Review

Efficacy of Pembrolizumab in Advanced Melanoma: A Narrative Review

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Review

Efficacy of pembrolizumab in advanced melanoma: a narrative review

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Abstract: (1) Introduction: pembrolizumab showed to increase survival in patients with metastatic melanoma, Considering the numerous oncoming studies, we decided to conduct a narrative review of the latest efficacy evidence regarding the use of pembrolizumab, alone or in combination, in patients with metastatic melanoma. (2) Methods: A search was conducted on Pubmed using "pembrolizumab," and "metastatic melanoma" as keywords, considering studies from 2022 onward. (3) results: we reviewed pembrolizumab and associations, cost-effectiveness, virus, advanced acral melanoma, long-term outcomes, real-life data, biomarkers, obesity, and vaccines (4) Conclusions: pembrolizumab is a fundamental option in the therapy of metastatic melanoma. However, a certain group of patients do not respond and therefore new combination options need to be evaluated. In particular, the use of vaccines tailored to tumor epitopes could represent a breakthrough in the treatment of resistant forms. Further studies with larger sample numbers are needed to confirm preliminary results.

Keywords: advanced melanoma; pembrolizumab; immunotherapy

1. Introduction

Melanoma is one of the most problematic malignancies for public health, with a progressive increase in new diagnoses. In addition, about 20% of patients may develop an advanced (unresectable/metastatic) form [1]. However, the possibility of evaluating new markers for lymph node involvement [2,3], and new therapeutic regimens for metastatic melanoma allowed a substantial reduction in mortality [4]. Immunologic check-point inhibitors (ICIs), particularly ipilimumab, pembrolizumab, and nivolumab, permitted a revolution of the approach to the metastatic patient, inducing a relevant increase in survival [5-7]. Ipilimumab, an inhibitor of Cytotoxic T-lymphocyte-associated protein-4 (CTLA-4), in monotherapy allowed to increase the 10-year survival of metastatic patients from 10% to 22% [8].

The programmed death (PD-1) inhibitors, nivolumab and pembrolizumab, also showed to increase survival in metastatic patients [9-13]. In three clinical trials in patients with advanced melanoma (KEYNOTE-001, KEYNOTE-002, and KEYNOTE-006), therapy with pembrolizumab alone resulted in 30-40% complete clinical response [9,11,14-17]. Specifically, in the KEYNOTE-001 study on advanced melanoma, pembrolizumab monotherapy resulted in a 3-year overall survival (OS) of 41% in patients previously treated with ipilimumab and 45% in therapy-naïve patients [13]. New studies are currently emerging in the literature regarding the efficacy of pembrolizumab in patients with metastatic melanoma, even considering the long-term efficacy in real-life.

Pembrolizumab is a humanized IgG4/kappa anti PD-1 monoclonal antibody produced in hamster ovarian cells. The PD-1 receptor is a negative regulator of T-cell activity that has been shown to

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be involved in the control of T-cell immune responses. KEYTRUDA potentiates T-cell responses, including antitumor responses, by blocking PD-1 binding to PD-L1 and PD-L2, which are expressed on antigen-presenting cells and may be expressed by tumors or other cells in the tumor microenvironment [18]. According to current indications, pembrolizumab is indicated as monotherapy in the treatment of adults and adolescents ≥ 12 years of age with unresectable or metastatic melanoma. In addition, pembrolizumab as monotherapy is indicated in the adjuvant treatment of adults and adolescents ≥ 12 years old with melanoma stage IIB, IIC, or III and who had complete tumor resection [18].

A recent meta-analysis [19] compared the use of pembrolizumab with conventional chemotherapy drugs for the treatment of advanced forms of melanoma, showing promising results of pembrolizumab (pembrolizumab monotherapy group 2 mg/kg with OS 13.4 months, 10 mg/kg group OS 14.7 months, chemotherapy group OS 11.0 months). However, there were insufficient data to confirm statistical significance.

In cutaneous melanoma, in addition to tumor mutahional burden (TMB), MHC-II expression, plodia, heterogeneity, and tumor purity were also associated with response to pembrolizumab. Specifically, MHC-II expression on tumor cells was found to be predictive of response to anti-PD1, hypothesizing that they represented a subset of tumors capable of stimulating CD4 T-cells or CD8 T-cells. In support of this, MHC-II transcriptomics was correlated with the expression of CD4, PRF1, and GZMA (cytolytic molecules) [20].

