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## **The safety of anti PD-1 therapeutics for the treatment of melanoma**

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## **Abstract**

**Introduction** The introduction of immunotherapies into clinical practice has substantially improved the prognosis of metastatic melanoma patients as well as patients suffering from other cancers. The two FDA-approved checkpoint inhibitors against PD-1 (nivolumab and pembrolizumab) have been shown to significantly improve patient survival while being less toxic than previous treatment options.

**Areas covered** The current scientific literature on safety and adverse events (AEs) related to anti-PD-1 therapies has been investigated with special attention to case reports and to the latest results announced at the major clinical cancer and melanoma meetings, including ASCO (American Society of Clinical Oncology), ESMO (European Society of medical Oncology) and EADO (European Association of Dermato-Oncology) annual meetings.

**Expert opinion** Even though anti-PD-1 therapies are better tolerated than conventional chemo- or other immune-therapies, they still induce a plethora of AEs. Given the mechanism of action, it is supposed that most if not all of them are immune related. Fortunately, the majority are mild and manageable. However, due to the increase in patients' life expectancy, there is a substantial need to understand and prevent severe cutaneous, pulmonary, neurological and other AEs which have major impact on the quality of life. The safety profile after long term use of these medications is still unclear. In addition, non-steroid based immune interventions to control autoimmunity are still to be developed.

**Keywords:** adverse events; anti-PD-1 antibody; immunotherapy, melanoma

## 1. Introduction

### 1.1 Immunotherapy for melanoma

The incidence rate of melanoma has been on the rise for the past decades and although it is not the most common skin cancer, it accounts for the major part of skin cancer related deaths.<sup>1</sup> Due to paucity of effective therapies, less than a decade ago, 3-year survival rates in patients with stage IV melanoma were 6-12 %.<sup>2</sup>

In the 90's the first therapies aimed at modulating the immune system in order to enhance endogenous anti-tumoral responses got approved by the Food and Drug Administration (FDA), the US regulatory body in charge of protecting and promoting public health and regulating commercialization of new drugs.

Interferon (IFN)- $\alpha$ , a cytokine, belonging to the type I IFN family, stimulates antitumor immunity by enhancing activation and maturation of dendritic cells (DCs) as well as promoting Th1 immune response.<sup>3</sup> The overall response rate in stage II-III high risk melanoma patients was 22%, however it was most efficient in those with smaller tumour burden.<sup>4 5</sup> Later, interleukin (IL)-2, known to promote proliferation and antitumor activities of T lymphocytes when administered in high doses, was introduced as cancer therapy and FDA approved for the treatment of metastatic melanoma in 1998.<sup>6</sup> It showed moderate efficacy with response rate of 16% and complete remission (CR) in 6% of melanoma patients. Furthermore, it could only be prescribed to patients in an overall healthy state due to the severe side effects that need to be endured.<sup>7</sup> It is only in early 2000's that a major breakthrough in immunotherapies was achieved thank to the development of drugs with more targeted mechanism of action and therefore, less side effects and better tolerated.

The first monoclonal Ab targeting the checkpoint receptor cytotoxic T lymphocyte-associated antigen (CTLA)-4, named ipilimumab (Yervoy, BMS-734016) got approved by the FDA and the European Medicines Agency (EMA) in 2011 for the treatment of patients with advanced melanoma.<sup>8,9</sup> Administered as monotherapy at a dose of 3 mg/kg every 3 weeks it resulted in 1-year and 2-year survival rates of 39.3% and 24.2%, respectively.<sup>10</sup>

Those unprecedented results represented the beginning of a new era in the field of oncology. The modulation of patient's immune system using checkpoint inhibitor is able to induce a much more durable response compared to conventional therapies (chemo-, targeted- therapies) which were able to suppress growth but not often eradicate the tumors.

Such evidences paved the way for the development of new therapeutic Abs aimed at blocking different checkpoint receptors. Building on previous studies done in the context of chronic viral infection, programmed death (PD)-1 pathway rapidly became an extremely interesting candidate to target for cancer therapy (see Mechanism of action). In September 2014, the first Ab against PD-1 was approved for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if *BRAF V600* mutation positive, a BRAF inhibitor.<sup>11</sup>

## 1.2 Pembrolizumab

Pembrolizumab (lambrolizumab, MK-3475, Keytruda) is a humanized monoclonal IgG4-κ ab with an approximate molecular weight of 149 kDa.<sup>12</sup> The recommended dosage is 2 mg/kg administered as an intravenous infusion over 30 minutes every 3 weeks until disease progression or unacceptable toxicity.<sup>12</sup> Pembrolizumab was compared (10 mg/kg every 2 or 3 weeks) to ipilimumab (3 mg/kg every 3 weeks) in a randomized clinical trial and showed an objective response rate (ORR) of 33% (independent from the dose) in comparison to 11.9% in the ipilimumab group.

