Review

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Melanoma Management

Immunotherapy in metastatic melanoma: a novel scenario of new toxicities and their management

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Checkpoint inhibitors can cause an imbalance in immune tolerance that may clinically manifest as immune-related adverse events (irAEs). These events may involve many organs and tissues, including the skin, gastrointestinal (GI) tract, liver, endocrine system, kidneys, central nervous system (CNS), eyes and lungs. The incidence of irAEs appears to be lower with anti-programmed death antigen-1/programmed death antigen-ligand-1 agents than with the anti-cytotoxic T-lymphocyte-associated protein-4 antibody ipilimumab. Combined immunotherapy does not appear to be associated with novel safety signals compared with monotherapy, but more organs may be involved. Increased experience and the use of algorithms for the most common irAEs have resulted in severe toxicity and related deaths being reduced. However, continuous vigilance, especially regarding less common events, is needed to better characterize the wide spectrum of clinical manifestations.

First draft submitted: 14 February 2019; Accepted for publication: 18 July 2019; Published online: 8 November 2019

Keywords: anti-CTLA-4 • anti-PD1 • checkpoint inhibitors • combination therapy • immune-related adverse events

Immune checkpoints represent a significant target in the treatment of metastatic melanoma. They have a crucial role in the maintenance of self-tolerance. Normally, they control the immune system through coinhibitory and costimulatory signals. Coinhibitory checkpoint molecules include CTLA-4, PD-1, LAG-3 and Tim-3, while CD137, OX40 and CD40 are examples of costimulatory molecules.

Checkpoint inhibitors allow the potentiation of T-cell specific immune responses against tumor cells. However, due to their mechanism of action, they can potentially cause an imbalance in immune tolerance that translates into uncontrolled immune reactions and may clinically manifest as autoimmune-like adverse events (AEs), called immune-related adverse events (irAEs) [1]. Such events may involve many organs and tissues, including the skin, gastroinestinal (GI) tract, liver, endocrine system and lungs. They are mainly caused by T cells, but also by antibodies secreted by B cells and cytokines produced by granulocytes [2–5].

These specific toxicities were first observed with the anti-CTLA-4 antibodies, ipilimumab and tremelimumab. Clinical studies with these agents as monotherapy showed a significant occurrence of dose-dependent grade 3–4 irAEs, ranging from 10 to 52% [6–9]. More recently, the anti-PD-1 agents nivolumab and pembrolizumab have shown greater clinical benefit compared with ipilimumab in two Phase III trials, Checkmate 066 and Keynote 006, respectively [10,11]. Both anti-PD1 antibodies have shown similar results, in terms of survival and toxicities, in patients with metastatic melanoma [10,11]. The incidence of grade 3–4 AEs with these drugs given as monotherapy is lower than with ipilimumab (7–12% vs 20%, respectively) [11,12]. Other checkpoint inhibitors being investigated in melanoma are directed against the main PD-1 ligand, PD-L1 [13–15], and include atezolizumab (MPDL3280A), durvalumab (MEDI4736) and avelumab.

Even in melanoma, the majority of patients still do not respond to checkpoint inhibitors. For this reason, trials of different immunotherapy combinations are being assessed. In particular, the combination of nivolumab and ipilimumab is very promising in metastastic melanoma, overcoming both anti-PD-1 and anti-CTLA-4 monotherapy

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in terms of progression-free survival (PFS) and response rate [13]. However, combination therapies have typically shown an increased incidence of toxicities, in some cases involving multiple organs at the same time.

AEs arising from checkpoint inhibitor monotherapy

Organ-specific irAEs have been described in clinical trials with ipilimumab and anti-PD-1/PD-L1 antibodies and mainly consist of dermatological, gastrointestinal (diarrhea, colitis), liver, endocrine (thyroid dysfunction, hypophysitis, adrenal crisis), renal, ocular and pulmonary toxicities.

Skin toxicity

Dermatological toxicities have been described in 44% of patients treated with ipilimumab [11], 34% of patients who received nivolumab [10–12] and 39% of patients who received pembrolizumab [11]. They include rash, pruritus and vitiligo. Rash can typically manifest as macupapular, but other types, including papulopustular, follicular, urticarial dermatitis and Sweet's syndrome, have also been described. The macupapular variant is the most common, and usually appears early on, after 3–6 weeks of treatment. Less common rashes of special interest have been observed in patients receiving anti-PD-1/PD-L1 therapy, such as lichenoid dermatitis [14], bullous pemphigoid [15], Steven Johnson syndrome and toxic epidermal necrolysis [16]. These events have been attributed to the blockade of coexpressed tumor antigens on the cells and may appear on different skin levels [17]. Pruritus, with or without rash, is also a very common event. It has been observed in nearly a quarter of patients treated with ipilimumab and in 10% of patients treated with anti-PD-1 agents [16]. Vitiligo is a dermatological side effect that is usually related to a good response to treatment, especially in melanoma patients treated with ipilimumab and anti-PD-1 drugs. It is characterized by immune-mediated destruction of melanocytes [18], and its etiology remains poorly understood. Skin-resident microbes have been recently demonstrated to be implicated in immune processes of the skin, including those that are associated with vitiligo progression. Identification of skin-resident microbes may be helpful for the development of immunotherapies to augment antimelanoma immune responses [19]. In melanoma patients, vitiligo is more frequent during treatment with pembrolizumab than with ipilimumab, occurring in nearly 10 versus 2% of patients [11]. Hua *et al.* reported prospective data on 67 patients with metastatic melanoma who received pembrolizumab treatment in a Phase I trial. Seventeen patients (25%) developed vitiligo during pembrolizumab treatment. Clinical benefit was demonstrated in patients who developed vitiligo, with these patients having a significantly higher objective response rate (partial or complete) compared with the 50 patients without vitiligo (71 vs 28%; $p = 0.002$). Of the 17 patients with vitiligo, three (18%) had a complete response, nine (53%) had a partial response, three (18%) had stable disease and two (12%) had progressive disease. All patients with vitiligo were alive at the time of analysis, with a median follow-up of 441 days [20].