Considering the numerous studies being published, we decided to conduct a narrative review of the literature reporting the latest efficacy evidence regarding the use of pembrolizumab, alone or in combination, in patients with metastatic melanoma.

2. Materials and Methods

A search was conducted on Pubmed using "pembrolizumab," and "metastatic melanoma" as keywords. Only English language studies were included in the narrative review. Studies from 2022 onward were included as a time limit. The purpose is to summarize the latest evidence produced regarding the efficacy of pembrolizumab in the patient with metastatic melanoma. Studies that did not evaluate the efficacy of pembrolizumab or that used a neoadjuvant/adjuvant setting and case reports were excluded.

3. Results

From our review of the literature on Pubmed, a total of 143 articles were found. After excluding studies that did not meet the search criteria, a total of 20 studies were reviewed.

3.1. Pembrolizumab and associations

In a multicenter, double-blind phase III study, the combination of pembrolizumab and talimogene laherparepvec (T-VEC) versus placebo and pembrolizumab was evaluated in 692 patients with metastatic/unresectable melanoma who were naive for anti PD-1. In both groups pembrolizumab was administered at 200 mg once every 3 weeks. T-VEC and pembrolizumab did not significantly improve progression-free survival (PFS) and OS compared with the placebo/pembrolizumab group. Thus, in this study, it appears that conbination therapy does not bring improvement in the parameters considered [21].

A recent phase I study evaluated the combination of pembrolizumab, vemurafenib, and cobimetinib as first-line in BRAF V600E / K mutated BRAF metastatic patients. There were 2 groups treated with pembrolizumab / vemurafenib and pembrolizumab / vemurafenib / cobimetinib, respectively. The median OS was 23.8 months for vemurafenib / pembrolizumab, while the triple combination did not allow calculation of OS as it was discontinued due to the occurrence of side effects, leading to the closure of the study [22]. BRAF inhibitors (BRAFi) appear to be able to modulate the tumor microenvironment (TME). In fact, administration of a BRAFi in a BRAF-mutant melanoma model resulted in increased expression of CD40 ligand and interferon-gamma on tumor-infiltrating CD4+ lymphocytes (TILs) on the one hand, and decreased T-reg and myeloid cells on the other, suggesting

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antitumor modulation of the TME [22]. Furthermore, an increase in T-cell exhaustion markers TIM-3 and PD1 was correlated with BRAFi in patients with metastatic melanoma [22]. However, the triple therapy proposed in this study did not allowed the progression of the trial due to the significant adverse events, as dermatitis (n=8), hepatitis (n=1), arthralgias (n=1), and QTc prolongation (n=1).

Despite the failure of previous combinations, a phase Ib/II study of 24 patients with metastatic melanoma evaluated the efficacy of pembrolizumab and all-trans retinoic acid (ATRA), achieving an overall response rate of 71%, of which 50% were in complete response, and a 1-year OS was 80%. The aim of this study was to target circulating myeloid-derived suppressor cells (MDSCs), seeking to enhance the action of immunotherapy. The promising results open the way for further studies and represents an interesting new therapeutic option [23]. MDSCs are cells characterized by high immune function. Being immature cells, MDSCs can change following exposure to agents such as ATRA, a derivative of vit. A. Specifically, ATRA appears to reduce both the frequency and functions of MDSCs through activation of ERK1/2, upregulation of glutathione synthase, and increased glutathione. The latter reduces ROS production and consequently results in terminal differentiation of MDSCs. Thus, it is hypothesized that combining Pembrolizumab with ATRA could reduce the frequency of MDSCs and improve the efficacy of pembrolizumab, particularly by reducing polymorphonucleate-MDSCs [23].