Furthermore, the 6-month progression free survival (PFS) was better in the pembrolizumab group with rates of 47% versus 26.5% for the ipilimumab group and an estimated 12-month overall survival (OS) rate around 70% in pembrolizumab treated group versus 58% in ipilimumab receiving patients.<sup>13</sup> The latest report from the longest ongoing study aimed at characterizing pembrolizumab ORR and OS, showed that it is associated with a 33% ORR, a 12-month PFS of 35% and a median OS of 23 months.<sup>14</sup>

### 1.3 Nivolumab

Almost concurrently (December 2014), a similar checkpoint inhibitor against PD-1, produced by a different company, was approved by the FDA for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if *BRAF V600* mutation positive, a BRAF inhibitor.<sup>15</sup>

Nivolumab (Opdivo, BMS-936558, MDX-1106) is a fully human IgG4-κ Ab. It was the first anti-PD1 checkpoint inhibitor to show significant clinical activity in several cancer types including melanoma.<sup>16,17</sup> The recommended dosage as a single agent is 3 mg/kg administered as an intravenous infusion over 60 minutes every 2 weeks until disease progression or unacceptable toxicity.<sup>18</sup>

Nivolumab was first compared to dacarbazine in a double blinded randomized clinical trial with *BRAF-wild type* (wt) melanoma patients. It showed a 1-year OS of 72.9% in nivolumab receiving patients, compared to 42.1% in the dacarbazine group<sup>19</sup>. The recently updated data for the longest study on nivolumab demonstrated that the median OS at 5 years is 34% and ORR was seen in 32% of the patients<sup>20</sup>.

Nivolumab was also compared to ipilimumab and to the ipilimumab/nivolumab combination in a double blinded prospective randomized trial and showed better PFS, as a monotherapy and in combination with ipilimumab, compared to ipilimumab monotherapy (ipilimumab: 2.9, nivolumab: 6.9, combination: 11.5 months).<sup>21</sup>

#### 1.4 Anti-PD-1 in melanoma therapy

According to the ESMO Clinical Practice Guidelines for diagnosis, anti-PD-1 therapy is recommended, according to the ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up of cutaneous melanoma, as the first-line therapy for patients with metastatic (stage IV) melanoma independent of the BRAF status, as well as a second-line treatment after failed response to ipilimumab or BRAF/MEK inhibitor therapies.<sup>22</sup> As a result, anti-PD-1 therapies are now being administered to a greater number of patients whose life expectancy is substantially increasing. Since, before the introduction of immune checkpoint inhibitors, the attention was mainly focused on managing the disease and hoping for a few more weeks or months survival, the quality of life of patients surviving for a longer period of time has not been addressed extensively. This is why we urgently need to rigorously and systematically document treatment related-AEs and find better ways to manage them.

Although clinical efficacy of checkpoint inhibitors is impressive, about 50% of the patients do not respond to anti-PD-1 therapy, without clear reason and of those who showed an objective response to the treatment, 25% experience a relapse at a median of 21 months after the start of the therapy.<sup>14</sup> As observed with targeted therapies before (such as BRAFi), the high plasticity of cancer cells enables them to evade the immune system<sup>23</sup>. For that reason, research is now focusing on combination treatment to prevent immune evasion which is known to happen faster under monotherapy. It was also shown that treatments with combination of drugs (such as ipilimumab and nivolumab) lead to more frequent and more severe AEs, strengthening the need to have a clear understanding of the AEs occurring with monotherapies in the first place.<sup>21</sup>

Nevertheless, other drugs targeting PD-1/PD-L1 axis are being designed and tested

on various types of cancers and despite some variation noticed in the different trials, all the drugs showed anti-tumor activity. This highlights the central role of PD-1 checkpoint in cancer and the importance of understanding its precise mechanisms of action in order to better comprehend the therapy-associated AEs.<sup>24</sup>

#### 1. 5 Anti-PD-1 therapy: mechanism of action

PD-1 is a transmembrane glycoprotein receptor which can be found on T-cells, B-cells, natural killer (NK) cells, DCs, and myeloid cells (See Figure 1).<sup>25</sup> It is a member of B7/CD28 family and binds to its ligands PD-L1 (B7-H1, CD274) and PD-L2 (B7-DC, CD273). PD-L1 expression may be induced or is constitutively expressed on various types of cells (hematopoietic and non-hematopoietic as well as some cancer cells)<sup>26</sup> while PD-L2 has a more restricted pattern of expression and can mainly be found on antigen presenting cells (APCs) upon inflammation, it has also been shown to be expressed by T-cells.<sup>27</sup> The T-cell response is tightly regulated by a range of co-stimulatory and co-inhibitory signals.