Gastrointestinal toxicity

The most frequent gastrointestinal events associated with checkpoint inhibitor treatment are diarrhea and colitis [6–8]. The incidence of grade 3–4 diarrhea and colitis is nearly 5% with ipilimumab and 1–3% with anti-PD-1/PD-L1 antibodies, with a median time of onset of 6–8 weeks [3,6,7,11,12,21,22]. Diarrhea is caused by infiltration of the intestinal mucosa by immune cells. Colitis is a severe consequence of diarrhea and cases of bowel perforation and deaths due to colitis have been described in the initial studies with ipilimumab. However, no cases of bowel perforation have been described with anti-PD-1/PD-L1 therapy [10–12].

Hepatic toxicity

Hepatic toxicity has been described in nearly 10% of patients treated with ipilimumab [6–9] and in 5% or less in those treated with anti-PD-1/PD-L1 agents [10–12]. Median time of onset is 8–12 weeks with ipilimumab and 89 days (range 13–140 days) with anti-PD-1/PD-L1 treatment [11,12,23]. Frequently, liver toxicity occurs with asymptomatic increases in AST and ALT. Histopathologic alterations, such as panlobular hepatitis, biliary ducts or perivenular infiltrates, have also been observed [21,23].

Endocrinopathies

Endocrine toxicities may include hypothyroidism, hyperthyroidism, thyroiditis, hypophysitis and adrenal insufficiency. These events usually appear 6 weeks or later from the start of treatment. They may take a long time to resolve and in most cases are irreversible [22,24]. Diagnosis may be challenging since they often manifest with generic symptoms such as headache or fatigue and laboratory test alterations can be necessary to confirm diagnosis. Some

events, such as hypophysitis, are also associated with a radiological finding of gland inflammation. According to a recent review summarizing large cohorts of malignant melanoma patients, ipilimumab was associated with an increased incidence of hypophysitis of approximately 10–15% [25]. This increase may be partly due to improvements in clinical recognition. Hypophysitis due to ipilimumab differs from the idiopathic autoimmune hypophysitis, as it is not characterized by optic chiasm compression [25,26] and visual alterations [25,26] and it is more frequent in males and older patients [27]. Two cases of diabetes insipidus have been reported during ipilimumab treatment [27,28].

The mechanisms of hypophysitis are not fully understood but may be mediated by complement activation subsequent to humoral immunity against the pituitary gland [29]. During hypophysitis, hormones released by the pituitary gland (i.e., adrenocorticotropic hormone [ACTH], thyroid-stimulating hormone [TSH], follicle-stimulating hormone, luteinizing hormone, growth hormone, prolactin) may be reduced. Suspected hypophysitis is usually associated with headache and fatigue. Enhancement and enlargement of the pituitary and biochemical evidence of pituitary dysfunction (low ACTH and TSH) may also occur [25,26].

In contrast, the incidence of anti-PD-1/PD-L1-induced hypophysitis is markedly lower (<1%) [30]. This may be attributed to functional differences in the processes of T-cell activation and the ectopic expression of CTLA-4 in the human pituitary gland that may be targeted by an anti-CTLA-4 antibody [29,30].

Thyroid dysfunction is more commonly due to the release of antibodies (antithyroglobulin, antithyroid peroxidase), even though they are not always found in patient's serum [27,30]. Shang *et al.* [30] investigated the incidence of endocrine events in patients treated with anti-PD-1 monotherapy and showed a significant increase of all grades of hypothyroidism (relative risk: 6.38; 95% CI: 3.78–10.77; $p < 0.001$) and hyperthyroidism (relative risk: 5.08; 95% CI: 2.55–10.14; $p < 0.001$) and a lower incidence of hypophysitis.

Pneumonitis

Pneumonitis has not been described in studies of ipilimumab monotherapy, although pulmonary events such as sarcoid-like reactions and obstructive pneumonia were observed [28]. The incidence of pneumonitis with anti-PD-1/PD-L1 drugs is about 0–10.6% for all grades and 0–4.3% for grade 3–4 events. Pneumonitis has been described more frequently in patients with lung or renal cancer than in melanoma patients (4.1 vs 1.6%; $p = 0.002$ and 1.8 vs 0.2%; $p < 0.001$) [31]. This toxicity resulted in five deaths in four trials; four patients with non-small-cell lung cancer (NSCLC) treated with monotherapy and one patient with melanoma treated with a combination of nivolumab and ipilimumab [31]. For this reason, pneumonitis has been identified as a serious and potentially life-threatening irAE of special interest. The different incidence of pneumonitis among tumor types, in particular the higher incidence in patients with NSCLC, has been attributed to the chronic exposure of pulmonary mucosa to different factors, such as smoke, obstructive pulmonary disease or fibrosis [32,33]. However, reasons for the higher incidence observed in patients with renal cancer remain unclear.

Renal toxicity

Cortazar *et al.* reported an overall incidence of acute renal injury of 2.2% among 3695 patients treated with immunotherapies in published Phase II and III trials [34]. The incidence of grade 3–4 AEs or need for dialysis was 0.6%. The incidence of renal toxicity was 2.0% with ipilimumab, 1.9% with nivolumab and 1.4% with pembrolizumab. However, the incidence of renal toxicity in clinical practice may be higher, with a recently reported rate of 13.9% [35]. Cases of renal dysfunction attributed to ipilimumab have been associated with electrolyte alterations [36,37]. Pathologically, renal toxicity may manifest as interstitial nephritis and, in some patients, may show pathologic characteristics similar to lupus or granulomatous nephritis [38–40]. In recent Phase I [41] and II trials [42] of pembrolizumab, nephritis was reported in 6.7% of patients [41]. In the KEYNOTE-1 trial, acute renal injury was reported in two out of 495 patients and hyperkalemia in four patients [41]. In a trial in patients with NSCLC, 1.7% of patients treated with pembrolizumab reported an increase of creatinine levels, while no alterations were described in patients who received chemotherapy [43]. Recently, four cases of biopsy-proven acute interstitial nephritis were described nearly 12 months after the start of pembrolizumab [44]. Three patients responded to steroids with complete remission and one patient required dialysis. Duration of steroid use was about 1–3 months. Renal toxicity attributed to nivolumab has become more frequent than described in early trials [45]. In fact, in recent clinical trials in NSCLC, the incidence reported was 3%, with cases of acute interstitial nephritis occurring later after the start of therapy, as seen with pembrolizumab [46].

