, more data is needed, and a randomised phase II trial of alternative dosing strategies of this combination is underway.

3.4 - Talimogene laherparepvec (T-VEC) plus immune checkpoint inhibitors

T-VEC is an oncolytic attenuated herpes simplex virus type 1 (HSV-1), engineered to express GM-CSF. To date is the only that has been approved for cancer treatment, specifically for advanced melanoma. Phase Ib clinical trial in 19 patients with advanced melanoma, intratumoral T-VEC administration followed by addition of a standard intravenous dose of the anti-CTLA4 antibody ipilimumab (3 mg/kg) showed promising results with acceptable toxicity.[45] These results supported a subsequent Phase II randomised clinical trial in 198 patients with unresectable stage IIIB–IV melanoma that compared combination treatment using T-VEC and ipilimumab to ipilimumab alone. It found a significant improvement in response rate (38% with the combination *vs* 18% with ipilimumab alone; OR 2.9; 95% CI 1.5–5.5; $p = 0.002$), with no increase in the incidence or severity of serious adverse effects with combination treatment, therefore showing that this combination had greater anti-tumour activity without additional safety concerns.[46] T-VEC and pembrolizumab combination has also been tested in a phase I study of 21 patients with unresectable stage IIIb/IV melanoma by injecting lesions and with no prior systemic therapy. Although the sample size was small, data showed 57.1% objective response, encouraging further studies.[47] A larger randomised phase III trial is currently underway to evaluate this combination in comparison to pembrolizumab alone (MASTERKEY-265).

3.5 NKTR-214 plus nivolumab

Cytokines, namely interleukin-2 (IL2) an endogenous agonist of the IL2 pathway and a well described stimulator of CD8+ T cell (CD8 T) and NK cells, which had been approved during the 1990s by FDA the for the treatment of metastatic melanoma, might also concur in this cytolysis activity. However, doses of IL2 needed to activate CD8 T cells and NK cells are high and associated with severe toxicity, which limited its use.[38] NKTR-214 is a clinical-stage biologic pro-drug that comprises IL2 protein bound by multiple releasable polyethylene glycol (PEG) chains. In this highly PEG-bound form, IL2 is inactive, but it expands directly in the tumor microenvironment and activates specific cancer-fighting T cells and natural killer (NK) cells and increases expression of cell-surface PD-1, with minimized unwanted systemic toxicity of IL-2.[38] Preliminary data from the ongoing PIVOT Phase I/II Study was presented at ASCO 2018, demonstrating safety and efficacy, and thus encouraging further phase III studies.[48]

4 - IMMUNE CHECKPOINT INHIBITORS IN AN ADJUVANT SETTING

The likelihood of systemic metastatic disease among patients with stage III melanoma correlates closely with microscopic versus palpable nodal disease and with the number of positive nodes. The population of patients with stage III melanoma is heterogeneous, with disease-specific survival rates of 78% among patients with stage IIIA disease, 59% among those with stage IIIB disease, and 40% among those with stage IIIC disease.[2,49] Patients with the largest metastasis of more than 1 mm have a significantly higher risk of recurrence or death than those with the smallest metastasis of 1 mm or less (50). In this setting, ipilimumab was investigated at a dose of 10 mg/kg (against the 3 mg/kg used in metastatic disease) every 3 weeks for four doses, then every 3 months for up to 3 years or until disease recurrence or an unacceptable level of toxic effects occurred in patients who had undergone complete resection of stage III melanoma in a phase 3 clinical trial.[51] In this study, recurrence free survival was the primary end point and overall survival (OS), distant metastasis-free survival, and safety were the secondary end points. At a median follow-up of 5.3 years the results showed significantly higher rates of recurrence-free survival, OS, and distant metastasis–free survival than placebo. The 5-year rate of recurrence-free survival was 40.8% in the ipilimumab group, as compared with 30.3% in the placebo group (HR for recurrence or death = 0.76; 95% CI: 0.64 to 0.89; $p < 0.001$). The rate of OS at 5 years was 65.4% in the ipilimumab group, as compared with 54.4% in the placebo group (HR for death = 0.72; 95.1% CI: 0.58 to 0.88; $p = 0.001$). The rate of distant metastasis-free