PD-1 plays an important role in maintaining peripheral tolerance. Its expression on lymphocytes is modulated by inflammatory cytokines: it is rapidly up-regulated upon cognate antigen recognition via the T cell receptor and then decrease after antigen clearance.<sup>28</sup> When PD-1 ligates with one of its ligand, it induces an co-inhibitory signal in the T-cell, preventing its proliferation, cytokine expression, cytolytic function and survival,<sup>25</sup> establishing a feedback loop that dampens the immune responses and limits bystander tissue damage unless the activation is overridden by strong co-stimulatory signals. For instance, PD-1 is essential for the regulatory T-cells (Tregs) to achieve their suppressive function and avoid reaction against self-antigens or against the foetuses in pregnant women.<sup>29</sup> In healthy individuals, the levels of PD-1<sup>+</sup>

T-cells varies greatly, depending on the ongoing response of their immune system and antigen load.<sup>30</sup>

Due to the fine tuning necessary for PD-1 to maintain an efficient immune response, it is understandable that when its expression is dysregulated the homeostasis cannot be maintained and it can lead to various diseases.<sup>31</sup> The study of viral infections provided the first great deal of insight into understanding PD-1 mechanisms. In chronic viral infection (unlike acute infection), T-cells functions are impaired and exhibit an exhausted phenotype; those anergic T-cell overexpress PD-1 and it was shown that blocking this pathway can improve their effector functions.<sup>32,33,34</sup>

Subsequently, evidences suggested that T-cell with similar characteristics can be found in the tumor environment as well.<sup>35</sup> Phenotypic analysis revealed that, in melanoma, antigen-specific tumor infiltrating lymphocytes (TILs) have a higher expression of PD-1 (and other co-inhibitory receptors such as CTLA-4) compared to circulating T-cells. Such cells had impaired effector functions and were unable to proliferate or differentiate into memory T-cell.<sup>36</sup> Thus, it became self-evident to test blocking the PD-1/PD-L1 pathway to restore T-cell function, as it was previously done in chronic viral infections.<sup>37</sup> Indeed, in 2007, Wong et al. showed that in melanoma, when the PD-1 pathway was disrupted, it enhanced proliferation and functions of antigen-specific T-cells.<sup>38</sup> Even though the mechanisms leading to T-cell exhaustion are not fully understood to date, prolonged and/or high PD-1 expression seems to be one of the main drivers.<sup>39</sup> Many studies have helped strengthened our understanding of PD-1's role in the alteration of the magnitude of response following T-cell activation.<sup>40, 41</sup> However, PD-1 mediated loss of function in T-cell occurring in tumors is the result of a complex interplay between PD-1 receptor, its two ligands PD-L1 and PD-L2, the different cell types expressing those proteins and the environment. PD-1 high expression is not sufficient to lead to T-cell exhaustion. As shown by

Duraiswamy et al., CD8<sup>+</sup>PD-1<sup>hi</sup> subset from a healthy person have a different phenotype and gene expression pattern than CD8<sup>+</sup>PD-1<sup>hi</sup> subset in the context of T-cell exhaustion. Indeed, most PD-1<sup>hi</sup> CD8 T-cells in healthy adults are effector memory T-cells rather than exhausted T-cells and their effector functions are not altered.<sup>30</sup>

PD-1/PD-L1 axis is more complex than envisaged; albeit having been discovered over 10 years ago, many uncertainties remain regarding its exact biological mechanisms.<sup>24</sup>

PD-1/PD-L1 pathway's most reported function in cancer mimics its protective physiological role. TILs expressing a high level of PD-1 because of chronic antigen exposure will ligate PD-L1 on tumor cells which will then deliver an inhibitory signal to the T-cell allowing the tumor cell to escape the immune response.<sup>42</sup> PD-1 signaling eventually leads to T-cell inhibition, however various mechanisms have been reported. The main pathway seems to be the inhibition of the Akt pathway via PI3K antagonization.<sup>43</sup> Nonetheless, it can also induce the expression of genes encoding molecules that inhibit T-cells such as basic leucine zipper transcription factor (BTAF).<sup>44</sup> More recently PD-1 signaling in activated T-cells has also been shown to induce metabolic changes which are able to abrogate effector functions.<sup>45</sup>

Besides playing a role in the tumor microenvironment, PD-1/PD-L1 axis is also involved in the shift of balance towards tolerance induction instead of effector differentiation during T-cell activation in the periphery. Indeed, when PD-1 on a naïve CD4<sup>+</sup> T-cell ligates with PD-L1 from an APC, it induces the development of a T<sub>reg</sub> by antagonizing the Akt–mTOR signaling cascade.<sup>46</sup> The T<sub>reg</sub> will in turn be able to dampen T-effector cells.<sup>47</sup> As well as expressing PD-1 receptor, T-cells can also harbor PD-L1. The latter was shown to interact with CD80 (receptor involved in CTLA-4 pathway) on an APC and similarly inhibit T-cell activation.<sup>48</sup> Moreover, PD-1

can also serve as checkpoint for other immune cell types such as NK cells, DCs or macrophages.<sup>49-51</sup> It is well known that tumor cells downregulate their major histocompatibility complex (MHC)-I expression to avoid recognition by T-cells. NK cells are usually responsible for the detection and killing of cells missing MHC-I on their surface, but if their activity is reduced, tumor cells could stay undetected and possibly even increase their invasive potential as shown by Garrido et al.<sup>52</sup> More studies are needed to confirm NK cell inhibition via -PD-1 in melanoma, but if it is verified it would be a very powerful weapon for tumor cells.

