

# Safety of Anti-PD1/PD-L1 Antibodies in Melanoma

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## ABSTRACT

Immune checkpoint inhibitors and in particular anti-PD-1/PD-L1 antibodies have remarkable efficacy in advanced melanoma, but like the vast majority of medications, they trade this outstanding efficacy for a safety cost. This chapter is a narrative review exploring the available safety data for the two anti-PD-1 antibodies, nivolumab and pembrolizumab, which have been commercially available for about a year in Europe, United States and other regions of the world. The main adverse effects associated with these agents, with frequency data and main features, the biological mechanisms involved where known, and their therapeutic management is discussed. Unlike anti-PD-1 antibodies, PD-L1 inhibitors are still in the middle of their clinical development, but limited preliminary data from a few clinical trials are available and have also been used for comparative purposes.

**Keywords:** Anti-PD1/PD-L1 antibodies; Melanoma; Pembrolizumab; Nivolumab; Adverse events; Immune checkpoints

**Abbreviations:** Programmed Cell Death Protein 1 (**PD1**); Programmed Death-Ligand 1 (**PD-L1**); Immune Checkpoint Inhibitor (**ICI**); Cytotoxic T-Lymphocyte-Associated Protein 4 (**CTLA-4**); immune related Adverse Event (**irAE**); Overall Survival (**OS**)

## INTRODUCTION

Immune checkpoints are important components of the self-tolerance system of animal organisms, a system that precludes autoimmune injuries to normal tissues of the body. Tumour cells often take over this mechanism and exploit it to evolve resistance to the immune system of the host, especially against the cytotoxic T cells [1]. As for numerous other cases of cell signaling, immune checkpoints work by a ligand-receptor interaction to send an immune inhibitory signal and Immune Checkpoint Inhibitors (**ICIs**) are pharmacological agents that impede them, this negation of negation being equivalent to a stimulatory boost on the immune system [1,2]. A number of immune checkpoints relevant to pharmacological modulation are currently actively explored in non-clinical and clinical settings, but three agents targeting two different immune checkpoints have by this time crossed the regulatory barrier to market approval in malignant melanoma: ipilimumab (targeting the CTLA-4 receptor), pembrolizumab, and nivolumab (targeting the PD-1 receptor) [2]. All three, as the majority of the ICIs currently under development are monoclonal antibodies, forming the so-called second-generation of antibodies, together with more sophisticated antibodies, such as those bi-or tri-specific (targeting simultaneously two or three antigens) and antibody-drug conjugates [3]. After ipilimumab kindled hopes for the unresectable or metastatic malignant melanoma, despite a relatively low response rate (estimated to about 11%), anti-PD-1 pembrolizumab and nivolumab followed soon with a higher response rate (25-33%) [4]. Anti-PD-L1 antibodies (targeting not the PD-1 receptor protein, but the PD-L1 ligand protein) have also been developed and clinically evaluated in various forms of cancer, including melanoma.

As the large majority of drugs, ICIs in general and anti-PD-1/PD-L1 inhibitors in particular, trade their therapeutic efficacy for a safety cost and as for many new drugs, discovering and understanding well the safety profile take a longer time than the first three phases of the clinical development, after which they reach the market. This chapter explores the safety data available for the two anti-PD-1 antibodies, which have been available on the market for about one year discussing the main adverse effects associated with these therapies, their potential mechanisms (where known) and the practical measures useful to minimize the risks in the clinical practice. Anti-PD-L1 antibodies have not yet attained their full clinical development and are not approved for use thus far, but data from the clinical trials with these antibodies, where available, have also been used here for comparative purposes.

## METHODS

This narrative review is based on a literature search carried out in PubMed for papers published up to May 10, 2016, using several alternative search terms: (“pembrolizumab” or “nivolumab” or “BMS-936559” or “atezolizumab”) and “adverse” and (“effects” or “events”) and “melanoma”; (“pembrolizumab” or “nivolumab” or “BMS-936559” or “atezolizumab”) and “safety” and “melanoma”; (“PD-1” or “PD-L1”) and “safety”, (“PD-1” or “PD-L1”) and (“adverse events” or “adverse effects”) and “melanoma”. No restrictions were applied within the search process and the papers returned were curated manually. Besides, we interrogated the FDA AERS data through the Open Vigil [5] interface for statistics on adverse reactions regarding the two approved antibodies.

## GENERAL SAFTEY PROFILE

Because in essence all ICI act by an indirect activation of the T-cells and immune system, the majority of their adverse effects are of an immunologic nature, dermatological toxicities being the most common immune-related AEs (**irAEs**) of these agents [6] (for a somewhat subtle distinction between “immune-mediate AEs” and “immune-related AEs”, see reference no. [7]). Nevertheless, a study evaluating the economic impact of adverse reactions accompanying treatments of metastatic melanoma in several countries (Australia, France, Germany and Italy), found that high grade irAEs of gastro-intestinal nature (associated to ipilimumab) had the highest costs [8]. Adverse events of an immunological etiology are often termed “select adverse events” in the scientific literature [9].

In a recent European observational study of patients with metastatic melanoma receiving one of the two authorized PD1 inhibitors, 138 out of 496 patients (27.82%) experienced adverse effects, with an average of 1.75 adverse effects per patient [10,11]. This is in a strong contrast with clinical trials, where adverse events (treatment related) were reported in a percentage of subjects varying between 70% [12] and 84% [13] for nivolumab and 47% [14] and 79.5% [15] for pembrolizumab. For atezolizumab (anti-PD-L1 antibody) the frequency of AEs of any grade was 70% [16], whereas for BMS-936559 (a second anti-PD-L1 inhibitor), the global rate of AEs was 91% [17].

In the phase III clinical trial comparing nivolumab and ipilimumab [18] the percentage of subjects experiencing adverse events with nivolumab, ipilimumab or their combination was 82.1%, 86.2%, and 95.5%, respectively; most of them were irAEs (62.0%, 73.6% and 87.9%, respectively) [18]. In an early phase trial (n=107) of nivolumab adverse events irrespective of seriousness were seen in 84% of the patients and 54% of all adverse events were considered to be irAEs [13]. A retrospective review of melanoma patients managed with nivolumab reported that 68.2% of patients had irAEs [19].

Treatment related serious adverse events occurred in 9.2%-16.3% of all patients treated with

nivolumab in phase III trials [13], and in 10.1-13.3% of all patients treated with pembrolizumab in a phase III trial [15]; their frequency was 5% in a phase I study of an anti-PD-L1 antibody [17] and 13% for another anti-PD-L1 antibody [16]. When looking also to the early phase trials, the frequency of high-grade (3-4) adverse events varied in clinical trials between 9.2% [18] and 22% [13] for nivolumab and between 10.1% [15] and 14% for pembrolizumab [14].