survival at 5 years was 48.3% in the ipilimumab group, as compared with 38.9% in the placebo group (HR for death or distant metastasis = 0.76; 95.8% CI: 0.64 to 0.92; p = 0.002). However with such a high dose of ipilimumab, adverse events, mostly immune-related of grade 3 or 4 events, were significantly higher in the ipilimumab group (41.6% *vs* 2.7%) and led to the discontinuation of treatment in approximately 40% of the patients by the end of the initial dosing period.

Recently, a phase III trial evaluated the efficacy of nivolumab versus ipilimumab for adjuvant therapy in patients with resected advanced melanoma.[52] Patients were randomly assigned (1:1) to receive an intravenous infusion of either nivolumab (3 mg/kg, every 2 weeks) or ipilimumab (10 mg/kg every 3 weeks for four doses and then every 12 weeks up to one year or until disease recurrence, unacceptable level of adverse effects or withdrawal of consent). The primary end point was recurrence-free survival. At a minimum follow-up of 18 months, the 12-month rate of recurrence-free survival was 70.5% (95% CI: 66.1 to 74.5) in the nivolumab group and 60.8% (95% CI: 56.0 to 65.2) in the ipilimumab group (HR for disease recurrence or death = 0.65; 97.56% CI: 0.51 to 0.83; $p < 0.001$).

Treatment related grade 3 or 4 adverse events were reported in 14.4% of the patients in the nivolumab group and in 45.9% in the ipilimumab group, which led to treatment descontinuation in 9.7% and 42.6% of the patients, respectively. Similar to previous study, these results may reflect the use of high doses of ipilimumab.

5 - IMMUNE CHECKPOINT INHIBITORS IN *BRAF* MUTATED MELANOMA

Activating mutation of the serine-threonine kinase *BRAF* gene is the most frequent genetic alteration in melanoma, observed in about 35%-50% of skin melanomas. The second most frequent genetic alteration activates the RAS oncogene and is observed in 10%-25% of the melanomas.[8,9]

Agents directly targeting the mutated *BRAF* (BRAF inhibitors) such as vemurafenib and dabrafenib have also shown to improve the outcomes of patients with advanced melanoma.[10,11] They classically induce earlier anti-tumour activity and, therefore, in most cases BRAF inhibitors are considered the preferred option to treat patients with rapidly growing tumors carrying this *BRAF* mutation. However, acquired resistance occurs in half of the patients after approximately six months of treatment.[10,11] The addition of a MEK inhibitor results in extension of the time to resistance, translating into longer median PFS of treated patients.[53,54]

On the other hand, treatment of melanoma with an ICI, has lower response rate but the response is much more durable, lasting for years.[55] For this reason, it was suggested that combination of BRAF/MEK inhibitor and ICI will significantly improve overall survival time.[56,57] However, an early phase clinical study evaluating the combination of vemurafenib with ipilimumab showed high toxicity (including hepatotoxicity) and required discontinuation of the study.[58]

Indeed, either target therapy or ICI can induce a good response in BRAF mutated melanoma, either as first line, or in previously treated patients. Nevertheless, in spite of the diverse treatment strategies, there is little insight into the most effective treatment for patients with *BRAF* mutant melanoma. Currently, phase III study results comparing the efficacy of targeted therapy and ICI in patients with *BRAF* mutation are still lacking, but it seems consensual that sequential therapy with ICI and BRAF/MEK inhibitor is beneficial, although the optimal sequence remains to be established.[59,60]

6 - BIOMARKERS UNDERLYING THE CONCEPT OF "CANCER IMMUNOGRAM"