PD-L2 is less well characterized than PD-L1 and its molecular pathways are not well understood yet. However, it was shown to play a role in the modulation of T-cell functions.<sup>53</sup> In addition, a new binding partner for PD-L2 was recently discovered; repulsive guidance molecule b (RGMb) engagement with PD-L2 promotes respiratory tolerance.<sup>54</sup> Xiao et al. suggest that PD-L2 promotes the development of respiratory tolerance by facilitating the initial T-cell expansion in draining lymph nodes.

And finally, PD-1 signaling can also play a role on B-cell modulation. As for T-cell inhibition, PD-1 expression on B-cells is able to inhibit their activation when engaging with its ligand. Comparable to what was demonstrated in T-cell, blocking PD-1 could enhance B-cell responses.<sup>55</sup>

In short, PD-1 immune checkpoint pathway plays an important role in inhibiting the anti-tumor response and cancer cells are able to take advantage of such mechanisms to avoid detection by the immune system. Two different mechanisms have been described: Intrinsic and adaptive immune resistance. Some tumor cells will constitutively have defense mechanisms against the immune system and for instance express PD-1 independently of external stimuli.<sup>56</sup> Others will react to the threat of being recognized, sense inflammatory cytokines (produced by NK or

activated CD8<sup>+</sup> and CD4<sup>+</sup> cells) and upregulate inhibitory factors such as PD-L1, IDO or support Treg activity.<sup>57, 58</sup>

The extend of the role PD-1 plays in every cell type interactions is far from being understood yet. However, it appears that the PD-1/PD-L1 pathway is, by various means, frequently hijacked by tumor cells, which probably accounts for the great results obtained with anti-PD-1 therapy.

The mechanism of action of anti-PD-1-Ab (as well as the other checkpoint inhibitors) are different from the ones seen with classic chemotherapy agents, such as dacarbazine (DTIC), or targeted therapies, such as BRAFi or MEKi. Hence, anti-PD-1 Abs are linked to a set of different, in many cases immune-related adverse events (irAEs). Fortunately, they are often mild in severity and easily manageable with little or no intervention, however, more serious and potentially fatal events have been reported.<sup>59</sup>

## 1.6 Future for immune-directed therapies

Melanoma is known to be the most mutated cancer and, due to tumor infiltrating lymphocytes, it is considered one of the most immunogenic tumors.<sup>60</sup> These qualities makes melanoma a good candidate for immune-directed therapies, other than mentioned above. Combination of approved therapies, adoptive T cell therapy, tumor vaccines with dendritic cells, attenuated herpes simplex virus-1 (Talimogene laherparepvec (T-VEC)) and other agents, are being investigated in clinical trials as well as in clinical setting.<sup>61-63</sup>

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## 2. Adverse events of anti-PD-1 therapies

### 2.1 General overview of AE

The first clinical trials of anti-PD-1 therapies having started over 5 years ago, we now have the advantage of hindsight and robust data regarding the different AEs associated with these therapies.

### Pembrolizumab

In the first expansion cohort of a phase I trial (KEYNOTE 001), efficacy and safety of pembrolizumab in patients with ipilimumab refractory advanced melanoma were investigated.<sup>64</sup> Patients were randomized to receive pembrolizumab at doses of 2 mg/kg or 10 mg/kg every 3 weeks. The overall AE rate was similar between the two groups, with 82% of the patients experiencing at least one. However, grade 3-4 AEs occurred in only 12% of all the patients (15% in 2 mg/kg and 8% in 10 mg/kg group), with the only severe AE reported in more than one patient being fatigue (3%).<sup>64</sup> A similar safety profile was demonstrated in the phase II trial (KEYNOTE 002) with AEs occurring in 68% of the 2 mg/kg group compared with 74% in the 10 mg/kg group. Grade 3-4 AEs were reported in 11% and in 14% of 2 mg/g and 10 mg/g groups respectively.<sup>65</sup> In Phase III trial (KEYNOTE 006) pembrolizumab at a dose of 10 mg/kg every 2 weeks or every 3 weeks was compared to ipilimumab at a dose of 3 mg/kg every 3 weeks. Although AEs of any grade occurred at a similar rate in all groups (79.5% vs 72.9% vs 73.0% respectively), grade 3-5 events were less common in patients, receiving pembrolizumab (13.3% in 10 mg/kg every 2 weeks and 10.1% 10 mg/kg every 3 weeks) when compared to ipilimumab (19.9%).<sup>13</sup> A systematic analysis of patients treated with pembrolizumab within 3 previously mentioned trials (n=1567) showed that AEs of any grade occurred at a rate of 77.9% and only 12.9% of the cumulated patient populations experienced grade 3-5 AEs.<sup>66</sup> Overall, pembrolizumab showed a better safety profile compared to the previously approved checkpoint inhibitor, ipilimumab. As a comparison, 84% of the patients

treated with ipilimumab experienced AEs of any grade and out of those, 28% were reported to have experienced grade 3-4 events.<sup>67</sup>