Finally, in a phase Ib study of patients with metastatic melanoma, the association between pembrolizumab (200 mg IV every 3 weeks) and escalating doses of IL-2 (6,000 or 60,000 or 600,000 IU/kg IV bolus every 8 hours up to 14 doses per cycle) was evaluated in cohorts of 3 patients. Nine patients were included, obtaining a partial response in 1 (11%), previously treated with pembrolizumab, with high doses of IL-2. Another patient showed a stable condition after 4.5 years of follow-up, even though he had not pembrolizumab treatment prior to the study. No patient showed dose-limiting toxicities. For this reason, treatment with pembrolizumab and IL-2 could be done, especially in those patients who did not respond to pembrolizumab therapy alone. However, evaluation on a larger sample is needed [24]. (Tab.1)

Table 1. Efficacy of pembrolizumab in association: summary of reviewed studies on metastatic/unresectable melanoma.

Study	Type of study	treatments	results
Chesney JA et al. 2023 [20]	double-blind phase III study 692 patients anti PD-1 naive	P/T-VEC vs P/placebo	T-VEC/pembrolizumab not significantly improve PFS and OS compared with placebo/pembrolizumab
Shaikh SS et al. 2022 [21]	phase I study BRAF V600E/K metastatic patients	P/ vemurafenib vs P/ vemurafenib /cobimetinib	median OS was 23.8 months P/vermurafenib P/ vemurafenib /cobimetinib discontinued for sides
Tobin RP et al 2023 [22]	phase Ib/II study 24 patients	P/ATRA	overall response rate 71%, of which 50% complete response 1-year OS was 80%.

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Silk AW et al 2023 [23]	phase Ib study 9 patients	P/ escalating doses of IL-2 (6,000 or 60,000 or 600,000 IU/kg IV bolus every 8 hours up to 14 doses per cycle)	partial response 1 (11%), with high doses IL-2 1 patient stable condition after 4.5 years of FU No dose-limiting toxicities. Evaluation on a larger sample is needed
Silk AW et al. 2023 [25]	phase 1b study 36 patients	Coxsackievirus A21 (V937) intratumorally + P	objective response in 47%, complete response in 22% of cases
Long GV et al. [41]	Phase II clinical trial KEYNOTE-D36	Pembrolizumab/EVX-01 (neoepitope vaccine)	ongoing
	KEYNOTE-942 clinical trial completely resected, high-risk melanoma 157 patients	mRNA-4157/pembrolizumab (107) or pembrolizumab alone (50)	After 18 months, RFS was 78.6% (Combination group) vs 62.2% (pembrolizumab alone). In addition, the combination group showed a reduction in the risk of recurrence or death by 44%

* Abbreviations: talimogene laherparepvec (T-VEC), progression-free survival (PFS), overall survival (OS), Pembrolizumab (P), all-trans retinoic acid (ATRA), recurrence free survival (RFS.

3.2. Pembrolizumab cost-effectiveness

A recent study based on a partitioned-survival model with a 1-week cycle length and a 20-year base-case time horizon evaluated the cost-effectiveness of pembrolizumab versus paclitaxel and carboplatin as first-line in patients with metastatic or unresectable melanoma. Considering that an increase of 2.63 life-years and an increase of 2.24 quality-adjusted life-years was estimated, pembrolizumab emerges as an effective treatment option in metastatic patients as first-line and also cost-effective, not exceeding the total cost of treatment the 3 times the per capita gross domestic Chinese [25]. This confirms that first-line therapy with pembrolizumab is not only an effective but also an economically viable option in countries with a per capita product similar to China.

3.3. Pembrolizumab and virus

Coxsackievirus A21 (V937) is an oncolytic virus that has been shown to be effective against metastatic melanoma. Oncolytic viruses are believed to act against tumours through two distinct mechanisms: direct lysis of tumour cells and activation of a specific anti-tumour immune response. Coxsackievirus is an RNA virus, which can cause mild upper respiratory symptoms, and V937 is a selected, genetically unmodified, oncolytic strain that can enter cells by binding to intracellular adhesion molecule-1 (ICAM-1) and decay-accelerating factor (DAF) receptors. Both of these receptors are specifically expressed on the surface of melanoma cells. From a pharmacodynamic point of view, oncolytic viruses result in increased production of interferon, CD8+ T cells and expression of programmed death ligand 1 (PD-L1) in the tumour microenvironment, as well as reduced populations of suppressor T cells. For this reason, it was assumed that they could improve responses when combined with inhibitors inhibiting PD-1/PD1-L or cytotoxic T lymphocyte antigen 4 (CTLA-4) [26].