Biomarkers in cancer research are defined as *"A biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease. A biomarker may be used to see how well the body responds to a treatment for a disease or condition."* (in NCI Dictionary of Cancer Terms, National Cancer Institute, 2015). Biomarkers can be classified as diagnostic (help to diagnose the current condition), prognostic (help to estimate whether the cancer will progress or will remain stable in the absence of treatment) or predictive (help to predict how well a patient will respond to treatment).[61]

Although ICI has marked efficacy in advance melanoma, only a subset of the patients has durable responses with immunotherapy.[62] Thus, which patients would benefit with ICI? Is it possible to individualize the therapeutic schedule according to the patient? In an attempt to collaborate in predictive biomarker research and treatment choice guidance, Blank CU et al proposed a framework called *"cancer immunogram"*, which describes the different interactions between cancer and the immune system.[63] To build this framework, the authors suggested the inclusion of seven classes of parameters (tumor foreignness; general immune status, immune cell infiltration, absence of checkpoints, absence of soluble inhibitors, absence of inhibitory tumor metabolism and tumor sensitivity to immune effectors) that characterize aspects of cancer-immune interactions. For each parameter possible biomarkers were assigned that should be obtained from the combination of tumor genomics, immunohistochemistry, and standard assays on the peripheral blood compartment (Fig. 2 A). Those parameters must be addressed and ideally scored in each patient. *"Cancer immunogram"* should also have value in relapsing cases as after restoring a parameter with a targeted therapy, a different one may be lost as a result of relapse (Fig. 2B).

Despite being urgently needed, to date none of these predictive biomarkers has been validated for immunotherapeutic agents in melanoma.[61,62]

7 - PARTICULAR SETTINGS: BRAIN METASTASIS AND MONITORING OF THERAPEUTIC RESPONSE

7.1 - Brain Metastasis

Brain metastases occur in up to 60% of patients with metastatic melanoma and classically confer a worse prognosis.[2]

Educação Médica Contínua

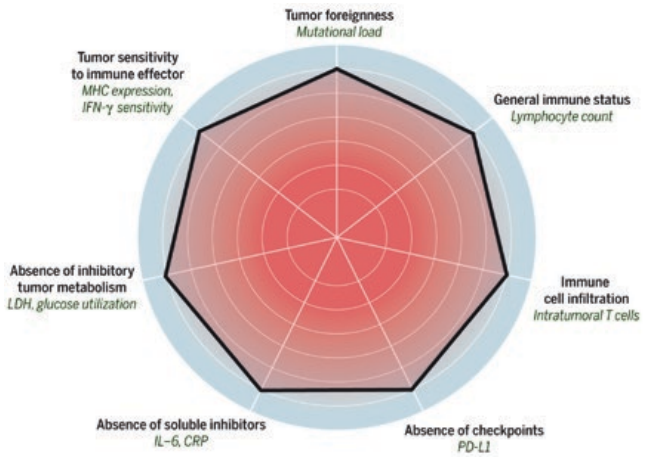

Figure 2A - (Adapted from Blank CU, et al. Science. 2016; 352:658-60[63]): The *"cancer immunogram"* proposed by Blank *et al*. All 7 parameters, and their potential biomarkers (in italic green) are represented in the circle. Desirable states are located in blue, thus in the ideal scenario the patient fits all parameters, and their link gave rise to a perfect *heptagon*. *Progressive* undesirable states are shown in the red gradient. The loss of one or more desirable states destabilizes the *heptagon* and indicates the need for a particular therapy (according to the parameter (s) lost) to restore the heptagon (desirable state).
PD - L1 (programmed cell death protein 1 ligand), MHC (major histocompatibility complex), IFN- γ (Interferon gamma), LDH (lactate dehydrogenase), IL-6 (Interleukin – 6), CRP (C-reactive protein).