Neurological toxicity

Neurological irAEs are rare but reports of severe syndromes have been described, such as immune polyneuropathies, Guillain-Barré syndrome, myasthenia gravis, posterior reversible encephalopathy syndrome (PRES), aseptic meningitis, enteric neuropathy, transverse myelitis and immune encephalitis.

The incidence of serious neurological toxicity is less than 1% in patients receiving ipilimumab 3 or 10 mg/kg [47]. The most common toxicities associated with ipilimumab are transient peripheral neuropathies, both sensory and motor, while more rare cases of Guillain-Barré-type syndrome, myasthenia gravis-type syndrome, aseptic meningitis, transverse myelitis and enteric neuropathy with severe constipation have also been reported [48]. Furthermore, two cases of encephalopathy during ipilimumab 10 mg/kg treatment have been reported, one of which was mild and was characterized by a reversible splenial lesion of corpus callosus (MERS) associated with neurogenic bladder that could be seen on brain MRI and typically resolved within 1–4 weeks [49]. The other event was PRES occurring in the setting of hypertension and acute renal failure, two possible other common causes of PRES [50]. PRES is similar to MERS, but with preferential involvement of the parietal and occipital lobes [50].

Anti-PD-1/PD-L1 agents may also cause neurological syndromes, usually presenting as specific symptoms and frequently involving peripheral nerves. They are described in less than 1% of patients treated with monotherapy [51]. Rarer and severe syndromes have also been described, such as eosinophilic fasciitis and acute encephalopathy reported in a patient with melanoma treated with pembrolizumab [52].

Ocular toxicity

Ocular toxicity has been described with ipilimumab and anti-PD-1 antibodies (1.3 and 1.6% of patients, respectively), frequently manifesting as uveitis and dry eyes. A systematic review on ocular toxicity during checkpoint inhibitor therapy reported a high frequency of ocular toxicity (odds ratio: 3.40; 95% CI: 1.32–8.71; p = 0.01) for all grade irAEs during treatment with ipilimumab, nivolumab or pembrolizumab across 11 trials involving 4965 patients with different solid tumors [53–55].

Cardiotoxicity & myositis

Other irAEs that are emerging with the use of anti-PD-1 inhibitors involve muscle tissue, in particular myositis and myocarditis. Clinical cases have been described and must be considered of clinical relevance, especially because some manifested as acute and fulminant events after one or two doses, while others are more insidious and occur later during therapy. If clinicians do not consider the possibility of such AEs, the consequences for patients can be severe and even fatal. Any muscle tissue may potentially be involved, although more often involvement of respiratory, cardiac and limb muscles has been described [56]. Myositis during nivolumab treatment has been reported in a patient concurrently taking atorvastatin [57]. Statins are known to cause myotoxicity that is likely specific to the HMG-CoA reductase pathway [57]. Also, pembrolizumab was associated with a case of rhabdomyolysis in a clinical trial [58]. In all cases, significant increases in creatine-phosphokinase were observed.

Cardiotoxicity associated with immune-checkpoint inhibitors may have different clinical manifestations, such as myocarditis, arrhythmia and pericardial disease. All are characterized by a typical T-cell infiltration at histopathologic examination [59,60].

The incidence of myocarditis has been estimated as approximately 0.09% and as being five-times higher in patients treated with concurrent anti-PD-1 and anti-CTLA-4 inhibitors. Cardiac biomarkers such as troponin and creatine kinase-MB are usually increased and abnormalities on electrocardiogram and echocardiography are frequent but not always present [61]. Additional serologic tests that may be of help for the diagnosis of cardiotoxicity include brain natriuretic peptide (BNP) and N-terminal pro-BNP (NT-proBNP) [62]. Cardiac magnetic resonance can also offer more accurate quantification of left ventricular ejection fraction and can assess the presence of myocardial inflammation [63]. The gold standard for diagnosis of myocarditis is endomyocardial biopsy using Dallas criteria [64]. Myocarditis is usually responsive to high doses of steroids [61–63] but in refractory cases, other immunosuppressive agents must be considered.

Arrhythmia usually represents a major complication and can manifest as atrial fibrillation, supraventricular/ventricular arrhythmias, conduction delays and, in some cases, can lead to complete heart block [65]. The timing of onset has not been well defined, but electrical alterations are reported in concert with suspected myocarditis [65]. Pericarditis, pericardial effusion and cardiac tamponade have been described later in the course of the treatment, from 10 to 24 weeks after the start of therapy [62,66].

Pancreatic toxicity & diabetes

Pancreatic toxicity has been most often described as asymptomatic elevations of lipase and amylase and more rarely as symptomatic pancreatitis [45].

Type 1 diabetes has been described during monotherapy with anti-PD-1/PD-L1 drugs and with combined ipilimumab and nivolumab [66,67]. It is difficult to estimate the true incidence of this phenomenon, as different factors may be implicated, such as genetic predisposition and previous history of hyperglycemia or Type 2 diabetes. Some patients developed acute severe hyperglycemia with ketoacidosis or low/undetectable C-peptide levels that may be considered as a new and insulin-deficient type of diabetes [66]. Furthermore, for some patients, a humoral (anti-GAD65 antibodies) and cellular autoimmunity (diabetes antigen-specific CD81 T cells, with majority of CCR72 or CD45RO1 effector or memory cells) has been demonstrated, but for some others, autoimmunity was not clearly shown [66–68]. These AEs may appear at different times, usually from 1 week to 5 months after the start of the treatment [69]. The duration of this event has not yet been defined.