### Nivolumab

Nivolumab and pembrolizumab are very similar drugs, however the trials, assessing their efficacy and safety have different ways of reporting the treatment-related AEs. In pembrolizumab trials (KEYNOTEs), AEs occurring at a rate of 1% or more were reported, while in nivolumab trials, a threshold of 3% was set.<sup>17, 68</sup>

In the phase I trial, assessing safety, antitumor activity and pharmacokinetics of nivolumab in patients with advanced melanoma, non-small-cell lung, prostate, renal cell or colorectal cancer at a doses of 0.1 to 10 mg/kg every 2 weeks, at least one AE was reported in 70% of patients, with 14% of them being of grade 3-4. In contrast to pembrolizumab trials, patients did not experience severe fatigue, however grade 3-4 events such as pneumonitis, increased lipase, and diarrhea were documented.<sup>17</sup>

In a randomized phase III clinical trial, previously untreated patients with advanced melanoma were assigned to receive nivolumab at a dose of 3 mg/kg every two weeks (plus dacarbazine-matched placebo) or dacarbazine at a dose of 1000 mg/m<sup>2</sup> (plus nivolumab matched placebo). Although reported rates of any treatment-related AEs were similar in both groups (74.3% vs 75.6% respectively), as expected, grade 3-4 events were less common in nivolumab (11.7%) than in dacarbazine (17.6%) receiving patients.<sup>19</sup>

In another randomized, double-blind, phase III clinical trial, where nivolumab was compared to a combination of nivolumab and ipilimumab and to ipilimumab as a monotherapy, treatment-related AE rates were shown to be the lowest in the nivolumab group (82.1% vs 95.5% vs 86.2% respectively). Furthermore, grade 3-4 treatment-related AEs, leading to discontinuation of therapy, were also less common in patients, receiving nivolumab monotherapy (5.1% vs 29.4% vs 13.2%

respectively).<sup>21</sup> Similar safety profile was reported in further trials with AEs occurring in 68-74.3% of patients, and only 9-11.7% being of grade 3-4.<sup>19, 68</sup> Unlike in ipilimumab trials in which 7 irAEs related deaths were reported,<sup>69</sup> in anti-PD-1 treated patients, no deaths induced by treatment-related AEs were reported.<sup>13, 64, 65, 68</sup>

AEs caused by anti-PD-1 therapy were documented in patients from 15 skin cancer centers in Germany and Switzerland. Out of 496 patients, receiving either pembrolizumab or nivolumab, 138 have been reported to experience AEs, most commonly skin AEs (n=43 patients), but also endocrine system, (n=30), respiratory tract (n=24), musculoskeletal system (n=21), gastrointestinal tract (n=21), nervous system (n=16), liver (n=11), pancreas (n=9), eyes (n=8), heart (n=5), blood (n=3) and renal system (n=2).<sup>59, 70</sup>

Overall, the safety profile of anti-PD-1 therapies is better than any other previous melanoma treatment available. A review of treatment-related AEs, reported in the biggest clinical trials, analyzing efficacy and safety of anti-PD-1 therapy is provided in Table 1.

Below, reviewed AEs are grouped by organ systems and presented according to reported frequency. Some of the AEs are demonstrated in Figures 2-4

## 2.2 Constitutional AEs

Constitutional symptoms are the most commonly reported AEs, which occur under therapy with anti-PD-1-Abs. They are also most common among grade 3-4 events.<sup>13, 64, 65</sup> Fatigue is reported in up to 37% of the patients, nausea (16.5%), asthenia (10%), decreased appetite (4-5.1%), headache (10%), other events were less common<sup>64, 65, 71, 72 19, 71, 73</sup>

## 2.3 Cutaneous AEs

Reported in up to 36% of the patients, skin reactions are the most common anti-PD-1 treatment-related AEs<sup>74</sup>. Cutaneous events, such as pruritus and rash, were reported

in 17-23% and 15-18% of the patients.<sup>19, 65</sup> Vitiligo was reported to develop in 9-25%,<sup>13, 19, 75</sup> and alopecia in 1.2%<sup>70</sup> of patients treated with anti-PD-1 therapy. Cases of less common skin diseases, such as bullous lichen planus-like reactions<sup>76</sup> or bullous pemphigoid<sup>77</sup> have also been described in the context of anti-PD1 therapy. In the pembrolizumab-treated patient population, severe skin reactions were reported at a rate of 1.2%, and it led to therapy discontinuation in only 1 patient. The other reported cutaneous reactions included: bullous dermatitis (grade 1; n=1), exfoliative dermatitis (grade 2; n=1), erythema multiforme (grade 3; n=1), exfoliative rash (grade 1; n=2), pruritus (grade 3; n=4), rash (including generalized rash and maculo-papular; grade 3; n=6), and Stevens-Johnson syndrome (grade 3; n=1).<sup>66</sup> 5.3% of patients had to discontinue the treatment with pembrolizumab because of severe cutaneous AEs.