Risk factors for melanoma brain metastasis (MBM) include male gender, primary disease of head or neck, presence of visceral or nodal metastases, increased serum lactate dehydrogenase (LDH) levels and high Clark's level/Breslow thickness of the primary disease.[2,64] Typically MBM present initially as headache, seizures, and neurological impairment.[65]

The central nervous system (CNS) is the major site of treatment failure and of the disease progression even when extracranial disease is controlled.[65] This may be due to the inability of drugs to target the CNS (blood-brain barrier), the abundance of ATP-binding cassette export pumps and also the unique microenvironment of the brain that shapes the transcriptional and phenotypic behavior of brain-resident melanoma cells.[65-67]

Until recently, therapeutic options for MBM were limited, largely consisting of surgery and radiation therapy (RT) in the form of stereotactic radiosurgery.[66]

It has been shown that the brain inflammatory microenvironment is critical for development of MBM, namely the presence of immune cells within the CNS, which promote cross talk, and key signaling events between metastatic cells and systemic immune cells. Immune cells such as glia (including microglia, oligodendrocytes and astrocytes) and macrophages create a suitable environment for development of brain metastasis by promoting growth through the production of various factors and chemokines. Regarding the close interaction between brain metastases and immune cells, ICI starts to be considered as potentially targeting also MBM.[65,66,68,69] ICI do not directly act on the tumor but they remove immunological "brakes" and allow activation of anti-tumour T cells, either during the priming phase (anti-CTLA4) or during the effector phase (anti-PD1). T cells are then able to cross the blood brain barrier and induce a response against MBM.[14,66] Ipilimumab was the first agent approved for MBM mainly based on improved outcomes in retrospective studies.[66,70] Pembrolizumab also showed activity against MBM.[71] Combination and sequential immunotherapy must be addressed as some patients with intracranial disease have responded to pembrolizumab after ipilimumab therapy whereas others have their disease stabilized following combination of pembrolizumab and ipilimumab after sequential failure of isolated ipilimumab and pembrolizumab.[66,72] Meanwhile, systematic reviews also support the combination of ICI with

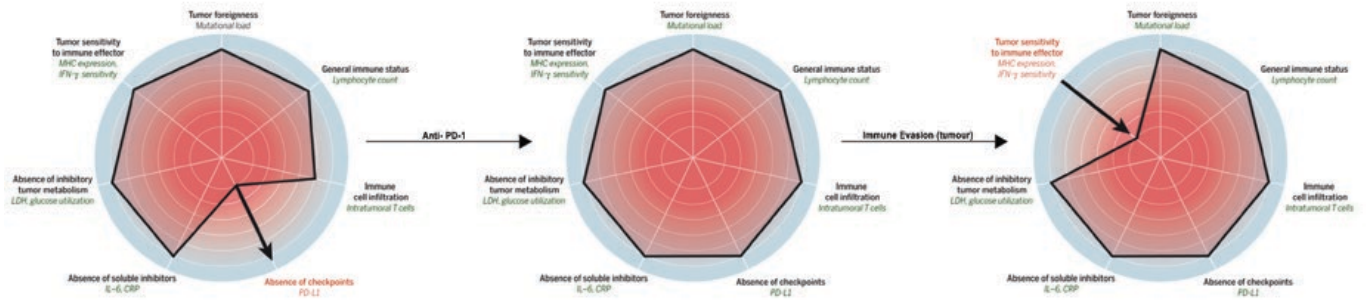

Figure 2B - (Adapted from Blank CU, et al. Science. 2016; 352:658-60[63]): *"Cancer immunogram"* is also useful in cases of non-responding patients, as shown in the following example: initially a patient with melanoma scored well with respect to all parameters in the cancer immunogram, except for strong expression of PD-L1 at the tumour site. Based on this analysis, a single agent PD-1 blockade was the treatment of choice and the patient experienced a clinical response after restoring this parameter by PD-1 blockade treatment (heptagon restored). However, mechanisms of immune evasion, led to tumour insensitivity to T cell effector mechanisms, and consequently to relapse. According to the *"cancer immunogram"* the relapsing patient scored unfavorably concerning tumour sensitivity of immune effector, a different pre-treatment scenario, and perhaps will benefit from another treatment option.