In a recent phase 1b study of 36 patients Coxsackievirus A21 (V937) was administered intratumorally in combination with pembrolizumab, achieving an objective response in 47% of cases, and a complete response in 22% of cases. Interestingly, the density of intratumoral CD3+CD8- T cells was lower in responders, suggesting that the combination of Coxsackievirus A21 (V937) and pembrolizumab might help overcome the limitations of a nonimmunologically "active" tumor environment

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[26]. Surprisingly, responses were not correlated with an increase in viral signalling proteins (RIG-I, TLR7 and TLR8), viral entry proteins (ICAM-1 and DAF), PD-L1, nor was an inflamed tumour microenvironment found. In fact, the levels of CD3+CD8- infiltrating T lymphocytes in the pre-treatment tumour samples were actually lower in responders than in non-responders. One hypothesis that might explain this is that the pre-treatment tumour samples of responders include fewer regulatory cells [26]. Another hypothesis that may explain how fewer tumour-infiltrating lymphocytes may favour a better response is that fewer immune cells were immediately available in the tumour to fight Coxsackievirus infection, resulting in more robust viral replication and an increased circulating viral load. A significant increase of IP-10/CXCL10 and a smaller increase of MDC/CCL22 was also observed in the serum of patients. CXCL10 plays an important function in the recruitment of antitumour T-cells in melanoma. Furthermore, CXCL10 via the CXCR3 receptor promotes the migration of lymphocytes to dendritic cells, which is necessary for the response to PD-1 blockade [27]. Clinically, it has been shown that elevated pre-treatment CXCL9 and CXCL10 levels correlate with response to anti-PD-(L)1 therapy in patients with non-small cell lung cancer , and CXCL9 and CXCL10 increase in the first months of treatment in melanoma patients responding to PD-1 inhibitor therapy. Finally, elevated pre-treatment CXCL9 and CXCL10 levels appear to correlate with response to anti-PD1 therapy in patients with non-small cell lung cancer [28], similarly we find an increase in CXCL9 and CXCL10 in the first months of treatment with PD-1 inhibitors in responders with melanoma.

In the light of these data, it is hypothesized that V937 infection increases the production of interferon-gamma and CXCL10 by immune cells in the tumor microenvironment, particularly macrophages, which promote the response to anti-PD-1 by properly guiding activated lymphocytes to dendritic cells [26].

In our opinion, the use of oncolytic viruses could be a promising solution, helping to overcome tumour immune escape mechanisms.

In a pilot study, the efficacy of intratumourally injected ONCOS-102, an oncolytic adenovirus expressing human GM-CSF, was evaluated in combination with pembrolizumab. 21 patients with advanced melanoma with disease progression after treatment with pembrolizumab were enrolled. The treatment was well-tolerated, with few adverse effects (pyrexia, nausea, chills), and no dose-limiting toxicities, being objectively effective in reducing the size of one or more non-injected metastases, highlighting the systemic action of the treatment. Serial analyses were also performed on tumours injected with ONCOS-102 using RNA expression and immunofluorescence, showing the correlation between immune cell level and persistence and patient outcome [29].

3.4. Pembrolizumab and advanced acral melanoma

A retrospective cohort study evaluated the efficacy in 325 patients with unresectable acral melanoma of PD-1 inhibitors, including pembrolizumab, in combination or not with ipilimumab. Included were anti-PD-1 184 (57%), anti-PD-1/ipilimumab 59 (18%), and 82 (25%) ipilimumab alone. The objective response rate (ORR) was significantly higher in the anti PD-1/ipilumumab combination group (43%) than anti PD-1 alone (26%) and ipilimumab alone (15%), respectively. However, anti PD-1/ipilimumab did not lead to a significant difference in OS and PFS compared with anti PD-1 alone [30]. For this reason, further trials considering the acral melanoma subgroup are needed to evaluate the best treatment approach.

3.5. Pembrolizumab, long-term outcomes and real-life data

Regarding long-term outcomes, 5-year OS data for pembrolizumab in metastatic melanoma have been published, with reference to the KEYNOTE-001 studies [31] e KEYNOTE-006 [32]. The results were comparable to those of nivolumab, showing a 5-year OS of 41% and 43% respectively in the two trials [33].