A possible link between development of cutaneous reactions and better treatment response has been postulated. In the systematic review of studies on melanoma immunotherapy which documented autoimmune toxicity and/or vitiligo, association between vitiligo development and better PFS as well as OS was found. This indicates that patients who developed vitiligo have less risk of disease progression and death, than patients who did not develop vitiligo.<sup>78</sup> Similar observations were made by Sanlorenzo et al., who reviewed data from 83 cancer patients, who received at least one dose of pembrolizumab. 42% of them developed skin AEs, (most commonly macular papular eruption (29%), pruritus (12%) and hypopigmentation (8%)) and showed significantly longer PFS compared to patients who did not experience cutaneous AEs.<sup>79</sup> In another study, the 25% of the patients who developed vitiligo have also been described to better respond to anti-PD-1 therapy, when compared to patients who did not develop it.<sup>75</sup>

Goldinger et al. reported, that the maculopapular rash, seen in anti-PD-1 receiving patients, demonstrates clinical, histologic, immunohistologic features as well as gene expression profile, resembling Stevens-Johnson syndrome/Toxic epidermal necrolysis and linked it to PD-1 role at regulating cytotoxic T-cell responses in the skin.<sup>80</sup>

## 2.4 Gastrointestinal tract

Gastrointestinal AEs are well known to occur under therapy with checkpoint inhibitors. These AEs require special attention given the impact they can have on quality of life as well as potential electrolyte disturbances, weight loss and risk of intestinal perforation in severe cases. Almost one third of the patients receiving ipilimumab experienced diarrhea, with grade 3-4 events being reported in 5.3% of them.<sup>69</sup> On the other hand, anti-PD-1 therapies are known to induce fewer and milder gastrointestinal events with diarrhea being reported in only 6%-18%.<sup>13, 17, 19</sup> With an occurrence rate of 2.5%, it is the only treatment related grade 3-4 event, reported in more than 1% of the patients under treatment.<sup>13, 19</sup>

Colitis and hepatitis account for minor part of gastrointestinal AEs, however they lead to treatment discontinuation in over a third these patients.<sup>66</sup> Colitis, described as diarrhoea associated with abdominal pain, per rectal bleeding or mucous, or large bowel inflammation, confirmed by imaging techniques (CT), is less common and causes less grade 3-4 events in patients receiving pembrolizumab (2.5%) than in the ones receiving ipilimumab (7%).<sup>13</sup> Yet, it is an AE, that requires treatment interruption in case of grade 2 and treatment discontinuation in case 3-4 events. In the three biggest trials analyzing the efficacy and safety of pembrolizumab, colitis was the cause of treatment discontinuation in 45,2% of the patients.<sup>66</sup>

Liver function tests are a part of the routine monitoring during treatment with checkpoint inhibitors. Since the majority of hepatitis cases (81.3%)<sup>66</sup> present as asymptomatic laboratory findings, it is also the main diagnostic tool in the case of treatment induced hepatitis. Hepatitis is reported to occur in less than 3% of the patients, however, most of them experienced grade 3-4 events.<sup>13, 71, 74</sup> In another study, 2.2% (n=11) of the patients developed hepatitis under treatment with pembrolizumab or nivolumab, all events were of grade 3-4 and led to treatment interruption or treatment discontinuation, in some cases additional treatment was also needed.<sup>70</sup>

## 2.5 Endocrine AEs

Endocrine disorders are a known irAEs caused by ipilimumab, with the most common being thyroid abnormalities (6%), hypophysitis (1.8%) and adrenal insufficiency (1.5%).<sup>81, 82</sup> Similar endocrinologic disorders are reported in 13% of the patients under anti-PD-1 therapy<sup>74</sup> and are one of the common AEs, defined as those having a potential immunologic cause and requiring frequent monitoring.

Hypothyroidism of grade 1-2 was reported in up to 10% of the patient population, whereas grade 3 events have only been described in 2 patients.<sup>65, 72, 83</sup> In case of hypothyroidism almost every patient required hormone replacement therapy but none of them had to stop the immunotherapy.<sup>66</sup>

Hyperthyroidism was less common with reported rate of 3.3-5% and two cases of grade 3 event, thyroiditis was reported in 0.8% patients.<sup>83</sup>

Thyroid disorders can, in most cases, be managed with hormone replacement and require no corticosteroid therapy or interruption of anti-PD-1 treatment. Unfortunately, many cases (81.1%) of thyroid abnormalities do not resolve, even after the treatment

has been stopped, and require subsequent hormone substitution or antithyroid medication.<sup>66</sup>

Painless thyroiditis syndrome is a condition, that usually presents either with transient thyrotoxicosis (initial phase of hyperthyroidism, followed by hypothyroidism) or with hypothyroidism without an initial thyrotoxic phase. In the study made by Orlov et al., all of the patients (n=10) who have been reported to experience painless thyroiditis syndrome under anti-PD-1 therapy, were positive for antithyroid Abs and negative for thyrotropin-binding inhibitory immunoglobulins.<sup>84</sup>

Although the rate of hypophysitis was only 0.8% (n=13), 6 patients were reported with grade 3-4 events and 4 had to stop to pembrolizumab treatment.<sup>66</sup> Given the unspecific clinical presentation of hypophysitis, with symptoms such as fatigue, nausea, anorexia, behavioral change, loss of libido and other, some cases of low grade hypophysitis may remain underreported. Hypophysitis was also reported to occur several months after treatment with ipilimumab, suggesting that a similar scenario is possible in the patient population treated with anti-PD-1 Abs.<sup>85</sup>