stereotactic radiosurgery or surgery.[73-75] Therefore, despite the shy results of the previous studies, prospective clinical trials must be conducted to accurately assess the efficacy of ICI on MBM and to determine the correct sequence of local and systemic therapies. Currently, there are many on-going clinical trials on this topic.[66]

7.2 - Monitoring therapeutic response

It is noteworthy that the unique anti-tumour mechanisms elicited by the ICI gave rise to a different response pattern which is no longer appropriately assessed by conventional tumor response criteria such as the WHO criteria[76] and Response Evaluation Criteria in Solid Tumours (RECIST).[77] Indeed, a subset of patients under ICI therapy develops an unconventional response pattern ("pseudoprogression") e.g. a response is detected only after a first increase in tumor burden or during/after new lesions have developed.[78] Pseudoprogression reflects T-cell infiltration driven by immune activation rather than tumor progression, can be confirmed only by serial imaging, and ICI therapy should not be stopped during this period.[79] Events like this would be classified as tumor progression according to the WHO or RECIST criteria and not as pseudoprogression. Consequently, new criteria for disease progression, the immune-related response criteria (irRC) were created, whose key features include: confirmation of disease progression on two consecutive scans (at least 4 weeks apart) and inclusion of new lesions in the sum of lesion measurements.[78,80] Regardless of clinical and radiologic monitoring, we should be aware that ICI therapy results from a complex interplay between the tumor and the immune system, and as such individual patterns of clinical response are expected.[80] Therefore *"cancer immunogram"*, mentioned above, would be crucial to evaluate therapeutic response and eventual relapses concerning ICI.[63] (Fig. 2B).

8 - FUTURE PERSPECTIVES: NEWLY EMERGING IMMUNE CHECKPOINT INHIBITORS

There has been an increasing interest in searching additional ICI that may affect novel therapeutic targets in cancer. Recent advances have identified several novel immune checkpoint targets, including indoleamine 2,3-dioxygenase 1 (IDO1), PD-1ligands (PD-L1 and PD-L2), lymphocyte activation gene-3 (LAG-3), B and T protein 3 (Tim-3), B- and T-lymphocyte attenuator (BLTA) and glucocorticoid-induced TNFR family related gene (GITR)[14,81-83] (Fig. 1).

8.1 - IDO1 inhibitors

Indoleamine 2,3-dioxygenase 1 (IDO1) is an intracellular cytosolic enzyme that regulates the degradation of tryptophan to N-formylkynurenine. Tryptophan depletion induces T-cell cycle arrest, increases their apoptosis by inhibiting the mechanistic target of rapamycin complex 1 (mTORC1), and induces a stress response that activates the general control nondepressible-2 (GCN2). Traditionally the immunosuppressive effect of IDO1 has been attributed mainly to reducing levels of tryptophan in the tumor microenvironment.[14,84,85]

Pre-clinical studies are now focusing on selective IDO inhibitors. Epacadostat is an orally available IDO1-selective inhibitor under active clinical investigation, and the most advanced in development. The lack of anticancer activity as a monotherapy has positioned epacadostat in combination with approved ICIs. Concerning melanoma, epacadostat combined with pembrolizumab is currently under a phase III clinical trial (NCT02752074).[84]

8.2 - PDL-1 inhibitors

PD-L1 and PD-L2 are PD-1 ligands expressed in multiple tissues. PD-L1 is widely expressed in a variety of hematopoietic and non-hematopoietic cells, and also in many human cancer types. PD-L2 tumor expression is less frequent than PD-L1 and is limited to antigen-presenting cells, macrophages, Th2 cells, and non-hematopoietic cells in the lung. Targeting PD-L1 instead of PD-1 offers a potential advantage in that PD-L2 remains uninhibited.[86]

Atezolizumab (a fully humanized engineered IgG1 monoclonal antibody against PD-L1 that contains a modified Fc receptor) and durvalumab (a Fc optimized monoclonal IgG1 directed against PD-L1) have shown encouraging results in phase I studies when combined with an anti-MEK or anti-BRAF in treatment of metastatic melanoma.[86-88] A phase III study has been designed to assess cobimetinib, vemurafenib and atezolizumab in combination versus cobimetinib plus vemurafenib (with placebo) for patients with previously untreated BRAF mutant metastatic melanoma (NCT02908672).[86]