There is convincing evidence that the safety profile of anti-PD1 inhibitors is superior to the one of ipilimumab [20]. In a comparative trial, high grade (3 or 4) AEs were more frequent among the patients treated with ipilimumab (27.3%) than among those receiving nivolumab (16.3%) [18]. Similarly, for pembrolizumab, high grade AEs were less frequent when compared with ipilimumab (10.1-13.3% versus 19.9%) [15]. However, in an observational study on 496 patients treated with pembrolizumab or nivolumab the percentage of grade 3 or 4 AEs was estimated to about 24% [11] (considerably higher than the rates reported in clinical trials, but the population and circumstances of administration may be different from those narrowly defined in clinical trials). Adverse reactions leading to treatment discontinuation (irrespective of the grade) were observed in a comparative phase III study in 7.7% of the nivolumab patients and in 14.8% of the ipilimumab group. In this trial the most frequent adverse events causing treatment interruption were of a gastro-intestinal nature, mainly diarrhoea (1.9% for nivolumab, 4.5% for ipilimumab) and colitis (0.6% for nivolumab, 7.7% for ipilimumab). In each of the monotherapy groups a death was recorded (caused by neutropenia in the nivolumab group and by cardiac arrest in the ipilimumab group), and no death was recorded in the combination arm [18]. The most common high grade adverse reactions of an immunological nature were also diarrhoea, colitis and an increase in the alanine aminotransferase level, generally favouring nivolumab over ipilimumab [18]. In this study, systemic or local immune modulatory therapies were administered in 47.0% of the patients receiving nivolumab and in 55.9% of those treated with ipilimumab to control the adverse events. Second-line immunosuppressive medicines such as infliximab were employed in 0.6% of the patients managed with nivolumab and 5.1% of those receiving ipilimumab (resolution rate for high grade events varied between 85 and 100%) [18]. In the phase III trial comparing pembrolizumab and ipilimumab 4.0% and 6.9% of those treated with the anti-PD1 agent discontinued treatment because of AEs, whereas 9.4% of those receiving ipilimumab did so [15]. Moderate to severe toxicities tend to have a later onset with pembrolizumab than with ipilimumab (median time 9 weeks versus 6 weeks) [21]. Moreover, in a phase I/II study in patients in whom ipilimumab therapy failed, 21 patients who had high grade toxicity to ipilimumab had no such problems with nivolumab [22]. On the whole, the types of AEs associated with nivolumab (and pembrolizumab) treatment are similar with those observed with ipilimumab; the two main exceptions are represented by pneumonitis and nephritis [7].

Anti-PD1 inhibitors have also better safety profiles than the former standard of treatment represented by dacarbazine. In the phase III trial comparing nivolumab and dacarbazine, the incidence of AEs irrespective of grade was similar in the two treatment arms (74.3% for nivolumab,

75.6% for dacarbazine), but those of high grade were less common in the nivolumab group than in the dacarbazine one (11.7% versus 17.6%) [23]. High grade serious adverse events related to the treatment were also similar in the two groups (5.8% versus 5.9%), but less patients in the nivolumab group discontinued their treatment because of AEs than those in the dacarbazine group (6.8% versus 11.7%) [23].

Unlike ipilimumab, PD-1 inhibitors are administered continuously for a longer time (in some studies for two years), and thus patients can develop irAEs late after the beginning of therapy, and even after the discontinuation of treatment [10]. The large majority of adverse events of anti-PD-1 inhibitors occur in the first 6 months of treatment; prolongation of administration after this time point does not seem to associate with additional toxicities [13].

In two early phase trials the spectrum of adverse events induced by anti-PD1 or anti-PD-L1 agents, as well as the frequency and severity seem to be unrelated to the dose administered (except for infusion reactions) [12,17], but in another early phase study the highest incidence of AEs was observed in patients receiving the highest dose at the shortest interval (10 mg/kg every 2 weeks) [24].

Systemic corticosteroids and other immune-suppressive agents used for the management of immune-related adverse events caused by anti-PD1 therapies do not seem to influence negatively tumor response, and therefore if the symptoms of the patient are serious or severe the management of AEs should be the first priority [25,26].

There is limited data suggesting that the safety profile of anti-PD1 antibodies is slightly better in patients with mutant BRAF status than in those with wild-type BRAF. A pooled analysis of four nivolumab clinical trials found that treatment-related AEs irrespective of grade was 68.3% in patients wild-type BRAF and 58.5% in those with mutant BRAF group. High grade (3 or 4) AEs in the two groups were 11.7% and 2.8%, respectively [27].

Although generally adverse events do not have benefits *per se*, a retrospective study of 148 patients administered nivolumab found a significant difference in Overall Survival (OS) between those experiencing irAEs of any grade as compared with those without AEs ( $p < 0.001$ ) and an OS advantage was reported particularly for those patients with three or more irAEs ( $p < 0.001$ ) [19].

In the following pages we will discuss each AE or group of related AEs with respect to their incidence, main characteristics and therapeutic management.

## GENERALIZED SYMPTOMS

General symptoms such as fatigue and asthenia, fever and chills, myalgias, and headaches are common, but of low grade in the large majority of cases (more than 95%) [24]. In an observational study fever and sweating were seen in 1.4% of the patients; no treatment or only symptomatic treatment was administered in these patients [11]. Headache was reported under pembrolizumab

with a frequency of 10%, all cases being low grade and chills with a frequency of 7% (no high grade case) [24]. In the FDA spontaneous system, of all AEs received for pembrolizumab (n=174), fatigue represents 8.6% (n=15), whereas asthenia cases represent only 1.72% (n=3).

Fatigue has been reported with a frequency higher than pruritus (19.9% for fatigue, 17.0% for pruritus in one nivolumab study) [23]. The incidence for fatigue varied for nivolumab between 19.9% [23] and 31.8% [13] in various melanoma studies (15.4% for a phase I trial in a variety of tumours including melanoma [28]) and between 16-37% in non-small cell lung carcinoma [29]; for pembrolizumab it varied between 19.1% [15] and 30% [24] in melanoma trials. Whereas fatigue was reported in one trial in 32% of the patients, grade 3 or 4 fatigue was only observed in less than 2% of the subjects [13,24,30]. Fatigue induced by anti-PD1 agents is typically minor and not connected with other general symptoms [29]. The mechanism involved in causing fatigue is not known so far; it may be partially an early expression of hypothyroidism, but not all patients with fatigue experience hypothyroidism [29]. Fatigue does not seem to be dose-related [29]. PD-L1 inhibitors seem to be associated with similar frequencies of fatigue as anti-PD-1 antibodies [29], but no direct comparison is available in humans so far. For intolerable cases, the use of short courses of oral corticosteroids (e.g. prednisolone, 10-20 mg) has been advised [21].

Asthenia (“lack of strength or feeling of inability to carry out daily tasks, which is more intense at the end of day, and usually improves after a period of sleep” [31]) was reported in a phase III trial by 10.2% of the patients treated with nivolumab, and in none it was grade 3 or 4 [23]. Similarly, in those treated with pembrolizumab asthenia had a frequency of 10% [24], 11.5% and 11.2% (administered at two weeks and three weeks, respectively) [15].

Pyrexia was reported in 4.7% of patients treated with nivolumab [13] and 7% of those treated with pembrolizumab [24] and in none it was high grade [13,24]. It is assumed to be caused by a nonspecific stimulation of the immune system and release of a variety of inflammatory proteins (cytokines)[29]. The therapeutic approach is symptomatic, with antipyretics (e.g. paracetamol) or NSAIDs (e.g. ibuprofen) [29]. In the AERS data, pyrexia cases represent 2.30% of all AEs received by FDA by spontaneous reporting.

## CUTANEOUS REACTIONS

In clinical trials 35-40% of melanoma patients receiving pembrolizumab or nivolumab generally suffer at least one cutaneous adverse effect, although in most of the cases these are benign and not challenging [6,13]. L. Hofmann et al. (2016) reported that 27.82% was the percentage of all patients experiencing at least one adverse effect and that those of a cutaneous nature represented only 8.7% of the patients in their sample [10]. On the other hand, a retrospective review of 83 patients treated with pembrolizumab found that 42% of patients developed treatment-related cutaneous AEs [32]. This same study also indicated that patients developing cutaneous AEs had advantages in terms of progression-free survival as compared with those not experiencing such

AEs [32]. Another retrospective study reported good correlations between rash and vitiligo on the one hand and OS on the other ( $p=0.004$  and  $0.028$ , respectively), whereas other irAEs (endocrinopathies, colitis, or pneumonitis) were not correlated with OS [19].

In one early phase trial ( $n=107$ ) only lichenoid skin reaction, lichen planus mucosa, and acute febrile neutrophilic dermatosis (Sweet's syndrome) were high grade (3), all others were assessed as grade 1 or 2 [13]. Permanent discontinuation of anti-PD1 antibodies because of cutaneous toxicities has been estimated to less than 5% of patients in clinical trials [29].