Other endocrine disorders, such diabetes mellitus and adrenal insufficiency were reported in less than 1% of the patients.<sup>66, 70</sup>

## 2.6 Respiratory tract

Respiratory AEs predominantly present as cough and dyspnea. Even though these symptoms have been reported in 18%-38% of the patients, only 3.7% of them were described as treatment related and no grade 3-4 events were documented.<sup>17, 74</sup>

Pneumonitis of immuno-allergic origin was reported in 1.6-2.0% of patients, receiving pembrolizumab or nivolumab, and was mild in most of them.<sup>59</sup> Yet, the condition did not resolve in up to 25% of the cases.<sup>66</sup>

In the patient population, analyzed by Zimmer et al., 1.6% of the patients experienced respiratory AEs, such as organizing inflammatory pneumonia, pneumonitis with sarcoid-like lesions and in one patient pneumonitis with subsequent lung fibrosis was documented.<sup>59</sup> Even though grade 1 pneumonitis can be managed with checkpoint inhibitor interruption and, if necessary, corticosteroid treatment, anti-PD-1 Ab therapy has to be permanently discontinued if the patient develops grade  $\geq 3$  pneumonitis or grade  $\geq 2$  event upon re-introduction of treatment.<sup>65</sup> A case of grade 4 pneumonitis, which required treatment with methylprednisolone and cyclophosphamide, as well as prolonged mechanical lung ventilation (65 days) and hospitalization (120 days), was reported by Watanabe et al.. The latter case reports, illustrate that even life threatening AEs can be managed with timely and adequate interventions.<sup>86</sup>

## 2.7 Neurologic and musculoskeletal AEs

In general, neurologic or musculoskeletal AEs are rare under therapy with anti-PD-1 Abs. Encephalopathy, hyperesthesia, paresthesia, peripheral neuropathy, Guillain-Barre syndrome, seizures and other neurological disorders were reported in less than 1% of the patient population.<sup>13, 59, 64, 65, 87</sup>

Given the substantial morbidity often caused by neurological events, they require prompt diagnosing and management. Neurological side effects can occur late after treatment initiation or even after its completion. Hence patients should be actively queried with regard to any abnormal neurological symptoms even after the end of the therapy.<sup>88</sup>

Muscular weakness and musculoskeletal pain, as well as myasthenic syndrome were also reported in less than 1% of patient population each.<sup>13, 19, 59, 65</sup> Although uncommon, such events have a significant impact on further therapy. A good

example is myositis, which was reported in only 0.4% of the pembrolizumab receiving patients and led to treatment discontinuation in 3 out of 6 of them.<sup>13, 66</sup>

## 2.8 Other and less frequent adverse events

Infrequent infusion reactions related to immunotherapies are not all well documented yet, mainly because clinical presentation varies greatly and can affect almost any organ system. They can be allergic or non-allergic and engender similar symptoms.<sup>89</sup> Such symptoms are reported in 0.1-5.6% of patients receiving pembrolizumab or nivolumab.<sup>66, 68, 74</sup>

Some rare events involve renal system, and include autoimmune nephritis, tubulointerstitial, acute interstitial nephritis and blood creatinine increase in 0.4%-1.5% of the patients<sup>66, 68</sup>

A case of autoimmune inner ear disease has been reported in a 82 year old male after two infusions of pembrolizumab. Clinically it presented as bilateral hearing loss, with decrease in word recognition scores down to 48% in the right ear and to 44% in the left ear. The patient was treated with intratympanic injections of dexamethasone solution, with no interruption of immunotherapy. After 6 intratympanic injections in the right and 4 in the left ear, the word recognition scores rose back to 88% and 84% in the right and left ear respectively.<sup>90</sup>

Furthermore, eosinophilic fasciitis, presenting as muscle pain, and cerebral vasculitis, presenting as physical and psychological alterations, have been reported to develop in a 51 year old woman who received pembrolizumab for metastatic melanoma. The AEs developed respectively at 18 months after treatment initiation and one month after the discontinuation of the therapy (treatment duration was 18 months). Biopsy from fascia confirmed the eosinophilic fasciitis, while cerebrospinal fluid analysis was

normal, suggesting a systemic, rather than a local immune reaction. Therapy with systemic steroids was initiated and the condition of the patient improved, yet she developed mild contractures and performance status deteriorated to 1 (compared to 0 before initiation of pembrolizumab therapy).<sup>88</sup>

Finally, 8 cases of immunotherapy (anti-CTLA-4, anti-PD-1 or combination therapy) related cardiotoxicities were reviewed by Heinzerling et al., 2 of the patients received anti-PD-1 Abs and developed myocarditis with cardiomyopathy and cardiac arrest which responded well to steroids and additional medication. Both patients had a history of cardiac comorbidities, which raises the question of whether cardiac AEs are caused or aggravated by the treatment.<sup>91</sup>

Such cases highlight the importance of close patient monitoring for rare or not yet reported AEs during and after treatment with checkpoint inhibitors. The more accurate our understanding of each and every AEs is, the more effective the patient care will be. Indeed, if AEs are detected very early or even anticipated there are more chances the treatment can be administered flawlessly and therefore be more efficient.