8.3 - Anti-LAG-3

LAG-3 is a CD4 homolog activation induced co-receptor of the T cell receptor that binds with high affinity to major histocompatibility complex (MHC) class II molecules.[69,86,87] Through its interaction with MHC class II, LAG-3 controls the response, activation, and growth of T-cells.[89]

Relatlimab is an anti-LAG-3 antibody, which is being investigated, alone and in combination with other treatments, in several types of cancer, including melanoma.[89] A phase I/II study (NCT01968109) is currently investigating the safety, tolerability, and effectiveness of relatlimab in combination with nivolumab in melanoma patients whose disease progressed after prior anti–PD-1 therapy. Initial encouraging results supported recruitment for a phase III study.[91]

8.4 - Anti-TIM-3

T-cell immunoglobulin mucin-3 (TIM-3) belongs to TIM proteins group, which are implicated in the regulation of innate and adaptive immune responses. Particularly, TIM-3 was originally identified as a negative immune regulator which is expressed on T helper 1 (Th1) cells. The interaction with its ligand galectin-9 inhibits Th1 responses and induces peripheral tolerance.[14,92,93] Further investigation indicated that TIM-3 is also expressed on multiple cells types.[93]

Concerning cancer, recent studies demonstrated that TIM-3 is highly expressed within the tumor microenvironment due to tumor associated antigens stimulation and suggest that this protein could play a direct role in the functional maturation of tumor-infiltrating Tregs and also in their immunosuppressive function within the tumor microenvironment.[87,93]

Pre-clinical studies found that coexpression of Tim-3 and PD-1 in CD8 T cells represent the most exhausted population of tumor-infiltrating Tregs and thus simultaneous blockade of Tim-3 and PD-1 could restore the anti-tumour activity of these exhausted T cells.[93,94] Indeed, later Koyama S *et al*[95] reported that the adaptive resistance to PD-1/PD-L1 ICI in lung cancer are due to upregulation of alternative immune checkpoints, notably TIM-3. Using a mouse model, they demonstrated that the addition of TIM-3 antibody overcomes resistance to PD-1 blockade. This study highlights the potential of using combinations of ICIs for enhancing the therapeutic success rates.

Currently, the humanized anti-TIM-3 antibody TSR-022 is under clinical trial, to be evaluated in monotherapy and in combination with an anti-PD-1 antibody in patients with advanced solid tumors and limited available treatment options (NCT02817633).

Pre-clinical studies have been shown that upon its administration, TSR-022 binds TIM-3 and activates antigen-specific T lymphocytes and cytotoxic T-cell mediated tumor lysis reducing tumour growth.[81]

8.5 - Anti-BLTA

BTLA is an immunoglobulin domain containing glycoprotein expressed on T cells, resting B cells, macrophages, dendritic cells and natural Killer cells that have been described as an inhibitory receptor on T cells with structural and functional similarities to CTLA-4 and PD-1.[14,82,96]

Unexpectedly herpesvirus entry mediator (HVEM), a tumor necrosis factor receptor, was identified as a natural ligand for BTLA in both mice and humans.[97]

In melanoma patients, tumor antigen–specific effector CD8+T cells persistently expressed high levels of BTLA and remained susceptible to functional inhibition by its ligand HVEM, which was also found to be highly expressed by melanomas.[97] L Derre *et al*[98] found that the addition of CpG oligodeoxynucleotides to the vaccine with Melan-AMART-1 formulation resulted in enhanced CD8+ T cell responses, due to downregulation of BTLA and resistance to BTLA--HVEM–mediated inhibition. Thus BTLA blockade by targeting BTLA and HVEM may provide an additional therapeutic option for melanoma, but it needs to be further elucidated.[82]