Hematological toxicity

Hematological AEs, including leucopenia, anemia and thrombocytopenia, have been reported during treatment with ipilimumab [70] and anti-PD1 antibodies [71–74]. In particular, autoimmune hemolytic anemia has been reported during treatment with nivolumab 3 mg/kg, both early on and later after the start of therapy [71–73]. Most reports were responsive to corticosteroids, but two had a fatal course [74,75]. One of these patients was treated with combined nivolumab and ipilimumab followed by nivolumab according to the CheckMate 401 study protocol [74]. In addition, a case of autoimmune anemia and pure red cell aplasia has been reported in a patient with mucosal melanoma treated with pembrolizumab [75].

Arthritis & arthralgia

Inflammatory arthritis and arthralgia during immune checkpoint inhibitor treatment have also been reported [76,77]. Cappelli *et al.* collected data from the literature, showing that immune checkpoint inhibitor-induced arthralgia is the more frequent, occurring in 1–43% of patients, while arthritis was reported in 1–7% of patients. Interestingly, patients with checkpoint inhibitor-induced inflammatory arthritis were seronegative for traditional antibodies associated with rheumatoid arthritis (rheumatoid factor or complement). These patients usually require high doses of steroids and some may need additional immunosuppression with methotrexate or TNF inhibitors [77]. Also, the anti-IL-6 receptor antibody, tocilizumab, that has been approved by the US FDA for the treatment of rheumatoid arthritis and juvenile idiopathic arthritis, has been used successfully in three patients who developed severe polyarthritis while receiving immune checkpoint inhibitors [78].

Other events

Other AEs described with immune checkpoint inhibitors include fatigue, pyrexia and infusion reactions. Fatigue is a very common event, the etiology of which is often unknown. In fact, the incidence reported in clinical trials was 16–37% with anti-PD-1 single agents [10–12] and 12–24% with anti-PD-L1 antibodies [79]. Fatigue also increased (21–71%) during combination immunotherapy, such as nivolumab and ipilimumab [80,81] or pembrolizumab and ipilimumab [82]. However, fatigue can be also a symptom related to undiagnosed hypophysitis. Fever and infusion reactions have been described in less than 1% of patients treated with anti-PD-1/PD-L1 therapy [11,12,79] and some grade 3 reactions have been described with ipilimumab [83].

AEs with combination immunotherapies

Combination treatment with anti-CTLA-4 and anti-PD-1 antibodies, most notably ipilimumab and nivolumab, has been widely studied and has received approval for the treatment of metastatic melanoma [80]. The irAEs described above may occur more frequently with combined ipilimumab and nivolumab, but these do not represent new safety signals compared with monotherapy. However, multiple irAEs may be more likely to occur in the same patient. In fact, 83–89% of patients developed irAEs and 36–47% discontinued combination treatment in clinical trials [80,81]. In the recent update of the CheckMate 067 study with a follow-up of 48 months, the rate of grade 3–4 toxicities was 59% in patients treated with the nivolumab and ipilimumab combination compared with 22 and 28% in patients treated with nivolumab or ipilimumab monotherapy, respectively. Grade 3 diarrhea was the most common event in the nivolumab plus ipilimumab group (29 [9%] out of 313) and in the nivolumab group (9 [3%]) and colitis was the most common event in the ipilimumab group (23 [7%] out of 311). Grade 4 increases of

Table 1. Incidence of adverse events in combination studies with anti-programmed death antigen-1/programmed death antigen-ligand-1 agents.

Combination	Trial	Number of total patients	All grades AEs (%)	Grade 3–4 AEs (%)	Patients discontinued for AEs (%)	Ref.
Nivolumab + ipilimumab	Phase III	945	96% (diarrhea: 44; fatigue: 35; pruritus: 33)†	58.5%	39.6%	[81]
Pembrolizumab + ipilimumab	Phase II	153	58% (diarrhea: 46; Pruritus: 39; rash: 39)†	42%	20%	[86]
Durvalumab(M) + trametinib (T) ± dabrafenib (D)	Phase I	50‡ Cohort A (M+T+D) Cohort B (M+T) Cohort C (T→M)	Pyrexia: 63% and fatigue: 54% Diarrhea: 30% and rash: 25% Vomiting: 67%	4%	4% (two pts with DLT)	[87]
Pembrolizumab + dabrafenib + trametinib	Phase I/II	15§	NR¶	67%	33% (three pts with DLT)	[88]
Atezolizumab (A) + vemurafenib (V)	Phase Ib	17#	NR	67% (A+V) 38% (V for 56 days→A) 33% (V for 28 days→A)	NR	[89]
Atezolizumab + cobimetinib	Phase Ib	22	NR	50% diarrhea and acneiform rash† (18% SAE)	14%	[90]
Atezolizumab + vemurafenib + cobimetinib	Phase Ib	11	>20% (elevation of AST and ALT)†	40% (three pts with SAE: one elevation of CPK; one sepsis; one diarrhea and ALT/AST elevation)	9%	[91]

†The most common.
‡As of December 2014.
§As of January 2016.
¶Not reported.
#As of November 2015.
AE: Adverse event; CPK: Creatine-phosphokinase; DLT: Dose-limiting toxicity; pt: Patient; SAE: Serious adverse event.

lipase were observed in all three groups (15 [5%] of the combination group, ten [3%] of nivolumab group and four [1%] of ipilimumab group). Further serious AEs were not observed during 4-year follow-up. Four treatment-related deaths occurred during the study: two in patients receiving nivolumab plus ipilimumab group (cardiomyopathy, liver necrosis), one in the nivolumab group (neutropenia) and one in the ipilimumab group (perforated colon) [81].