The most frequent cutaneous reactions are nonspecific macular popular rash, pruritus and hypopigmentation, but alopecia, a distinguishing lichenoid dermatitis, psoriasis, vitiligo, bullous disorders such as bullous pemphigoid, Stevens Johnson syndrome, toxic epidermal necrolysis, and mucosal adverse reactions (including xerostomia and oral lichenoid mucositis, oral mucositis, gingivitis, and sicca syndrome-like symptoms), also do occur, although less frequently [6,10,29,32]. With respect to dermatologic adverse reactions, the two main antibodies, pembrolizumab and nivolumab do not seem to have important differences, but to date exposure data has been limited [6].

Mild cutaneous reactions (the majority) can be managed with topical corticoids, systemic antihistamines (or where relevant, other antipruritic agents, including GABA agonists, NK-1 receptor antagonists, antidepressants) or without treatment (in one study a single patient with pruritus needed systemic corticoid therapy) [10,29]. In some cases the treatment had to be discontinued for a while, whereas in others the AEs improved or disappeared with continuation of the PD-1 inhibitor treatment. For the lichenoid skin reaction, oral prednisolone (1 mg/kg, orally) may be administered [10]. For any atypical rash, those not diminishing after intervention, that severe or involving oral mucosa, early dermatological assessment, clinical evaluation is advised, accompanied or not by skin biopsy and laboratory tests for assessing the renal and hepatic function, serum levels of tryptase, and immunoglobulin E [29]. Interface dermatitis, perivascular and periadnexal dermatitis with lymphocytic infiltrate, rare plasma cells and eosinophils are often seen in histological examinations [29].

Pruritus was reported in 9% (phase I trial [12]), 13.1% (another early phase trial [13]) and 17% (a phase III trial [23]) of the patients treated with nivolumab. In patients treated with pembrolizumab the 14.1 and 14.4% (a phase III trial [15]) or 21% (a phase 1 expansion study,  $n=135$  [24]) of the patients experienced pruritus. In a phase I trial of an anti-PD-L1 antibody the percentage of patients affected by pruritus was only 6% [17]. High grade pruritus was observed in up to 1% of the subjects [12,24,29]. In the AERS data, of the 174 spontaneous AE reported for pembrolizumab thus far, 4 (2.30%) refer to pruritus or generalized pruritus. In a systematic review and meta-analysis, the incidence of all grade pruritus was 13.2% (95% CI: 8.9 - 19.2%) for nivolumab and 20.2% (95% CI: 14.8 - 26.9%) for pembrolizumab. The Relative Risk (RR) computed against standard chemotherapy was 34.5 and 49.9, respectively ( $p<0.02$  in both cases) [33].

Rash was experienced by a percentage varying between 12% and 23% of patients [12,13,23] in various clinical trials performed with nivolumab and between 13.4% and 21% in clinical trials conducted with pembrolizumab [15,24]; In a phase I trial of an anti-PD-L1 antibody 7% of patients experienced rash [17]. In the AERS data, rash seems to be reported more often than pruritus, a number of 13 cases (7.47%) refer to various forms of rash, making this AE one of the most often reported spontaneously. In a systematic review and meta-analysis it was found that incidence for all-grade rash was 14.3% (95% CI: 8.7 - 22.7%) for nivolumab and 16.7% (95% CI: 11.9 to 23.0%) for pembrolizumab. RR computed against standard chemotherapy was 2.5 and 2.6, for nivolumab and pembrolizumab, respectively ( $p < 0.01$  in both cases) [33].

High grade rash was seen in 2% of the patients in one early phase trial of pembrolizumab [24], 0.5% in a phase III trial [23] and 0% in one phase I trial of nivolumab [12]. Skin rash is thought the most frequent irAE of immune checkpoint inhibitors, occurring usually after the second cycle of treatment [29]. It may assume a wide spectrum of presentations: maculopapular, papulopustular, follicular dermatitis, urticarial dermatitis or Sweet's syndrome [29]. In the anti-PD-L1 treatment a few cases of macular rash (1%), erythema (1%) and erythematous rash were reported [17].

Vitiligo has been reported relatively often in melanoma, but has not been observed in other solid tumours treated with PD1/PD-L1 inhibitors [6,10]. It has been recorded in 3% [12] -9.3% [13] -10.7% [23] of the patients treated with nivolumab, none of grade 3 or 4 [13]. Its frequency in patients treated with pembrolizumab varied between 9% [24] and 11.2% [15], but in the phase III trial in the ipilimumab arm it was only 1.6%, suggesting a stronger association with the anti-PD1 agents [15]. In an anti-PD-L1 antibody trial, the vitiligo incidence was only 2% [17]. No vitiligo AE has been reported as such in AERS, but two cases of "skin discolouration" were reported (1.15%). In a systematic review and meta-analysis, the incidence of vitiligo was 7.5% (95% CI: 5.9 - 9.5%) for nivolumab and 8.3% (95% CI: 4.4 - 15.2%) for pembrolizumab. The RR against standard chemotherapy was 14.6 and 17.5 ( $p = 0.058$  and  $0.48$ , respectively) [33]. This AE may be permanent, but treatment discontinuation or other therapeutic approach is not necessary [9].

Three cases of *lichenoid dermatitis* were published in patients under pembrolizumab treatment, two in advanced age subjects (80 and 79 years), the third in a 64-year old man, all relatively mild, two of them also under lisinopril treatment (known to be associated with lichenoid reactions), but the lichenoid dermatitis had its onset only after the introduction of pembrolizumab [34]. They are assumed to be T-cell mediated, as indicated by the dense histopathological presence of CD4-positive and CD8-positive T cells. In one case topical steroids diminished both the pruritus and the rash, in another case it diminished pruritus but not the rash, and in a third case no treatment was deemed necessary [34].

Dermatitis acneiform was reported in one nivolumab trial in 5.6% of cases and photosensitivity reactions in 3.7% of cases [13]. However, dermatitis acneiform was not always reported as such but rather under the umbrella term of "rash", as employed by the summary of product characteristics [35]. Dermatitis acneiform has been described as "uncommon" for pembrolizumab [36].



A case of bullous pemphigoid (an autoimmune skin disease with blisters caused by autoantibodies against epithelial basement membrane [37]) was reported in a 75-year-old patient in whom administration of pembrolizumab was halted after 6 cycles of therapy because of disease progression. At the 30-day follow-up the patient had full symptoms of bullous pemphigoid (especially at knee and elbow level), confirmed by skin biopsies. It improved rapidly after one week of oral prednisone in decreasing doses [38]. Another case was reported in a 42-year old male patient after 8 months of pembrolizumab treatment [37]. In a retrospective study, of 124 patients treated with pembrolizumab for bullous pemphigoid for metastatic melanoma three patients developed bullous pemphigoid (of 260 melanoma patients managed with BRAF inhibitors none experienced this adverse effect), treated promptly with topical and systemic steroids [39].

Generally patients already diagnosed with autoimmune conditions have not been included in trials for ICIs because of fears that these agents might aggravate the preexistent autoimmune disease. A patient with pre-existing severe autoimmune bullous pemphigoid was reported to have been treated with pembrolizumab for five cycles. The bullous pemphigoid tended to become manifest under pembrolizumab and was partially controlled with topical clobetazol; after the fourth and the fifth cycles the bullous pemphigoid was severe necessitating the interruption of treatment and systemic prednisone, but the authors argued that the therapy could be managed even in such patients with careful measures [40].

Alopecia was reported in 2% of the patients treated with nivolumab and 0.9% of those treated with pembrolizumab [33].

In a Japanese patient (80 years of age) a psoriasiform eruption was reported after the fourth dose (12<sup>th</sup> week of treatment) of nivolumab, and this coincided with the regression of melanoma lesions [41].

Oral mucositis and xerostomia have been reported more frequently with PD-1 inhibitors than with ipilimumab [9].

Other dermatological reactions in patients treated with nivolumab include xeroderma (dry skin) (5.3%), stomatitis (1.5%), urticaria (1.4%), photoallergic reactions (1.4%), excessive perspiration (0.9%), and skin exfoliation (0.7%). For pembrolizumab, other reactions include impaired healing (14%), xerosis (2.4%), alterations of hair colour (1.1%), and alopecia (0.9%) [33].