8.6 - GITR agonists

GITR is a costimulatory TNF receptor super family member, which expands CD8+ T effector memory cell population while promoting the loss or inhibition of Tregs.[83] BMS-986156 is a fully human IgG1 agonist monoclonal antibody that binds GITR and promotes effector T-cell activation and possible reduction/inactivation of regulatory Tcells. In this setting, a phase I/IIa study of BMS-986156 alone and in combination with nivolumab in patients with advanced solid tumors (NCT02598960) is ongoing. Preliminary data demonstrated a good safety profile in both groups, but anti--tumour activity was only observed with BMS-986156 plus nivolumab at doses predicted to be biologically active. Further evaluation of this combination in patients with advanced solid tumors is underway.[99]

9 - CONCLUSION

Immunotherapy with checkpoint inhibitors revolutionised the treatment of the patients with unresectable stage III or stage IV melanoma wild-type for BRAF.[100] The percentage of patients with poor prognostic markers, such as elevated serum LDH, impaired overall performance, has differed significantly between the trials, which may have influenced the patients' survival outcomes. Of note, as previously mentioned, those patients with MBM, given their worse prognosis, were not considered in most of the studies. However, globally, the studies demonstrated robust data on efficacy of anti-CTLA-4 and anti-PD1 on advanced melanoma, regarding the durability of their responses and potential for long-term survival, despite the concerning autoimmune side effects. Considering the distinct immune regulating roles of PD-1 and CTL-4, differences in outcomes and drug safety profile are expected. CTLA-4 primarily regulates effector T cell responses within the peripheral tissues.[101] On the other hand, PD-1 is a related inhibitory T cell receptor, in which its ligands, namely PD-L1, are expressed locally within the tumour microenvironment by stromal, immune cells, and by the tumour itself. Thus, targeting this pathway may have a more selective anti-tumour response and potentially fewer adverse effects.[102] A recent meta-analysis from the main trials representative of the new treatment options in advanced metastatic melanoma confirmed that anti-PD-1 agents have higher response rates[103] (Table 1).

As immune checkpoints play a crucial role in maintaining tolerance to self-antigens, treatment with their inhibitors is associated with organ-specific autoimmune phenomenon. The majority (> 70%) of patients treated with ipilimumab developed some form of immune-related adverse events with a quarter of cases considered severe.[104] The majority of events involve the skin (rash, pruritus, vitiligo), gastrointestinal tract (diarrhoea, colitis), endocrine system (thyroid disorders, hypophysitis) and the liver (elevated liver enzymes, hepatitis), and are dose-related. PD-1 inhibitors are much better tolerated than ipilimumab. Fatigue is a common problem, and immune-related adverse events occur less frequently and with lesser severity, but involved organs are similar.[105]

Further, regarding the distinct immune response when targeting PDL-1 or CTLA-4, the combined checkpoint blockade results in higher and more sustained responses; however, toxicity is substantially higher. In this setting combined synergistic therapy seems to be the next step in the treatment of advanced melanoma according to the recent results

concerning the newly emerging immune checkpoints.[14,81-83] In BRAF mutated melanoma the sequential therapy with ICI and target therapy seems to extend the therapeutic response, although the optimal sequential schedule remains to be elucidated.[59,60]

It is well demonstrated that immunotherapy with ICI have yielded promising results in advanced melanoma. PD-1 inhibitors are now the standard of care in melanoma due to their better safety profile and prolonged OS and PFS. Currently, in Portuguese Institute of Oncology, Lisbon, immunotherapy with pembrolizumab is the therapeutic of choice for patients with BRAF wild-type advanced melanoma. Despite the remarkable success of these new immunotherapies in melanoma patients, the efficacy of these agents varies among patients with similar tumor characteristics and similar burden and, at this moment, we have no clear identification of which patients will have a clinical benefit and a complete response after therapy. Similarly, after obtaining a complete response, there are no rules for maintenance therapy or for stopping and waiting to see. All patients under nivolumab and pembrolizumab who obtain clinical benefit maintain the treatment until disease progression or unacceptable toxicity. Some authors advocate the possibility of stopping at 24 months or earlier if a complete response is attained, since over 90% of patients who attain complete response continue to respond even after stopping therapy and have the ability of responding again on re-challenging. Indeed, due to melanoma heterogeneity, potential adverse side effects and high costs of immunotherapy, suitable predictive biomarkers to assess patient risk-benefit management with ICI are urgently needed, and should encompass the cell mediated immune system and the tumor infiltrating immune cells as well as the serum circulating factors.