A summary of rare severe and  $\geq 3$  grade AEs is provided in Table 2. Established and proposed management strategies of selected grade 3-4 AEs are provided in Table 3.

### **3. Expert opinion**

The crosstalk between tumor cells, APCs and other cellular components of the innate and adaptive immune system of the tumor microenvironment is crucial for the anti-tumor immune responses and the clinical outcome of immunotherapies. In most if not all cancer types, these interactions often create a local immunosuppressive milieu

which allows tumor cells to escape the immune-surveillance as predicted by the immune-editing paradigm.

Established tumors have obviously managed to successfully avoid being recognized by the immune system. With the introduction of PD-1 Abs which block the PD-1/PD-L1 pathway, the balance can be shifted to a new elimination phase that may, in the best case scenario, result in tumor clearance or at least in a stable equilibrium.<sup>92</sup>

These are the reasons why checkpoint inhibitors such as Abs against PD-1, achieve significant clinical benefits and metastatic tumor regression. Although anti-PD-1 Ab therapy is safe and well tolerated in melanoma patients,<sup>64, 73, 74, 93</sup> adverse cutaneous reactions have been frequently reported<sup>72, 73, 77, 94, 95</sup> as predicted by animal models in contrast to other autoimmune manifestations including cardiomyopathy and glomerulonephritis.<sup>96, 97</sup> Overall, the AE profile of anti PD-1 therapies is definitely milder than the AE frequency and severity induced by anti CTLA-4 therapy, ipilimumab.<sup>13</sup>

If we compare anti-PD-1 therapy to the combination therapy consisting of BRAFi and MEKi, the latter is also well tolerated, and achieved similar 1-year, 2-year and 3-year survival rates, it however has the advantage of being an oral medication. It's main drawback is that the response rate in patient population *BRAF* wt is not as high as in the *BRAF* mutated patient population.<sup>98 99</sup> Therefore, international guidelines recommend both treatment approaches as a 1<sup>st</sup> line in stage IV melanoma, independent on *BRAF* mutation status.<sup>22</sup>

Immunotherapies using Ab against PD-1 (i.e. pembrolizumab and nivolumab) caused a plethora of AEs. We assume, based on the mechanism of action, that most if not all of them are immune related. Fortunately, the majority are mild and manageable. However, since an increasing number of patients are now contemplating a rather

long life expectancy, there is a substantial need to understand and prevent severe AEs. Ocular, cutaneous, pulmonary and neurological AEs, but also the less frequently reported ones, can have a major impact on the patient's quality of life. Today, the recommended management strategy for any severe AE is treatment withholding and administration of corticosteroids, which is obviously a poor strategy. There is urgent need for consensus in management strategies using more sophisticated immunomodulatory drugs such as: TNF blockers, intravenous immunoglobulins, hydroxychloroquin or methotrexate, none of which are allowed in the registered trials, but may even support antitumor responses while controlling autoimmunity.

Until today, the available data sets today are restricted to clinical trial populations. Only scarce information is available regarding patients with reduced performance status, significant co-morbidities such as heart or liver disease, renal deficiency, co-existing auto-inflammatory and auto immune diseases including asthma, rheumatoid arthritis, lupus erythematosus or psoriasis. This data is urgently needed with the perspective that many more cancer entities will be treated with these immunotherapies in the near future.

In addition, there is little information on the frequency and the specific patterns of AEs after long term exposure to anti-PD-1 therapy.

Our own research has suggested a role for PD-L1 when expressed by keratinocytes in cutaneous eruptions associated with anti PD-1 therapy.<sup>80</sup> Getting a better insight into the role of PD-1/PD-L1 interaction in healthy and inflamed tissues is a required step towards a more general understanding of the role of lymphocytes in inflammatory processes such as cutaneous drug eruptions, inflammatory thyroid disease, arthritis or pneumonitis.

Furthermore, it will be critical to develop biomarkers able to identify patients at higher risk of developing a specific severe AE. Common ways of predicting patient's predisposition towards a specific drug can certainly be used. Those include: genome wide association studies or HLA constellation, certain co-medications, known preexisting drug sensitivity or pre-existing autoimmune disease such as rheumatoid arthritis or pemphigoid vulgaris.

In order to overcome those multiple challenges, interdisciplinary specialized immunology boards supported by clinical and basic immunology experts will be required. The field of immuno-oncology would greatly benefit from such boards being institutionalized in every major cancer centers in order to systematically report AEs. However, there is a threat that due to the general good tolerability of anti-PD-1 therapies, patients will often be treated in private offices or in small regional hospitals. Since doctors in those institutions may have insufficient experience in recent and rapidly evolving the field of immunotherapies, they could fail to report and/or pay less attention to unusual AEs.

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