## **GASTROINTESTINAL IRAES**

Gastrointestinal AEs such as diarrhoea have been reported in various studies with a frequency varying between 6.0 and 18.0%, whereas the incidence of high grade AEs is between 1% and 2.2% [10,12,13,23]. In their retrospective study, L. Hoffmann, et al. (2016), which generally reported lower rates of adverse events than in clinical trials published previously, reported a rate of 4.2% gastrointestinal AEs: diarrhoea, abdominal pain, coprostasis, xerostomia, colitis, and oesophagitis (in decreasing order of their occurrence frequency) [10]. In this study, only four cases of grade

3 gastrointestinal AEs were observed (all represented by diarrhoea) [10]. The frequency and severity of gastrointestinal AEs induced by anti-PD1 agents are substantially lower than with ipilimumab, but anti-PD1 antibodies may also cause intestinal perforations, although such cases are rarer [10].

In an early trial of pembrolizumab diarrhoea was experienced by about 20% of the patients and it was grade 3 in one single patient [24]. In the phase III trial of this antibody diarrhoea was reported in 14.4% and 16.9% (administered at three and two week interval, respectively), and it was grade 3 or 4 in 1.1% and 2.5%, respectively [15]. Pooled data from 1567 patients indicate a frequency of 15% for diarrhoea in patients treated with pembrolizumab [36]. For nivolumab, diarrhoea was reported in early phase trials in 11%-17.8% (grade 3 or 4 in 1%-1.9%) [12,13] and in the phase III melanoma trial in 16% of the patients and in 1% it was grade 3 or 4 [23]. Pooled data from several trials indicate a frequency of 13% for diarrhoea in patients treated with nivolumab [35]. In AERS, diarrhoea was reported in 6 out of 174 pembrolizumab cases (3.44%). In the phase III trial comparing nivolumab and ipilimumab, diarrhoea occurred less often in the patients treated with nivolumab than those treated with ipilimumab (2.2% for nivolumab, 6.1% for ipilimumab) [18]. In a phase I trial of an anti-PD-L1 antibody, diarrhoea was experienced by 9% of the patients and in none it was high grade [17]. It has been stated that whereas for ipilimumab and nivolumab the median time to the onset of diarrhoea is 7 weeks, for pembrolizumab it is about 6 months [21].

Colitis (defined as symptoms of abdominal pain accompanied by either clinical signs or imaging proof of colon inflammation [29]) occurred as a high grade AE in 1.4 and 2.5% of the patients treated with pembrolizumab in the phase III trial [15]. Colitis seems considerably less frequent with nivolumab than with ipilimumab (in the direct comparison 0.6% for the former, 8.7% for the latter) [18]. A case of collagenous colitis, where a thickened apical subepithelial collagen layer thicker than 25  $\mu\text{m}$  was detected by histological analysis was recently reported in a patient after 14 cycles of treatment with pembrolizumab [42]. Calprotectin, a protein produced by neutrophils and elevated in inflammatory bowel disease has been suggested to be a useful and early marker of severe auto-immune colitis, its rapid increase in feces being correlated with high likelihood of autoimmune colitis occurrence, as indicated by a small study (n=56), in which patients treated with anti-PD1 agents were underrepresented (n=4) [10,43]. Fluorescein-guided confocal laser endomicroscopy was proposed as a non-invasive technique to be used for the detection of ipilimumab-induced colitis [44], but in the consideration of the similar mechanism involved, it was also suggested for the anti-PD1 agents [10].

At least in some cases the occurrence of an adverse event with ipilimumab does not necessarily imply the occurrence of the same adverse event with an anti-PD1 agent. A case report described a patient who had experienced grade 3 colitis under ipilimumab and was thereafter treated with pembrolizumab over 20 months without any salient adverse effect [45].

Constipation was experienced by 10.7% of the patients treated with nivolumab in one phase III trial, and in none it was high grade [23].

Nausea occurred in 8.4% of patients in one early phase trial [13] and 16.5% in the phase III study [23], whereas it was grade 3 or 4 in 0.9% of the patient in the former [13] and in none of the patients in the latter trial [23]. It had a frequency of 10.1 and 11.2% in patients treated with pembrolizumab (at two and three week intervals, respectively) [15] and 10% in an early phase trial [24].

Vomiting affected 4.7% of the patients in one early phase nivolumab trial [13] and 6.3% in one phase III study [23] and it was grade 3 or 4 in 0.9% [13] or 0.5% [23]. Vomiting is described as “common” in the summary of product characteristics for both nivolumab [35] and pembrolizumab [36].

Abdominal pain occurred in 7.5% of subjects receiving nivolumab and it was grade 3 or 4 in 1.9% [13]. In pembrolizumab-treated patients abdominal pain occurred with a frequency of 5% in one trial and it was high grade in 1% [24]. Abdominal pain is described as “common” in the summary of product characteristics for both anti-PD1 agents [35,36].

Xerostomia (dry mouth) is described as “common” for both nivolumab [35] and pembrolizumab [36]. In one nivolumab trial it was experienced by 6.5% of the patients and in 0.9% it was grade 3 or 4 [13].

Gastro-intestinal adverse events are generally reversible and are managed by treatment discontinuation and, where needed, by administration of glucocorticoids [12,23] (e.g. prednisolone 1-2 mg/kg, p.o. or i.v.) [10]. For mild forms (grade 1), defined as less than 4 stools above the normal stool frequency per day for a patient, treatment continues to be applied and corticosteroids are not used, but a number of laboratory investigations are recommended: stool testing to detect *Clostridium difficile*, fecal lactoferrin (although its value is limited), ova and parasites, a Blood Count, Metabolic Panel (CMP), and magnesium and phosphorus tests, the latter to allow electrolyte replacement [9]. For mild (grade 1) colitis appropriate diet and antidiarrhoeal medicines have been recommended, such as atropine or diphenoxylate [29], but other opinions are against the use of antidiarrhoeal agents, because of fear that these could mask higher-grade toxicity [9]. In a case of colitis it is essential to intervene early, because deferred reporting, non-adherence to the antidiarrhoeal treatment and not stopping drug administration are associated with increased mortality due to colitis [29]. For moderate forms (grade 2) the ICI agent is put on hold, potential infection or infestation are ruled out by appropriate tests and steroids are used only after stool results become available [9]. Other authors have recommended the initiation of steroids when the patient has more than 6 stools per day or when diarrhoea is accompanied by abdominal pain [21].

Grade 3 diarrhoea has in some cases been controlled by symptomatic treatment [24], but in other patients infliximab (5 mg/kg body weight i.v.) has also been administered [10] (infliximab

is generally recommended if i.v. corticosteroids for three days have been ineffective and a second dose may be repeated after two weeks if the symptoms continue to manifest) [29,46]. Other institutions use infliximab only after 5-7 days of high dose steroids and for selected patients mycophenolate mofetil or tacrolimus may also be employed [9,21]. If diarrhoea worsens or lingers for four days or more, investigations to exclude a pathogenic cause are recommended [29]. Although budesonide was proved to be ineffective in preventing diarrhoea induced by ipilimumab [47], it is not known if the same is true for anti-PD1 or anti-PD-L1 antibodies and it has been stated that in some patients with mild diarrhoea budesonide may provide symptom relief [29].