The increased incidence of irAEs with combination therapy may depend on different factors, such as patient population, dose and schedule. In fact, combined administration of ipilimumab 3 mg/kg and nivolumab 3 mg/kg was poorly tolerated and exceeded the maximum tolerated dose (MTD) [84]. Recently, Checkmate 511 has demonstrated the better safety profile of the schedule nivolumab 3 mg/kg plus ipilimumab 1 mg/kg (NIVO3 + IPI1) compared with the approved combination (NIVO1 + IPI3). Incidence of treatment-related grade 3–5 AEs was 34% with NIVO3 + IPI1 versus 48% with NIVO1 + IPI3 ($p = 0.006$). There were no significant differences in terms of response (45.6% in the NIVO3 + IPI1 group and 50.6% in the NIVO1 + IPI3 group) and survival (PFS was 9.9 months in the NIVO3 + IPI1 group and 8.9 months in the NIVO1 + IPI3 group, overall survival [OS] was not reached in either group) between the two treatment groups [85]. Incidence of grade 3–4 irAEs for the approved schedule in this trial was less than that described in the Checkmate 067 trial (48 vs 59%), probably due to improved management of irAEs [85].

Pembrolizumab and ipilimumab combination has shown good tolerability. Preliminary results of the Phase Ib Keynote 029 study on 153 patients showed only 20% (31/153) of patients with severe AEs. Fatigue was the most common event (46%), then pruritus (39%), rash (39%; grade 3–4, 3%), diarrhea (24%; grade 3–4, <1%), increase of lipases (18%; grade 3–4, 14%) and vitiligo (18%). General incidence of grade 3–4 AEs was 42% [86].

The increased incidence of AEs with combination immunotherapies is shown in Table 1.

AEs with combined immunotherapy & targeted therapy

The combination of immunotherapy and targeted therapy is not standardized in the treatment of BRAF-mutated melanoma patients, but ongoing clinical trials are evaluating the efficacy and safety of such regimens.

A Phase I trial with combined vemurafenib and ipilimumab was stopped earlier because of hepatotoxicity [92]. In this trial, vemurafenib and ipilimumab were administered concurrently at standard doses and dose reduction was planned in the event of dose-limiting toxicity (DLT). However, hepatotoxicity was still observed despite a reduction in the dose of vemurafenib. Another report on ten patients with very advanced melanoma treated with ipilimumab and vemurafenib [93] has shown a lower incidence of AEs. The treatment schedule was different from the Ribas *et al.*'s trial, with vemurafenib first started at standard dose and ipilimumab at 3 mg/kg added when symptoms improved (between 3 and 30 weeks from the start of vemurafenib, median of 11.5 weeks). Treatment-related side effects were frequently of grade 1–2, and included rash, diarrhea, arthralgia and elevation of liver transaminases. As expected, elevation of liver transaminases was more frequent during the combination phase. In the sequential Phase II study (NCT01673854), patients received vemurafenib for 6 weeks and then switched to ipilimumab at the dose of 10 mg/kg; results from this trial are pending.

A Phase I trial with a different combination, dabrafenib with or without trametinib and ipilimumab, was recently reported [94]. Eight patients received dabrafenib and ipilimumab and seven received dabrafenib, trametinib and ipilimumab. While the double combination was very well tolerated with no DLT and only one grade 3 event (elevation of transaminases), the triple combination was more toxic causing two cases of perforating colitis that led to early termination of the trial [94].

Combination of targeted therapy with an anti-PD-1 or anti-PD-L1 antibody rather than ipilimumab seems better tolerated [87–89]. Dabrafenib/trametinib combined with PD-1/PD-L1 agents is under investigation in Phase I trials (NCT02027961, NCT02130466, NCT02027962). The Phase I, multicenter, open-label study (NCT02027961) is evaluating the safety and efficacy of durvalumab at 3 or 10 mg/kg every 2 weeks in combination with dabrafenib 150 mg twice daily plus trametinib 2 mg daily, or trametinib alone in patients with metastatic melanoma. Patients were enrolled by BRAF status into dose escalation cohorts (cohort A, durvalumab + dabrafenib + trametinib; cohort B, durvalumab + trametinib; or cohort C, sequential trametinib followed by durvalumab). Preliminary data showed DLT in one patient in cohort A with reversible grade 3 thrombocytopenia and one patient in cohort B with reversible grade 3 choroidal effusion. No MTD was identified and durvalumab 10 mg/kg every 2 weeks was selected for expansion in all cohorts. The most frequent drug-related AEs observed were pyrexia (63%) and fatigue (54%) (cohort A); diarrhea (30%) and rash (25%) (cohort B); and vomiting (67%) (cohort C). Two patients were discontinued due to drug-related AEs. To date, durvalumab can be safely combined with trametinib with or without dabrafenib at full doses and shows clinical activity in BRAF-mutant and wild-type melanoma patients [87].

KEYNOTE-022 (NCT02130466) is an ongoing multicenter Phase I/II study of pembrolizumab in combination with dabrafenib and trametinib as first-line therapy for BRAF-mutant melanoma patients. Pembrolizumab is administered at 2 mg/kg every 3 weeks with dabrafenib and trametinib at standard daily doses. Preliminary data on 15 patients showed DLTs in three patients: grade 4 neutropenia, grade 4 increase of ALT and grade 3 increase of ALT, AST and gamma-glutamyltransferase. All events resolved. Ten (67%) patients experienced grade 3–4 treatment-related AEs. Based on these results, the recommended Phase II regimen is pembrolizumab 2 mg/kg every 3 weeks plus dabrafenib at 150 mg twice daily and trametinib 2 mg once daily [88].

Furthermore, the combination of vemurafenib with the anti-PD-L1 atezolizumab has shown good activity and tolerability. In a Phase Ib study (NCT01656642), patients received atezolizumab combined with vemurafenib concurrently or after a run-in period with vemurafenib alone for 28 or 56 days. Atezolizumab was administered intravenously every 3 weeks at 20 or 15 mg/kg or 1200 mg fixed dose. Vemurafenib was given twice daily at 960 mg during the run-in period and at 720 mg during the combination. No dose-limiting AEs or atezolizumab-related treatment discontinuations were observed. In the overall study population, grade 3 AEs related to atezolizumab occurred in 41% of patients and grade 3 AEs related to vemurafenib occurred in 59% of patients. Sixty-seven percent of the concurrent cohort experienced a grade 3 AE, with lower rates of 33 and 38% experienced by the 28-day and 56-day vemurafenib run-in cohorts, respectively. Serious AEs included pyrexia and dehydration, which were manageable. There were no treatment-related grade 4 AEs or deaths [89].