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Educação Médica Contínua

VERIFIQUE O QUE APRENDEU

1. Which of the following is true of cutaneous melanoma:

a) Cutaneous melanoma represents a minority of all skin cancers although it is responsible for 50 percent of skin cancer deaths.
b) The risk of metastasis correlates directly with primary tumour thickness, but not with ulceration.
c) The incidence of melanoma is increasing however this trend should stabilize throughout the next two decades.
d) Thirty-five to 50% of patients with cutaneous melanoma harbour a *BRAF 600* mutation.
e) Conventional chemotherapy, has been largely effective in patients with unresectable or metastatic melanoma, but immunotherapy using immune system checkpoint inhibitors are now recommended for BRAF wild-type melanoma patients in advance stages.

2. Which of the following is the wrong sentence about immune checkpoint inhibitors (ICI):

a) Ipilimumab is a fully human monoclonal immunoglobulin G1 antibody against cytotoxic T lymphocyte antigen – 4 (CTLA-4).
b) Nivolumab, is a fully human monoclonal immunoglobulin G4 antibody against programmed death – 1 (PD-1).
c) Pembrolizumab is a fully human monoclonal immunoglobulin G4 antibody against programmed death – 1 (PD-1).
d) Nivolumab was the first drug introduced as immunotherapy for melanoma.
e) Pembrolizumab has been better tolerated than ipilimumab.

3. All of the following are possible adverse effects of immune checkpoint inhibitors (ICI), except:

a) Vitiligo
b) Non melanoma skin cancer
c) Colitis
d) Hypophysitis
e) Fatigue

4. Please point out the false sentence:

a) Targeting different checkpoint inhibition pathways triggers a different set of immune responses, thus combined therapy with anti-CTL-4 and anti-PD-1 may result in better outcomes.
b) Based on data from phase 3 studies, the FDA approved combined use of ipilimumab and nivolumab for the treatment of stage IV melanoma.
c) The population of patients with stage III melanoma is homogenous and they also benefit from adjuvant treatment with immune checkpoint inhibitors.
d) The use of higher doses of ipilimumab as adjuvant therapy for stage III melanoma resulted in high percentage (40-45%) of treatment related grade 3 or 4 adverse events.
e) As immune checkpoints play a crucial role in maintaining tolerance to self-antigens, treatment with their inhibitors is associated with organ-specific autoimmune phenomenon.

5. Which of the following is true regarding the therapy of advance melanoma

a) Immune checkpoints inhibitors have no activity in BRAF mutated melanomas.
b) BRAF inhibitors are linked with acquired resistance, occurring in half of the patients after approximately six months of treatment.
c) Prognostic biomarkers are urgently needed to assess the risk-benefit management with immune checkpoints inhibitors in patients with advanced melanoma.
d) CTLA-4 primarily regulates effector T cell responses within the tumour microenvironment and PD-1 is an inhibitory T cell receptor within the peripheral tissues.
e) To date only CTLA-4 and PD-1 have been identified as immune checkpoint targets.

6. Currently, in Portuguese Institute of Oncology, Lisbon, what is the therapy of choice for patients with BRAF wild-type advanced melanoma?

a) Nivolumab
b) Ipilimumab
c) Pembrolizumab
c) Nivolumab + Ipilimumab
d) Pembrolizumab + Ipilimumab

Chave: 1. d); 2. d); 3. b); 4. c); 5. b); 6. c)