## HEPATIC AES

These consist most often of increases in the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), not accompanied by symptoms [29]. High grade elevated levels of alanine aminotransferase were seen in 1.0%-1.3% of patients treated with nivolumab [18,23], whereas all grade increases in Alanine Aminotransferase (**ALT**) were reported in 1.5% [23]-4.0% [12]-4.7% [13] of the subjects. In a study of nivolumab, ALT increased in 4.7%, and AST increased in 3.7% of patients [13]. 8% of the patients treated with pembrolizumab had elevated levels of ALT (and 10% of AST) [24], while high-grade elevated levels of alanine aminotransferase were observed in 1% [24]. In the phase III pembrolizumab trial 1.1% and 1.8% of patients were reported to have developed hepatitis, all cases being high grade [15]. In the observational study of L. Hofmann et al. (2016), 2.2% of the patients developed hepatitis with the two anti-PD1 agents and all cases were high grade [10]. In other forms of cancer (hepatocellular carcinoma, renal cell carcinoma) abnormal increases of AST and ALT were seen more frequently than with monotherapy in melanoma, and in hepatocellular melanoma even with monotherapy such increases were seen in about 20% of patients [29]. Regarding increases in ALT, a slight difference was seen in the direct comparison between nivolumab and ipilimumab in the favour of the former, but the differences is not as large as the one seen for diarrhoea or colitis (1.3% for nivolumab, 1.6% for ipilimumab) [18].

In a phase I trial of an anti-PD-L1 antibody increased alanine amino-transferase was seen in only 1% of the patients and in no patient it was grade 3 or 4 [17].

When an ascendant trend is observed for the routine hepatic tests, a careful evaluation to exclude other causes (infectious, non-infectious, malignant) is recommended, as well as a number of additional laboratory tests such as ANA, SMA (smooth muscle antibody), LKM (antiliver-kidney microsome), CBC with differential, CMP, bilirubin (direct and indirect), Gamma-Glutamyl Transferase (GGT) and, depending on the risk assessment, computer tomography and liver biopsy [9]. If hepatotoxicity is suspected, carrying out liver functioning tests every three days is recommended [9].

Hepatic AEs are generally reversible and are managed by treatment discontinuation and where needed, by administration of systemic glucocorticoids [12,23]; in some patients oral mycophenolate mofetil (500 mg or 1.0 g twice daily) has also been added to the corticoids [10,29]. Steroid initiation is recommended when the hepatic enzyme values exceed 5 times the upper limit of normal levels [21]. Because obtaining serological results may involve a certain delay, it has been recommended to use steroids if no apparent cause for the increase in hepatic enzymes has been detected and the health of patient does not preclude it [21]. Influximab is not recommended for the management of hepatitis caused by ICIs because it has its own risk of hepatotoxicity [29], but oral mycophenolate mofetil (500 mg twice daily), with appropriate antibacterial and antiviral prevention may be used if no response is obtained by 3-5 days of systemic steroids [9,46]. Other potentially toxic agents such as paracetamol (acetaminophen), rifampicin, isoniazid, amiodarone or alcohol should also not be used wherever possible [9].

## PANCREATIC AES

Similarly to ipilimumab, treatment with anti-PD1 antibodies is associated with a grade 3 or 4 increase in serum amylase and lipase, which is usually observed in 1-2% of the patients [10,30]. These changes in laboratory values are typically asymptomatic and do not require immunosuppressive therapy, the mere monitoring being usually sufficient [29]. Pancreatitis is uncommon with nivolumab [36] or pembrolizumab [36] and for this reason routine monitoring of amylase and lipase in patients treated with these agents is not recommended, except for the patients that are part of a clinical trial [29]. Asymptomatic but severe (grade 3-4) increases in pancreatic enzymes require treatment discontinuation, at least until normalization [9].

## ENDOCRINE ADVERSE EVENTS

Endocrinopathies are relatively well known for anti-CTLA-4 therapies and have also been recognized in some patients treated with anti-PD1 or anti-PDL-1 therapies; although with these agents they tend to be rarer [29]. Diagnosing endocrine disturbances caused by these therapies is far from being easy, because they may express by generic symptoms such as fatigue or headache [29]. Endocrinopathies have been reported in up to 13% of the patients treated with nivolumab [13]. Because the majority of endocrinopathies may be treated appropriately with hormone replacement, the treatment is not commonly interrupted [29], but on the other hand, endocrinopathies are among the few irAEs caused by anti-PD-1 inhibitors that may be irreversible (and hormone replacement therapy may be needed on a permanent basis) [26].

Hypothyroidism affects about 8.6% of patients treated with nivolumab [35], but in a phase 1 study it occurred in 2% and in less than 1% it was high grade [12], in another it was reported in 5.6% of patients and in 0.9% it was high grade [13]. In patients treated with pembrolizumab the hypothyroidism incidence varied between about 8% [24] and 10.1% [15]. In the phase III trial of pembrolizumab, hypothyroidism was recorded in 3.2% and 6.5% of the patients administered the antibody at three and two weeks, respectively [15]. Increases in Thyroid Stimulating Hormone

**(TSH)** were reported in 3% in a phase 1 nivolumab trial (<1% were high grade) [12] and in 5.6% in a phase III trial (0.9% high grade) [13]. In a small observational study, of 14 patients treated with nivolumab, three developed hypothyroidism, suggesting a higher incidence than previously known [48], but this still has to be interpreted cautiously because the study size is very limited. Anti-PDL-1 antibodies administration is also associated with hypothyroidism (3%), as well as rarer cases of autoimmune thyroiditis (1%) and adrenal insufficiency (1%) [17]. Hypothyroidism is managed with thyroid-replacement therapy [17,24,29]. In most, if not all studies, the majority of endocrine events did not resolve [18]. In one male patient (age 46) hypothyroidism was also accompanied by rhabdomyolysis, which seemed to be related to the thyroid AE, because normalization of thyroid function tests was also associated with normalization of the Creatin Kinase **(CK)** levels [49].

The thyroid dysfunction is often initially asymptomatic, but it evolves to a symptomatic form, needing hormone replacement therapy. In one patient after two additional courses of treatment the thyroid dysfunction was accompanied by myalgia and fever >39.5°C, brusquely deteriorating the quality of life of the patient [48].

The timing of onset for thyroid dysfunctions following administration of anti-PD1/PD-L1 therapies has not been reported in publications thus far [29]. In one case hypothyroidism occurred after the sixth dose (18<sup>th</sup> week of treatment) [49]. Elevated anti-TPO and anti-thyroglobulin antibodies were reported in patients treated with ipilimumab [49], but it is not known if the same is true for the anti-PD-1/PD-L1 agents, although considering the common aspects of the two classes, it has been suggested so [29]. In two patients treated with nivolumab serological aggravation of autoimmune thyroid disease was reported; one patient had Hashimoto disease and the other was likely to have a subclinical form of the disease. The authors concluded that serology should be used to anticipate potential thyroid AEs in patients with subclinical autoimmune thyroid disease [50]. Treatment with the anti-PD1 inhibitor is usually continued, with appropriate hormone replacement (levothyroxine) therapy [9].

Hyperthyroidism cases were also reported in one nivolumab trial in 1% of the patients, and not all were high grade [12]. Such patients are treated with standard antithyroid agents (e.g. carbimazole) [21,29]. If a patient develops thyroiditis, this may be expressed initially by hyperthyroidism, followed later by hypothyroidism; in such a case, the initial phase is treated with beta-blockers (for tachycardia and tremor) and the late phase by thyroid hormone replacement [21,29]. It has been stated that many patients have a subclinical thyroiditis for which no specific therapy is needed [21].