Another Phase Ib trial (NCT01988896) enrolled patients to receive cobimetinib and atezolizumab at various doses. Cobimetinib was escalated from 20 to 60 mg daily for the first 21 days of a 28-day cycle. Atezolizumab was given at 800 mg every 2 weeks. The 60-mg daily dose of cobimetinib was discovered to be the MTD. After

a median follow-up of 12.7 months, half of the patients had treatment-related grade 3–4 AEs, with diarrhea and dermatitis acneiform as the most common. There were no treatment-related deaths and 18% of patients had treatment-related serious AEs; 14% discontinued treatment due to AEs [90]. A Phase III study has been designed to explore the combination of atezolizumab and cobimetinib compared with atezolizumab alone in patients with untreated BRAF wild-type unresectable melanoma.

This study was amended and included another cohort (cohort 4) where patients could receive the triplet with vemurafenib, atezolizumab and cobimetinib. Vemurafenib, during a 28-day run-in period, was administered at 960-mg twice daily on days 1–21. After the run-in, the vemurafenib dose was reduced to 720-mg twice daily and atezolizumab was administered at 800 mg every 2 weeks. Cobimetinib was given at 60 mg daily on days 1–21 across both phases of the study. Preliminary data of atezolizumab plus cobimetinib plus vemurafenib combination therapy showed a manageable safety profile and promising antitumor activity in patients with BRAFV600-mutant metastatic melanoma [91]. All grade AEs occurred in more than 20% patients and included nausea, fatigue, flu-like symptoms, photosensitivity, maculopapular rash, elevated ALT/AST (the most common) and bilirubin, mucosal inflammation and arthralgia. Grade 3–4 AEs were experienced by 40% of patients treated with the combination, of which 27% were atezolizumab-related. There were three treatment-related serious AEs, which included one patient with elevated creatine-phosphokinase, one with sepsis and one with diarrhea and ALT/AST elevations. These events were resolved with dose interruptions and/or dose reductions. Six patients had cobimetinib and/or vemurafenib-related grade 3–4 AEs during the run-in period, and five had atezolizumab and/or cobimetinib and/or vemurafenib-related grade 3–4 AEs during the triple combination period; there were no unexpected AEs or deaths. No atezolizumab-related serious AEs occurred. One patient discontinued because of elevated ALT/AST ratio. Based on these findings, a Phase III study (TRILOGY) is ongoing to further explore cobimetinib, vemurafenib and atezolizumab versus cobimetinib, vemurafenib and placebo for patients with previously untreated BRAF-mutant metastatic melanoma (NCT02908672).

AEs with combination immunotherapy & radiotherapy

Combination immunotherapy and radiotherapy (RT) toxicity in metastatic melanoma patients is unknown. Six retrospective studies [95–100] and two case reports [101,102] have examined stereotactic radiotherapy (SRT) concurrent with ipilimumab in melanoma brain metastases. The median SRT dose ranged from 14 to 60 Gy in one to five fractions and median follow-up ranged between 7.3 and 33.1 months. Concurrent SRT and ipilimumab did not show an increase of toxicity compared with ipilimumab alone [98]. Another study showed two cases of grade 3 seizures and two cases of grade 3 central nervous system (CNS) hemorrhages (13%) related to SRT alone or concurrent treatment [95] and there was a trend toward increased CNS toxicity in the concurrent therapy group. Symptomatic radiation necrosis was also described for the combination therapy [97]. Other studies did not find increased toxicity after combination treatment [96,97,99,100].

Combination of anti-PD-1 agents and RT in melanoma patients with brain metastases was recently analyzed by Kaidar-Person *et al.* [101]. In a retrospective study on 58 patients, where 29 received anti-PD-1 agent combined to SRT, they reported a higher incidence of intracranial complications in patients treated with nivolumab or pembrolizumab and SRT (eight cases of radiation necrosis and seven of hemorrhage, $p = 0.08$). However, patients treated with immunotherapy and SRT had a significant OS advantage compared with SRT without immunotherapy (15 vs 6 months; $p = 0.0013$) [101]. Also, Long *et al.* conducted a trial of combined anti-PD-1 antibody and RT (intracranial SRT, extracranial and whole brain) in patients with metastatic melanoma and brain metastases [102]. They analyzed 53 patients, among whom 35 received extracranial RT or intracranial SRT and 21 received whole brain RT. Eleven patients were treated with extracranial RT sequentially (RT then anti-PD1), 16 concurrently and 15 received RT on lesions progressing on anti-PD1 therapy. No potentiation of toxicity was observed in patients receiving extracranial RT. Grade 3 radiation necrosis was the main side effect in a patient treated with SRT. One patient treated with whole-brain RT developed Stevens-Johnson syndrome, and another developed acute neurocognitive decline. Also, one patient developed severe cerebral edema. This study concluded that RT and anti-PD-1 antibodies could be safely combined, without increasing AEs in extracranial sites. The combination with whole-brain RT was also well tolerated, even though rare toxicities, which could not be attributed to anti-PD-1 therapy or RT, were detected.

Very limited data are available on the combination of immunotherapy and extracranial SRT. Two case reports of SRT concurrent with ipilimumab in liver metastases from NSCLC and melanoma did not show any significant toxicity [101,102].

An ongoing Phase II study (NCT02821182) has been recently designed to evaluate prospectively the efficacy and toxicity of the combination of anti-PD-1 therapy (pembrolizumab or nivolumab) and SRT [103].

General management of immunotherapy AEs

IrAEs have modified the traditional clinical approach of physicians to the management of toxicities in patients with cancer. Anti-CTLA-4 therapy in melanoma was the first to show potential difficulties, with drug-related deaths being described in the early trials [104,105]. Subsequently, the increased experience of physicians and the development of algorithms for each of the most common irAEs with well-described supportive measures have resulted in a reduction of severe toxicities and related deaths. Furthermore, a multidisciplinary approach that includes collaboration between oncologists and other specialists has become a successful strategy for the management of irAEs.