In a study of 103 Chinese patients with metastatic melanoma, 3-year follow-up was performed after failure of first treatment and second treatment with pembrolizumab 2 mg/kg every 3 weeks for ≤35 cycles. The median OS was 13.2 months and the 36-month OS rate was 22.3%. It is interesting that

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median OS data by melanoma subtype are reported, specifically 14.8 months for acral, with a significant difference between acral PD-L1-positive disease (median OS 22.8 months) and acral PD-L1-negative disease (8.4 months). In addition, pembrolizumab therapy as a second-line therapy was shown to have good tolerability [34]. These studies suggest that a good therapeutic response is maintained even in long-term follow-up, partly due to the tolerability of the treatment.

In a retrospective study on 132 patients with metastatic melanoma treated with ICIs, including pembrolizumab, [35] Perez et al. report that 34.8% achieved a complete clinical response, while PFS was 97.5% at 1 year and 94.7% at 3 years after treatment discontinuation. Thus, this real-life study suggests that discontinuation of ICIs in those metastatic patients with complete remission of disease is feasible, keeping the clinical condition stable at 3 years. However, it is also necessary to evaluate these aspects in studies with a larger sample size.

In another national retrospective study of 5097 New Zealand patients with metastatic melanoma treated with ICIs, including pembrolizumab, a 1-year OS of 72% was reported, while the 2-year OS was 60%, confirming data from previous clinical trials [36].

Finally, a recent study of 1037 patients with metastatic melanoma compared the efficacy of nivolumab and pembrolizumab. Both have approval for the treatment of metastatic melanoma, but the choice between the two is often left to the clinician. The results of this study showed that the median OS was 17.4 months for pembrolizumab and 20 months for nivolumab, while the estimated OS at 2 and 3 years was 42/34% for pembrolizumab and 47/37% with nivolumab. The differences in OS, PFS, and overall response were not statistically significant in naive patients with metastatic melanoma, confirming the clinician's role in treatment choice [37].

3.6. Pembrolizumab and biomarkers

Possible new markers of therapeutic response to pembrolizumab recently emerged in a study of 46 metastatic melanoma patients. CD103 expressed on CD8+ T lymphocytes is associated with a complete response to pembrolizumab with statistical significance when the density (p=0.04) and proportions (p=0.012) of this T-cell population increased. Improved survival correlated with the increased proportion of CD8+ CD103+ T-cells (P = 0.0085) and also with reduced expression of periplakin (P = 0.012) and periplakin + SOX10 (P = 0.0012) [38]. This preliminary study highlights that the role of certain cellular markers could help select those metastatic patients most promising to respond to pembrolizumab monotherapy, however further studies on a larger sample are needed.

In a study of 68 metastatic melanoma patients, tryptophan and glucose metabolism was evaluated using C11-labeled α-methyl tryptophan (C11-AMT) and fluorodeoxyglucose (FDG) PET imaging of tumor lesions. In addition, the expression of tryptophan-metabolizing enzymes (TMEs; TPH1, TPH2, TDO2, IDO1) and the tryptophan transporter, LAT1, were evaluated at immunohistochemistry. In this study, it was found that elevated tryptophan metabolism in metastatic melanoma is a predictor of poor response to pembrolizumab [39].

Interestingly, some soluble biomarkers have also been proposed. In a recent study of 41 patients with metastatic melanoma, plasma levels of soluble PD-1 (sPD-1), soluble programmed cell death ligand 1 (sPD-L1), butyrophilins (BTNs) 2A1, 3A1, and body mass index (BMI) were evaluated as predictors of efficacy of anti PD-1 therapy. Low levels of sPD-1 plus high levels of sBTN2A1 were associated with a better overall response rate. In addition, patients with BMI $\geqslant$ 25 and sPD-1 < 11.24 ng/ml had longer time to treatment failure ($p < 0.001$) [40].