Hypophysitis confirmed by biochemical measurements of prolactin, T4, TSH, LH, FSH, ACTH, cortisol and in certain cases by radiological proof of hypophysis inflammation [29]. Although it has been stated that “the incidence of hypophysitis with single-agent anti-PD-1/PD-L1 mAb therapy ranges from 1% to 6%” [29], this seem to be an overestimation (and the authors making the

claim do not support it with data). Hypophysitis was seen in 0.4% and 0.7% of the patients treated with pembrolizumab, and in two of three cases it was grade 3 or 4 [23]. In a nivolumab study hypophysitis was under 1% [12], in an early phase study it was not mentioned at all (probably not detected) [13], and in the phase III trial comparing nivolumab and ipilimumab, a frequency of 0.6% was seen for hypophysitis in the nivolumab arm (whereas in the ipilimumab it was 3.9% and for the ipilimumab and nivolumab combination it was 7.7%) [18]. According to the Summary of Product Characteristics for nivolumab, hypophysitis is an “uncommon” AE, i.e. it occurs with a frequency higher than 1/1,000 and lower than 1/100 [35]. In AERS no hypophysitis AE has been reported for pembrolizumab so far. Non-clinical data in mice and clinical data in a small number of patients indicated that antipituitary antibodies develop following repeated administration of ipilimumab [51] and it is assumed that a similar mechanism might explain the occurrence of the rare forms of hypophysitis in the case of anti-PD1/PD-L1 therapies [29]. The therapeutic management of hypophysitis involves treatment discontinuation (permanent for grade 3 or 4), systemic corticosteroids (i.v. followed by a switch to oral route after symptom improvement), a formal endocrinology consultation and hormone replacement therapy [9].

Adrenocortical insufficiency is also uncommon (with an incidence less than 1%) and is treated with glucocorticoid replacement therapy, possibly requiring hospitalization [29].

## **PNEUMONITIS**

Pneumonitis (inflammation of the lung parenchyma [29]) has been one of the first AEs to raise concerns regarding the safety of anti-PD1 inhibitors [52]. It has been reported in one nivolumab study in 2% of the patients [13], and in 3% in another [12], high grade pneumonitis occurring in less than 1% of the patients [12,13], whereas in some studies no case of pneumonitis was reported [23]. In pembrolizumab the percentage of patients developing pneumonitis has been estimated to 2% based on pooled data from clinical trials [36]. It seems that in melanoma patient’s pneumonitis occur less frequently than in those with renal cancer and NSCLC [21]. The occurrence of this event seems not to be related to the tumor type, dose or length of exposure. Low-grade pneumonitis seems reversible by treatment interruption and use of corticosteroid, but in about 1% of patients death due to drug-induced pneumonitis was reported [12]. In an early trial of pembrolizumab treatment-related pneumonitis was recorded in 4% of the subjects, and one 96-year patient died after acute bronchopneumonia and pneumothorax developed because of diagnostic procedures (bronchoscopy, biopsy) [24] and in a trial in patients with several forms of cancer three patients (1%) died because of pneumonitis, none with melanoma (two with lung cancer and one with colorectal cancer) [12]. One case was reported where pneumonia occurred after 9 weeks of treatment with nivolumab and a decrease in the number of metastatic lesions was found following improvement of the pneumonia [25]. In the observational study of Hofmann/Zimmer (2016), 1.6% of patients had autoimmune-related pneumonitis, in some with sarcoid-like lesions and one patient later developed lung fibrosis [11]. Evaluation of the first nine cases of

pneumonitis caused by nivolumab did not discover any connection between the manifestation of this AE and the type of tumour, the dose or the total exposure to the anti-PDL1 inhibitor [12,53].

The risk of pneumonitis seems to be similar for anti-CTLA-4 (ipilimumab) and anti-PD1/PD-L1 (pembrolizumab, nivolumab) agents, both for all grade and high grade pneumonitis, as indicated by a meta-analysis of the available data published so far (OR for PD-1 inhibitors versus ipilimumab monotherapy, all grade pneumonitis: 1.26; 95% CI: 0.44-3.63;  $p = 0.66$ ; OR, high grade pneumonitis: 0.71; 95% CI: 0.10-5.08;  $p = 0.74$ ) [54]. The percentage of patients with high grade pneumonitis among those treated with nivolumab seems lower when compared with some chemotherapies or TKIs [53]. Combination of anti-PD-1/PD-L1 antibodies with other pharmacological agents for cancer treatment (chemotherapy, targeted therapies) seems to associate with an increased risk of pneumonitis [29]. There seems to be a wide window in the time between the antibody administration and onset of pneumonitis, in a small series of three patients this varying between 7.4 to 24.3 months. The age range of the three patients was very wide as well, from 38 to 70. Based on the radiologic (CT) imaging, the conditions of the patients were consistent with acute interstitial pneumonia as a form of acute respiratory distress syndrome in two of the patients and to a nonspecific interstitial pneumonia in the third [55].

The diagnosis involves first a CT scan, but in more serious cases (grade 2 or higher) it is recommended to consider consultations by an infectionist and a pneumologist, so as to exclude lung infections or malignant infiltration, as well as to evaluate lung function and perform bronchoscopy [11,29]. Mild (grade 1) pneumonitis may be managed by careful observation (no drug withholding). For higher grade, drug discontinuation and, if needed, administration of immune modulatory agents (mostly systemic steroids) is recommended. The majority of the cases resolve with corticosteroids (high dose prednisolone, 1-2 mg/kg bw, tapering over weeks to months for full resolution), only rarely being necessary the addition of further immune modulatory agents, such as azathioprine, cyclosporine [54], infliximab or mycophenolate mofetil [11,29]. In the observational study of Hofmann/Zimmer et al. (2016), treatment was interrupted only in grade 3 or 4 pneumonitis, but steroids were administered in both grade 2 and grade 1 cases [11]. Steroid tapering is recommended to take place over at least 1.5 months. If the symptoms last after 48 hours, aggravate, or reappear during this period, additional immunosuppressants may be administered [9].

Other lung AEs are also possible: in patients treated with pembrolizumab 8% [24] had cough as an adverse event and 4% [24] dyspnoea, whereas in a nivolumab trial cough was reported in 3% [12] -3.7% [13] and dyspnoea in 2% [12] of the patients. Although it has been stated that cough and dyspnoea have been recorded in 18-38% of patients [11], these high percentages refer to all events irrespective of causality, while the anti-PD-1-related AEs have been in clinical trials under 10%. In the light of the life-threatening or fatal cases of pneumonitis, such respiratory symptoms (cough, dyspnoea) have to be followed carefully [11].



## MUSCULO-SKELETAL EVENTS

If the first clinical trial with nivolumab reported an incidence of 15.4% of musculoskeletal events [28], in a moderate-size observational study 4.23% of patients developed musculo-skeletal adverse events and 5 of them (1, 00%) were grade 3 or 4 [11].

Arthralgia has been reported with frequencies of 6.5 [13], 7.7% [18] and 11% [35] in patients treated with nivolumab and with frequencies of 7% [14], 9.4 and 11.6% [15] and 12% [24,36] in patients treated with pembrolizumab. In patients treated with an anti-PD-L1 antibody (BMS-936559) the frequency of arthralgia was 7% [17]. In a small number of patients (0%-0.4%) arthralgia was high grade (>3) [15,23]. In the observational study of Zimmer, et al. (2016) 1.6% of patients experienced arthralgia [11]. In AERS, the number of arthralgia cases reported for pembrolizumab was the same as those for diarrhoea (n=6, 3.44% of all AEs reported for pembrolizumab). In a patient developing both uveitis and arthralgia with nivolumab, the grade 2 uveitis disappeared following intraocular steroid administration, but semi-rigid swan neck deformities developed gradually in the two hands (Jaccoud's arthropathy), indicating that in some patients such changes may be irreversible [11,56]. Arthralgia (as myalgia) do not seem to be dose-dependent, as in a clinical trial the frequency of myalgia was slightly higher with the dose of 2 mg/kg (7%) than 10 mg/kg (6%) [14]. In the first clinical trial with nivolumab two patients developing polyarticular arthropaties had predisposing factors: one a history of Lyme arthritis and polymyalgia rheumatic, the second a titer of the Antinuclear Antibody (**ANA**) > 1: 1000 [28]. Two other cases have been reported with synovitis and tenosynovitis (as well as bone marrow edema and myositis in the first), but no obvious predisposing factor was identified, and in the second patient C - reactive protein (**CRP**) and Erythrocyte Sedimentation Rate (**ESR**) had normal values, unlike the first one [57].