Considering each toxicity, there is a consensus between different guidelines for management of irAEs (ESMO, ASCO, SITC) [106–108]. These have entered clinical practice and are very useful to clinicians in the management of irAEs. They use Common Terminology Criteria for Adverse Events criteria (version 4.0) [109] with grades of toxicity defined from 1 (mild and asymptomatic) to 2 (moderate with possible symptoms) and 3–4 (severe/life threatening with symptoms and possible clinical deterioration). Usually for grade 1 AEs, immunotherapy is continued, but close monitoring of symptoms is indicated, and if the event worsens, treatment as per a higher grade is indicated. For grade 2 AEs, immunotherapy is withheld with daily monitoring of symptoms and the use of oral corticosteroids; if symptoms improve to grade 1 or better, immunotherapy can be resumed. However, if the AE is persistent beyond 3 days, immunotherapy must be discontinued and, after symptoms have improved, corticosteroids must be tapered over $\geq$4 weeks. For grade 3–4 toxicity, immunotherapy must be discontinued; hospitalization should be considered and intravenous administration of high doses of corticosteroids is indicated along with the prophylactic use of antibiotics. If symptoms improve to $\leq$ grade 1 or baseline, tapering of corticosteroids over $\geq$6 weeks is indicated, but if they worsen within 48 h, additional immunosuppressive agents must be considered.

The first step for the correct management of irAEs is the education of patients and their caregivers, who must be informed about the risk of such events. Information is necessary for the early recognition of symptoms and for a timely diagnosis and an early start of specific treatment. This approach is necessary to avoid severe grades of toxicity and lethal complications.

A relevant issue regarding the management of irAEs is the use of immunosuppressive agents. Corticosteroids have a central role in the management of irAEs. Theoretically, any corticosteroid may be used, but in clinical practice, prednisone, methylprednisolone and dexamethasone are the most commonly used. Doses and administration sites depend on the grade of toxicity. Usually, oral prednisone 1 mg/kg per day is given for moderate grades while intravenous methylprednisolone 2–4 mg/kg per day is administered for severe grades.

The prophylactic use of corticosteroids to avoid irAEs is not indicated, as demonstrated in the trial with budesonide to prevent diarrhea [110]. For prolonged use of corticosteroids (>2 weeks) or during the use of other immunosuppressive drugs, prophylactic antifungal and antibiotic agents are indicated to prevent opportunistic infections. Also, calcium/vitamin D should be considered in case of prolonged use of corticosteroids (usually >4 weeks). Glycemia levels should be monitored during the use of steroids.

Other immunosuppressive agents include infliximab, mycophenolate mofetil, tacrolimus and cyclophosphamide. More rarely, other approaches can include intravenous immunoglobulin or plasmapheresis. Infliximab is recommended in corticosteroid-refractory diarrhea at the dose of 5 mg/kg and it can be repeated after 15 days if symptoms persist. It is not indicated in liver toxicity as it may confer its own risk of hepatotoxicity. For hepatic toxicity, mycophenolate mofetil at a dose of 500–1000 mg twice daily until resolution is recommended. Tacrolimus or cyclophosphamide may also be used. Intravenous immunoglobulin has also been successfully used in one report of hepatitis [111] and has also been used in some cases of severe neurologic syndromes [111,112]. The use of corticosteroids and of other immunosuppressive agents may switch-off inflammation, and thus improve resolution of side effects, without eliminating antitumor responses [112]. The administration of other immunosuppressive agents should be done through specialist consultation, depending on the type of toxicity. Specialist consultation may be required for each toxicity, in particular when a diagnosis is less common and more insidious, such as neurological, musculoskeletal or endocrine syndromes. Sometimes diagnosis of an immune-mediated AE may be difficult as it is not always associated with the presence of specific antibodies in the serum. A biopsy should be always considered in this case to exclude other causes. Biochemical tests are very useful for diagnosis of most common toxicities, such as hypo-hyperthyroidism (TSH, T4), hypophysitis (prolactin, TSH, T4, luteinizing hormone, follicle-stimulating hormone, ACTH and cortisol) and adrenal insufficiency (ACTH, cortisol). Radiology or endoscopy may also

contribute to diagnosis, such as specific MRI for the study of pituitary gland (thin cuts study), which can show an enlargement in case of hypophysitis or 'empty sella syndrome' in case of a late evolution. Colonoscopy or x-ray and/or high-resolution CT chest scan are also performed in cases of suspected colitis and interstitial pneumonitis, respectively. Other supportive medications are also important to improve some clinical conditions and symptoms, such as topical or oral antihistamines, Gamma aminobutyric acid (GABA) agonists and antidepressants. These drugs may be particularly useful for pruritus or skin rash.

In case of endocrinopathies, long-term hormone replacement may be necessary, as these toxicities may become permanent. Supplementation of affected hormones is requested in case of secondary hypothyroidism (levothyroxine 0.5–1.5 μg/kg every day) or secondary hypoadrenalism (hydrocortisone, usually 20 mg each morning and 10 mg each afternoon and evening) [17,107,108]. In this type of toxicity, immune checkpoints inhibitors treatment can usually be safely continued.

Discussion

Immune-related AEs are very specific side effects of checkpoint inhibitors. A clear knowledge of these drugs and their associated irAEs is crucial for the safety of patients. Anti-CTLA-4 and anti-PD-1/PD-L1 antibodies may be responsible for organ-specific inflammatory conditions that can be variable and more or less frequent according to the type of agent, the dose, the schedule, patient characteristics and the type of neoplasia. There is a difference between the incidence of AEs with ipilimumab and with the anti-PD-1s, nivolumab or pembrolizumab. Anti-PD-1 agents generally appear less toxic than ipilimumab and they also have a slightly different safety profile (pneumonitis or thyroiditis are more typical for anti-PD-1 inhibitors while colitis or hypophysitis are most frequently associated with ipilimumab). Dose and schedule may influence toxicity and, in particular, combination immunotherapies such as ipilimumab plus nivolumab rather than single agents or sequential schedules may result in the highest incidence of multiple AEs [80,81].