Regarding imaging tecnique, another study used positron emission tomography (PET) with zirconium-89 (89Zr)-labeled pembrolizumab prior to pembrolizumab treatment to evaluate the predictive ability of response to anti PD-1. In 11 patients with metastatic melanoma 89Zr-pembrolizumab tumor uptake was calculated, obtaining a statistically significant direct correlation with clinical response, OS, and PFS. Although this method is interesting in the landscape of pembrolizumab efficacy markers, the small sample size requires further studies to confirm its clinical utility [41]. (Tab.2)

Texture analysis features at contrast medium CT of melanoma metastases also appear to be a predictor of response to treatment with anti PD-1. In a study of 127 metastases, 3 items were identified

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as independent predictors of response to therapy: skewness at fine texture scale, skewness at medium texture scale, and variation of entropy at fine texture scale. Although further studies are needed to confirm their validity, CT methods may also be an aid in assessing response to anti PD-1 immunotherapy [42].

Table 2. Pembrolizumab and biomarkers: summary of reviewed studies on metastatic/unresectable melanoma.

Study	Type of study	Biomarkers	results
Edmonds NL et al. 2022 [34]	preliminary study 46 patients	CD103 Periplakin periplakin + SOX10	increased density/proportions CD103 CD8+ T associated with complete response to P Reduced expression of periplakin and periplakin + SOX10 associated with improved survival during P
Oldan JD et al. 2023 [35]	Prospective clinical trial 68 patients	C11-AMT FDG PET tryptophan-metabolizing enzymes	elevated tryptophan metabolism predictor of poor response to P.
Incorvaia L. et al 2023 [36]	Prospective cohort study 41 patients	sPD-1 sPD-L1 BTNs 2A1, 3A1	Low levels of sPD-1 + high levels of sBTN2A1 associated with a better overall response rate. patients with BMI ⩾ 25 and sPD-1 < 11.24 ng/ml had longer time to treatment failure.
Kok IC et al 2022 [37]	Prospective study 11 patients	(89Zr)-labeled P PET	89Zr-P tumor uptake significant direct correlate with clinical response, OS, and PFS
Bonnin A et al. 2022 [38]	Retrospective study 127 metastases	Texture analysis features at contrast medium CT of melanoma metastases	3 items predictors of favorable response to P 1. skewness at FTS, 2. skewness at MTS, 3. variation of entropy at FTS

* Abbreviations: Pembrolizumab (P), C11-labeled α-methyl tryptophan (C11-AMT), fluorodeoxyglucose (FDG), tryptophan-metabolizing enzymes (TMEs; TPH1, TPH2, TDO2, IDO1), soluble PD-1 (sPD-1), soluble programmed cell death ligand 1 (sPD-L1), butyrophilins (BTNs), zirconium-89 (89Zr), progression-free survival (PFS), overall survival (OS), fine texture scale (FTS), Medium texture scale.

3.7. Pembrolizumab and obesity

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Although obesity is a major risk factor for cancer mortality [43], an "obesity paradox" emerges as obesity correlates with better clinical outcomes in patients with metastatic melanoma, especially when treated with anti PD-1. In a retrospective study of 266 patients with metastatic melanoma treated with pembrolizumab or nivolumab, it emerges that obesity had a protective role, specifically a 10 cm2/m2 increase in visceral fat index (VFI) was associated with longer overall survival after adjusting for covariates, but this was not maintained when adjusted for systemic immune-inflammation index. Thus, the prognostic role of visceral obesity is conditional on inflammatory status. Obesity appears to determine an increase in PD-1 expression, induces T-cells to release more PD-1 protein, and increases the secretion of adiponectin and leptin from adipose tissue. These alterations result in increased T-cell depletion and dysfunction, which promote tumour progression. On the other hand, this provides a better understanding of the relationship between obesity and ICIs, as these agents remove the inhibitory signals of T-cell activation and promote an effective anti-tumour response. [44]

3.8. Pembrolizumab and vaccines

The phase II KEYNOTE-D36 clinical trial will evaluate the efficacy and safety of the combination of pembrolizumab and EVX-01, a personalized neoepitope vaccine, in patients with metastatic or unresectable melanoma. EVX-01 consists of multiple peptides that represent a neoepitope expressed only in the patient's specific tumor, allowing a tumor-specific immune response [45].