Several mechanisms have been postulated to explain the occurrence of arthritis cases in patients treated with anti-PD1 therapies: (a) arthritogenic T-cell clones previously inactive, may expand and become noticeable under the ICI agent; (b) development of arthritogenic T-cell clones leading to ensuing arthritis, because under the influence of anti-PD1 agents autoantigens are newly presented to the helper T cells without appropriate suppression or regulation for tolerance and; (c) a direct effect of the antibody on the synovial tissue resulting in metabolic changes, inflammation and consequent fibrosis [57].

The incidence of myalgia in clinical trials has been 3.7% (0% grade 3-4) [13] for nivolumab, and 2.2 and 6.8% (0.4% grade 3-5) [15], 4% (1% grade 3) [14], 12% (0% grade 3-5) [24] for pembrolizumab. The official prescribing information does not use the term "myalgia", but the more comprehensive phrase "musculoskeletal pain", defined (for nivolumab and slightly differently but similar for pembrolizumab) as "a composite term which includes back pain, bone pain, musculoskeletal chest pain, musculoskeletal discomfort, myalgia, neck pain, pain in extremity, and spinal pain" and described as common (i.e. with a frequency lower than 10% but higher than 1%). Myositis, described as "myalgia, myopathy, polymyalgia rheumatica and rhabdomyolysis"

in the official prescribing information for pembrolizumab is also common. An isolated case in an elderly patient (84 years) treated with atorvastatin for 10 years suggests that in association with statins there is an increased risk of myositis; in this case, it led to dyspnoea manifested when speaking, resolved in a few weeks after treatment discontinuation (but not helped by 5-day treatment with prednisolone) [58]. In an observational study 1.4% of patients were affected by myositis/myalgia, and this limited their ability to walk, as well as having a large impact on their quality of life [11]. It has been suggested that at least in some patient's rhabdomyolysis might be a consequence of hypothyroidism [49].

In the observational study of Zimmer, et al. (2016) 0.4% of the patients (two each) were affected by polymyalgia rheumatica and muscle spasms or muscle weakness, one patient (0.2%) suffered from wrist synovitis and in one psoriasis arthritis was exacerbated [11].

Symptomatic treatment with NSAIDs is considered most often sufficient for musculo-skeletal AEs, although in some cases low doses of corticosteroids administered on a long term may be needed [11,21]. Myositis of various degrees of severity has been reported to respond well and rapidly to systemic corticosteroids [11].

## RENAL ADVERSE EVENTS

There are suspicions that treatment with PD-1 inhibitors, both as mono-therapy [24] or in combination with anti-CTLA-4 [59] may trigger renal adverse events such as acute interstitial nephritis [29,60]. In one early phase trial, two cases of grade 3 renal failure were recorded, both considered potentially immune-mediated; the renal function of the patients ameliorated with treatment discontinuation and addition of glucocorticoid therapy [24]. In one study, 2% of patients treated with pembrolizumab had renal failure and in 1% this was judged to be a high grade AE [24]. Most patients with renal failure induced by anti-PD-1 therapies are asymptomatic, with progressive increases in creatinine values. Administration of corticosteroids usually leads to improvements in creatinine levels [29]. A female patient (58 years) was recently reported to have developed acute renal failure and hyponatremia starting four days after the first dose of nivolumab and culminating at 9 days post-infusion. This indicates that although usually renal dysfunction develops after at least one month if not several, very early onset cases are also possible. In this case a rapid response to prednisone was seen, with improvement of the renal function within 24 hours, to progress to grade 1 nephritis after three days [61].

## CARDIOVASCULAR EVENTS

This group includes flushing and hypotension, which were reported with an equal frequency of 4.7% in the case of nivolumab [13]. Other cardiac AEs associated with nivolumab or pembrolizumab seem to be uncommon or rare. Tachycardia is reported by the product information for nivolumab as an uncommon AE (frequency lower than 1%, higher than 0.1%) [35], but no such AE is stated by the pembrolizumab product information [36]. A case of acute heart failure caused by immune-mediated myocarditis, manifested by progressive dyspnea, was reported in a 73-year old female

patient after five cycles of pembrolizumab. The patient had been free of any heart diseases or cardiac symptoms [62]. This finding, although rare, is consistent with non-clinical data in mice deficient for PD1 (Pdcd1-/-), who die of autoimmune dilated cardiomyopathy, because of auto antibodies developed against cardiac troponin I [62,63]. In an observational study in patients treated with nivolumab or pembrolizumab, five patients (1%) were affected by eight cardiac AEs, five high grades (one grade 5, ventricular arrhythmia) [11]. One patient treated with pembrolizumab developed atrial flutter and heart failure with reduced ejection fraction and died because of ventricular arrhythmia, despite discontinuation of pembrolizumab and treatment with prednisolone; the autopsy revealed myocarditis with cardiomyopathy [11]. One patient developed sinus tachycardia resolved with oral metoprolol, another developed hypertension and a third one angina pectoris, all under treatment with pembrolizumab [11]. Angina pectoris in the latter patient resolved only by treatment discontinuation. One patient treated with nivolumab had sudden asystolia and required resuscitation [11].

## NEUROLOGICAL AES

A very small number of neurological AEs have been reported for anti-PD-1 agents: demyelination, Guillain-Barré syndrome, neuropathy, seizures, paresis, cerebral oedema, dysarthria, dysgeusia, hypogeusia, insomnia and hypersomnia, lethargy, memory disturbances, vertigo, restless leg syndrome, and tremor [11].

A case of subacute multifocal Central Nervous System (**CNS**) demyelination was reported in a patient treated initially with ipilimumab and later with nivolumab, whose course was fatal after 4 months, despite nivolumab discontinuation and administration of immunosuppressants [64]. Rare cases (less than 1 in 1000) of Guillain-Barré Syndrome (**GBS**) were detected under treatment with pembrolizumab [36] or nivolumab [35]; in the case of the combination nivolumab-ipilimumab the frequency is higher than 1 in 1000 but lower than 1 in 100 [35]. In the observational study of L. Zimmer et al, the frequency of the Guillan-Barré syndrome was 0.2% [11]. Besides treatment discontinuation, immunoglobulins and prednisolone have been attempted for GBS induced by nivolumab; this led to symptom disappearance, but with sequelae [11].

Low grade (1 or 2) peripheral neuropathy and bilateral optic neuritis have been reported in patients treated with nivolumab [11,65,66]. One 66-year-old male patient developed partial motor convulsive status under treatment with nivolumab, although the dose was low (2 mg/kg) and administered at 3 week-intervals. These symptoms and corresponding radiologic findings disappeared slowly when the antibody was discontinued [67]. In an observational study an incidence of 0.4% was reported for seizures, which were successfully treated with oral levetiracetam (500 mg twice daily) or lorazepam [11].

Paraesthesia, paresis/paralysis, and polyneuropathy were observed in 0.6% of patients treated with nivolumab [11]. In this same study aphasia, a parkinsonoid syndrome associated with bradykinesia, and (meningo)-radiculitis were seen in 0.2% of patients [11]. Attempts to treat

paraesthesia or polyneuropathy with systemic corticosteroids, pregabalin (oral) or magnesium, did not lead to symptomatic improvement, and they have been reported to remain untreated. Two of three patients with paresis had their symptoms resolved after treatment discontinuation and steroid administration for 4 weeks, but in a third patient this approach, as well as the use of pyridostigmine, was not successful [11]. The symptoms of (meningo-) radiculitis were ameliorated by oral administration of dexamethasone (4 mg four times daily) [11].