However, efficacy and safety may be also dose dependent, at least for ipilimumab, as shown by a comparison of ipilimumab 10 and 3 mg/kg [8]. In this study in 727 patients, median OS was superior in patients treated with the higher ipilimumab dose, with OS of 15.7 months (95% CI: 11.6–17.8) for ipilimumab at 10 mg/kg and 11.5 months (9.9–13.3) for ipilimumab at 3 mg/kg (hazard ratio: 0.84; 95% CI: 0.70–0.99; $p = 0.04$). The most common grade 3–4 treatment-related AEs were diarrhea (10 vs 6%), colitis (5 vs 2%), increased AST (3 vs 1%) and hypophysitis (3 vs 2%). Treatment-related serious AEs were reported in 37% of patients in the 10 mg/kg group and 18% in the 3 mg/kg group; 1 versus less than 1% of patients died from treatment-related AEs. The BMS-CA209-511 trial (NCT02714218) recently demonstrated that combination nivolumab at 3 mg/kg with ipilimumab at 1 mg/kg was less toxic than nivolumab at 1 mg/kg with ipilimumab at 3 mg/kg, but showed similar efficacy [85].

An interesting issue is the relationship between the higher grade 3–4 irAEs incidence and longer survival. No definite conclusions can be drawn, but preliminary data suggest that patients developing some irAEs-like skin reactions may experience a longer progression-free interval [113]. Recently, uveitis has been associated with responses in two patients treated with pembrolizumab [114]. In a pooled analysis of two studies (CheckMate 067 and 069), 176 patients treated with nivolumab plus ipilimumab who were discontinued from treatment due to AEs, showed longer median PFS compared with patients without AEs (16.7 vs 10.8 months; $p < 0.04$) [115]. Although waiting for definitive data, the idea of a correlation between response and toxicity may serve as significant comfort to patients who consider toxicity a surrogate marker of treatment response.

Novel combinations of immunotherapy with targeted therapy or RT must be monitored carefully. In fact, both targeted therapy and RT may potentiate the immune response, as demonstrated in preclinical trials [116,117]. Both may result in proimmunogenic effects through the increase of antigen presentation and the recruitment of effector T cells to the tumor microenvironment [116,117]. Combining these approaches may produce a synergism of action that translates into a potentiation of antitumor response, also in sites that are not irradiated (abscopal effect) [118]. However, toxicity may also be potentiated, even though no definite data are available. Ongoing clinical trials will clarify the safety of these strategies.

Patient characteristics may be relevant to the appearance of irAEs. To date, we do not have biomarkers that predict immune system changes during toxicity or that help to select patients to a treatment. Biomarkers would be useful to avoid severe toxicities.

Special populations of patients include those with hepatitis B, C or HIV and with autoimmune diseases. They, notably, are excluded from clinical trials, because there is a concern of worsening the preexisting disease. However,

trials on hepatocellular carcinoma with nivolumab are ongoing and include patients with hepatitis B and C; one study has shown no increase of toxicity in these patients [119,120]. Other ongoing trials are also evaluating the safety and activity in patients with HIV- and HPV-associated tumors (NCT2408861 and NCT02255097). Regarding autoimmune diseases, no conclusions on toxicity can be drawn as very few data are available.

Conclusion

Toxicities related to immune checkpoint inhibitors have changed the clinical scenario in oncology. With the increasing use of these drugs, correct management is necessary to avoid morbidity and mortality caused by severe irAEs. Clinicians, patients and their caregivers must be aware of possible AEs, including rare events. Future research is also required to better understand the mechanisms that may be responsible of severe and life-threatening AEs and to find biomarkers for patient selection. Meanwhile, oncologists should strengthen their collaboration with several specialties and maintain vigilance for early detection of toxicities.

Future perspective

IrAE's management remains a crucial issue for clinicians. Checkpoints inhibitors alone or in combination with other conventional and innovative treatments may improve survival of metastatic melanoma patients but also expose them to novel toxicities. To date, no biomarkers are available to select patients for the best therapy. For this reason, the knowledge and most correct management of possible immune-mediated AEs that involve each organ and sometimes present in insidious ways, are fundamental to avoid lethal complications. Collaboration among physicians, patients and their caregivers is the start point. Acute and chronic events may change patients' life, like hypophysitis, thyroiditis and diabetes as they may be permanent and necessitate long-life replacement therapy.

Probably, in the next 5 years we will have managed to improve not all, but most of the main irAE-related issues and we hope in this way we will have saved more lives.

Executive summary

- Checkpoint inhibitors (anti-cytotoxic T-lymphocyte-associated protein-4 [anti-CTLA-4] and anti-programmed death antigen-1/programmed death antigen-ligand-1 [anti-PD-1/PD-L1] antibodies) can potentially cause an imbalance in immune tolerance that translates into uncontrolled immune reactions and may clinically manifest as immune-related adverse events (irAEs).
- These events may involve many organs and tissues, including the skin, GI tract, liver, endocrine system, kidneys, CNS, eyes and lungs. The incidence of irAEs appear to be lower with anti-PD-1/PD-L1 agents than with the anti-CTLA-4 antibody ipilimumab. Combined anti-CTLA-4 and anti-PD-1 immunotherapy does not appear to be associated with novel safety signals compared with single-agent therapy, but a greater number of organs and tissues may be involved in some patients.
- Increased experience and the development of algorithms for the most common irAEs, including those arising from the use of corticosteroids and other immunosuppressive agents, have resulted in severe toxicity and related deaths being reduced or avoided.
- Clinicians, patients and their caregivers must be aware of possible irAEs, including the possibility of very rare events that may require complex diagnosis and therapy. A multidisciplinary approach that includes the collaboration between oncologists and other specialists is necessary to allow the best diagnosis of irAEs and the most appropriate use of medications. A higher incidence of irAEs may be related to improved response and outcomes in some patients, although further investigation into this matter is required.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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