Regarding the use of vaccines in combination with pembrolizumab, data were recently presented from a KEYNOTE-942 clinical trial in patients with completely resected, high-risk melanoma. Patients were treated with mRNA-4157/pembrolizumab (107) or pembrolizumab alone (50). 9 doses of mRNA-4157 (1mg) were administered every 3 weeks, while pembrolizumab 200mg was administered intravenously for 18 cycles. After 18 months, recurrence free survival (RFS) was 78.6% in the combination group versus 62.2% in the group with pembrolizumab alone. In addition, the combination group showed a reduction in the risk of recurrence or death by 44% (HR = 0.561; 95% CI: (0.309, 1.017). These results are very promising, highlighting the therapeutic possibilities of the combination of adjuvant standard of care and a vaccine tailored to specific tumor neoantigens, however, a phase III study with larger sample size is needed to confirm the results [46,47]. In particular, this mRNA vaccine encodes up to 34 tumour neo-antigens and is produced based on the specific mutation sequence of the patient's tumour. Once administered into the body, the neo-antigen sequences encoded by RNA are translated endogenously and subjected to natural antigen processing and presentation by antigen-presentig cells, a crucial step in adaptive immunity.

4. Discussion

Current therapeutic options in advanced melanoma include PD-1 inhibitors associated or not with CTLA-4 or lymphocyte-activating gene (LAG)-3 inhibitors, and in cases of BRAF V600E/K mutated BRAF inhibitors, called targeted therapy [11,48]. However, the DREAMseq study showed that immunotherapy followed by target therapy in case of progression increased OS of 20% compared with reversing the two treatments [49]. This is because BRAF inhibitors are supposed to modulate the tumor immune microenvironment by increasing interferon gamma production on intratumoral CD4+ T lymphocytes and to increase CD8+ tumor-infiltrating lymphocytes (TILs) [50,51].

Currently, guidelines suggest in patients with metastatic melanoma, regardless of BRAF status, the use of an ICIs with anti PD-1, in association or not with anti-CTLA4, allowing long-lasting responses and long-time survival in responders [52].

Specifically, clinical trials of ICIs in patients with metastatic melanoma report a 5-year OS of up to 60% and a 5-year ongoing response rate of up to 62% [49,53]. However, about 40-60% of treated patients do not respond to ICIs, and about 30% of treated patients relapse in the following 2 years [7,11,54-56].

Therefore, the use of vaccines in combination with pembrolizumab could be a solution for all those patients who do not respond to immunotherapy due to immune escape. Clinical trial data show that 50 to 64% of melanoma patients, even if on immunotherapy, will experience disease progression

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after 1 to 5 years [48]. The mechanisms of resistance may be related to several aspects. Reduced expression of molecular targets, particularly PD-L1, on neoplastic cells results in primary tumor resistance to pembrolizumab [57, 58]

Other causes of resistance to immunotherapy are low neoantigen load on tumor cells, allowing them to overcome immune surveillance [59], and immunosuppression brought about by the tumor microenvironment [60-63].

Another aspect to be considered is the use of markers that could help predict response to pembrolizumab, thus allowing selection of patients who might benefit from association with other treatments, such as personalized incoming vaccinations. In our opinion, the use of texture analysis features to contrast medium CT of melanoma metastases could be a method that could be easily integrated into the staging of the metastatic patient, however, further studies are needed to confirm its usefulness [39].

Furthermore, evaluation of the tumor microenvironment, in addition to being useful in assessing the prognosis of melanoma [64-68], also appears to be able to give insight into the efficacy of pembrolizumab therapy [35], although the data need further confirmation on larger samples.

It is important to notiche that therapy with pembrolizumab can be combined with BRAF inhibitors. The BRAF V600 mutation is found in approximately 35-50% of metastatic melanomas, and is associated with increased disease aggressiveness and reduced survival [69,70]. A recent multicenter, double-blind phase III trial (STARBOARD) of 624 patients with BRAF V600E/K metastatic melanoma compared the efficacy of triple therapy (encorafenib, binimetinib and pembrolizumab) versus placebo plus PD-1 monotherapy (pembrolizumab). However, data from this study are not currently available.

5. Conclusions

In conclusion, pembrolizumab represents a fundamental option in the therapy of metastatic melanoma, as confirmed by data in real-life and in long-term follow-up. However, a certain proportion of patients do not respond to this therapy and therefore new combination options need to be evaluated. Among the new treatments, the use of vaccinations tailored to tumor epitopes could represent a breakthrough in the treatment of resistant forms, but further studies with larger sample numbers are needed to confirm preliminary results.

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