In patients using combinations of anti-PD-1/PD-L1 therapies and ipilimumab or tremelimumab, isolated cases of myasthenia gravis have been detected [29]. Although more evidence is available indicating the ipilimumab is the main contributing factor, more recently isolated case of myasthenia gravis associated with nivolumab monotherapy have been reported, with dyspnoea as the earliest symptom (one in a melanoma patient [68], the other in a lung cancer patient [69]). Other two case reports showed a worsening of pre-existing myasthenia gravis under nivolumab [70] or pembrolizumab [71] treatment. In both cases, an increase in the acetylcholine receptor antibody titer was observed [68,70], but in a different patient no increase in the levels of these antibodies was detected [11]. Re-introduction of anti-PD-1 therapy has been recommended with careful observation when the myasthenia gravis symptoms have improved to a mild level, such as NCI-CTCAE Grade 1 [70]. Otherwise, for the management of drug-induced myasthenia gravis in this case administration of corticosteroids, intravenous immunoglobulin or plasmapheresis has been advised, as well as neurologic consultation [29]. In general the same measures are applied for most neurological AEs; however, except for grade 1 AEs, the administration of the monoclonal antibody should be interrupted until the nature of the event is figured out and prompt consultation of a neurologist is recommended [21, 29].

A case of eosinophilic fasciitis and acute encephalopathy was reported in a patient one month after the last administration of pembrolizumab (18 months from the initiation of treatment), with acute confusion and weakness, believed to be caused by intracranial vasculitis. Fasciitis was treated with high-dose steroids and for the supposed vasculitis aspirin was administered, leading to the disappearance of the neurologic symptoms [72].

## OCULAR TOXICITY

This is typically expressed by uveitis (covering iritis and iridocyclitis) [36], which has been reported in patients treated with both anti-PD-1/PD-L1 monotherapy or combination of such agents and anti-CTLA-4 antibodies [29,35, 36], but the frequency is higher with the combination: whereas as monotherapy uveitis is possible but uncommon (frequency lower than 1%), in combination with ipilimumab uveitis and blurred vision are common (frequency lower than 10% but higher than 1%) [35]. A case was reported in a 63-year-old female, who developed severe uveitis and hypotony following pembrolizumab treatment, which needed prolonged treatment with corticosteroids; in less than three months, this patient also developed advanced bilateral posterior subcapsular cataracts. Despite improvement of uveitis with oral steroids and of cystoid

macular edema with intravitreal steroids, hypotony persisted and led to reduced visual acuity [73]. In an 82-year-old patient, bilateral severe anterior uveitis and papillitis developed after two months of treatment with pembrolizumab administered every three weeks. Topical steroid administration and discontinuation of treatment led to rapid and complete recovery, but uveitis occurred again when treatment with pemrbolizumab was resumed [74]. For the management of uveitis topical corticosteroids (eye drops) are usually applied and consulting an ophthalmologist is recommended. For high grade uveitis oral corticosteroids should be considered [21,29]. Dry eye is also a common AE observed with both pembrolizumab and nivolumab. The prescribing information for nivolumab mentions blurred vision as a common AE, whereas for pembrolizumab no such AE is mentioned [35,36]. In a medium-sized observational study 1.6% of patients had ocular side effects: blurred vision, conjunctivitis, iritis, uveitis, and dry eyes [11].

## **EAR TOXICITY**

A first case of autoimmune inner ear disease was recently reported in an 82 year old male treated initially with ipilimumab and after the failure of this, with pembrolizumab. After two doses of the latter, the patient experienced bilateral hearing loss, which improved after intratympanic dexamethasone injections. The authors reporting the case speculated that this was caused by a cross-reactive autoimmune response of the T-cells of the patient to inner ear melanocytes [75].

## **METABOLISM AND NUTRITION-RELATED AES**

Decreased appetite was reported in one study with pembrolizumab in 4%-6.5% of the patients and in about 1% it was high grade [13,24]. This adverse event was described in up to 15% of nivolumab patients [35]. Of the 174 AEs received by FDA by spontaneous reporting for pembrolizumab only 2 (1.14% of all reports for this agent) refer to decreased appetite. Weight decrease is common (i.e. more than 1%, less than 10%) with nivolumab [35], in one study it was reported in 5.6% of patients [13], but no such adverse reaction is mentioned by the official prescribing information for pembrolizumab [36]. Hyperuricemia was experienced by 3.7% of patients and hypophosphatemia by 3.7% of patients treated with nivolumab in one study [13].

## **BLOOD AND LYMPHATIC SYSTEM DISORDERS**

6.5% of patients had lymphopenia in one early phase study with nivolumab [13], but in the phase III trial comparing nivolumab and dacarbazine, neutropenia and thrombocytopenia were reported in the dacarbazine arm, but no such case occurred in the nivolumab arm [13]. In an observational study three patients (0.6%) experienced changes in blood count, of whom two had anaemia and one grade 3 leucopenia [11]. In what was probably the first clinical trial with nivolumab in a variety of tumours, the most frequent AE was a decrease in CD4+ lymphocyte counts (35.9%) and lymphopenia (25.6%) [28], but later clinical trials have not reported such high percentages of lymphopenia. In one study WBC was reduced in 3.7% of patients, in 4.7% haemoglobin and platelet count decreased [13], but high grade anaemia was seen in less than 1%

of the patients treated with nivolumab in another study [30]. Anaemia is described as “common” for pembrolizumab [36] and “very common” for nivolumab [35]. In the spontaneous reporting system of AERS, anemia has been more frequently reported for pembrolizumab than other forms of cytopenia (4 cases of anemia, one of thrombocytopenia and one of pancytopenia).

## INFUSION-RELATED REACTIONS

These were seen in 5.6% of the subjects in one nivolumab trial [13], in 9.0% in another [12] and in 10% in an anti-PD-L1 antibody phase I trial [17]. They are described as “common” in the prescribing information for both nivolumab [35] and pembrolizumab [36]. In cases of high grade infusion reactions, therapeutic management may involve reducing the infusion rate and administration of antihistamines and i.v. corticosteroids, as suggested by the ipilimumab experience [17,29].

## OTHER SAFETY ASPECTS OF PD-1/PD-L1 INHIBITORS

Administering BRAF antagonists after PD-1 inhibitors may associate with a higher risk of severe cutaneous and systemic toxicity, with extensive morbiliform eruptions, hypotension, multiorgan injury and fever, possibly by a priming of the immune system by the PD-1 inhibitors. In one patient ongoing administration of vemurafenib led to severe neurologic and systemic toxicities (including acute inflammatory demyelinating polyneuropathy) and in another to anaphylaxis [76]. In three patients treated sequentially with ipilimumab (3 mg/kg) after nivolumab (3 mg/kg) or pembrolizumab (10 mg/kg) unusually severe irAE were reported, with very early onset and pleiomorphic expression [77].

Using steroids or other immune modulating therapies on a long-term basis for the management of anti-PD1 toxicities may bring their own adverse effects, both short-term and long term. The former include opportunistic infections, sleep disturbances (insomnia), mood swings, irritability, gastritis, diabetes and hypertension; the latter include osteoporosis [21]. No evidence-based guidelines are available, but prevention of *Pneumocystis jirovecii* pneumonia has been advised in those patients receiving more than 25 mg prednisolone per day for longer than 4 weeks, as well as intermittent glycaemia monitoring for diabetes and appropriate agents to prevent or diminish gastric adverse effects (heartburn) [21]. If steroid treatment is used for more than 3 months, vitamin D supplements, calcium, bisphosphonates and education for minimizing the risk of osteoporosis are necessary [21,78].

## CONCLUSION

Although anti-PD1/PD-L1 inhibitors have a more favorable safety profile when compared with CTLA-4 inhibitors, they are not devoid of adverse events, which may occur long after treatment initiation, are most often immune-related and in some cases (especially for dermatological AEs) are associated with therapeutic response. Therapeutic management of AEs caused by these agents involves treatment interruption or discontinuation (except for mild forms) and immune

modulating agents, especially systemic high dose or where relevant, topical steroids, infliximab and mycophenolate mofetil. Large knowledge gaps continue to surround the mechanisms and biological signalling pathways underlying many of these AEs. Discovering practical means to identify the patients susceptible to such AEs (e.g. risk factors, biomarkers, SNPs or genotypes etc) as well as predictors of response (allowing to avoid exposing patients unnecessarily to potential adverse events) should remain a priority for the future research in the field.

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