Immune-related adverse events in patients with solid-organ tumours treated with immunotherapy: a 3-year study of 102 cases from a single centre

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Abstract

Objective

Immune checkpoint blockade therapy (ICBT) increases the anti-tumoural function of the immune system, but it can also induce immune-related adverse events (irAEs). Our aim was to assess the irAEs due to ICBT in patients from a single centre of Northern Spain.

Methods

We set up an observational study of patients treated in monotherapy with ICBT targeted against cytotoxic T-lymphocyte antigen 4 (CTLA-4), programmed cell death 1 (PD-1) or its ligand (PD-L1) for solid organ tumours. All patients were followed up in a single University Hospital from March 2015 to September 2018.

Results

We studied 102 patients (63 men/39 women); mean age 60.6±9.7 years, with lung (n=63), melanoma (n=21), kidney (n=11), gastric (n=3), colon (n=3) or bladder (n=1) cancer. Only 7 patients had a previous diagnosis of an immune-mediated disease, specifically: psoriasis (n=2), psoriatic arthritis (n=1), systemic lupus erythematosus (n=1), spondyloarthitis (n=1), rheumatoid arthritis (n=1) and cutaneous lupus (n=1). One of the following ICBT was administered: nivolumab (n=52), pembrolizumab (n=35), atezolizumab (n=10) and ipilimumab (n=5). After a mean follow-up time of 14.4±7.7 months since ICBT onset, 87 (85.3%) patients had experienced irAEs, mostly gastrointestinal, thyroid and musculskeletal manifestations including inflammatory arthralgia (n= 8), arthritis (n= 6) and myositis (n=2). ICBT was discontinued in 41 patients but it was reintroduced in 30 of them after resolution of the adverse event, with a good tolerance in all cases. Thirty-six (41.4%) of the 87 patients required specific treatment (prednisone, levothyroxine, and thiamazol) for the irAEs.

Conclusion

irAEs are frequent in patients undergoing ICBT. Almost half of the patients that have irAEs require treatment. Musculoskeletal manifestations are not uncommon.

Key words

immune checkpoint blockade therapy, immunotherapy, immune-related adverse events, nivolumab, pembrolizumab, atezolizumab, ipilimumab

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Introduction

A number of new different therapies against cancer have been developed in the last years. The use of these therapies has improved the overall survival, reducing the frequency of adverse events (AEs). One of them is the immunotherapy, especially the so-called immune checkpoint blockade therapy (ICBT). It increases anti-tumour immune function by blocking the intrinsic down-regulators of immunity (1). The two main types of ICBT are targeted against cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed cell death 1 (PD-1) or its ligand (PD-L1). CTLA-4 inhibits T-cell activation at a proximal step in the immune response (2, 3). By contrast, PD-1 inhibits T cells at later stages, in the peripheral tissues (4, 5).

ICBT enhances the activity of the immune system through the blockade of these down-regulators, leading to inflammatory/immune adverse events (AEs), which are commonly known as immune-related adverse events (irAEs). Any organ or system can be potentially involved, although the most common are gastrointestinal tract, endocrine glands, skin, liver, and joints (6). The precise pathophysiology of these AEs remains unclear, but it is believed to be related to the role that immune checkpoints play in maintaining immunologic homeostasis. The management of these irAEs represents a challenge, since no prospective clinical trials have defined the best treatment approaches, and recommendations are mainly based on expert opinions. To date, most studies on irAEs are based on small case-series with short-term follow-up or reporting particular patients or specific complications.

Taking all these considerations into account, the aim of this study was to assess the incidence, clinical features, treatment and outcome of the different irAEs in an unselected large series of patients with different types of solid-organ tumours who received ICBT in a single University centre.

Material and methods

Design, enrolment criteria and definitions

We set up an observational study that included all consecutive patients that started ICBT as monotherapy due to solid-organ tumours from a single tertiary-care University Hospital in Northern Spain between March 2015 and September 2018. All the patients were followed-up for at least 6 months. Before starting ICBT, a complete clinical evaluation, including assessment of history of previous autoimmune diseases, physical examination, data on baseline laboratory tests and radiological procedures, was performed. All the patients were diagnosed with solid-organ tumours and treated with ICBT as monotherapy, either as first or successive line. For the purpose of this study, we decided not to include patients with more than one agent simultaneously as this could prevent us from identifying the actual agent responsible for the irAE. A history of a previous autoimmune disease was not considered as an exclusion criterion for ICBT, and the therapeutic decision in every patient was individualised.

Patients were treated with one of the following ICBTs: pembrolizumab (anti-PD1), nivolumab (anti-PD1), atezolizumab (anti-PD-L1) or ipilimumab (anti-CTLA-4), according to the European Medicines Agency (EMA) indications and the European Society for Medical Oncology (ESMO). In most cases, the ICBT was not used as the first-line therapy. Conventional drugs used before ICBT were the following: platin-derivatives, vinorelbine, bevacizumab, gemcitabine, pemetrexed, abraxane, docetaxel, zoledronate, afatinib, gefitinib, interferon, sunitinib, everolimus, pazopanib. The therapeutic response to ICBT was defined according to 4 categories: disease, progression, complete remission, partial remission and stable disease. Progression was established by using imaging tests [computerised tomography [CT], magnetic resonance imaging [MRI], positron emission tomography [PET] and/or ultrasonography], according to RECIST 1.1 criteria (7). A controlled disease was defined when there was no progression, which would imply either stable disease or partial/complete remission.

Severity and treatment of immune-related adverse events

The severity of irAEs was defined according to the 2018 American Society of Clinical Oncology (ASCO) guidelines (8) and classified in 4 grades, from 1 to 4. Treatment of irAEs was based on the degree of the toxicity. CBT maintenance was based on the response of the disease and the severity of irAEs. With respect to this, ICBT was continued if the oncologic disease was controlled and the irAEs were mild. By contrast, ICBT was temporal or permanently discontinued when irAEs were severe, very disturbing for the patient or they were not controlled with the therapy. Glucocorticoids and non-steroidal anti-inflammatory drugs (NSAIDs) were used as a symptomatic treatment for the different irAEs. No immunosuppressive agent was used for this purpose. This study was approved by the Ethics Committee of Cantabria (Spain).

Statistical analysis

Results were expressed as numbers and percentage, mean ± standard deviation (SD). Factors associated with tumour progression were analysed using logistic regressión; its results are presented as odds ratios with 95% confidence intervals. The probability of developing an irAEs according to the ICBT taken was estimated with Kaplan-Meier method. Factors associated with irAEs were studied via Cox regression, were failure was the first appearance of an irAEs and follow-up time was the time from starting ICBT until irAEs appearance.

Results

Baseline characteristics

We included 102 patients (63 men/39 women; mean age, 60.6±9.7 years), treated with ICBT as monotherapy for a solid-organ tumour. They received the following ICBT: nivolumab (n=52), pembrolizumab (n=35), atezolizumab (n=10) and ipilimumab (n=5). The underlying solid tumours were: lung (n=63), melanoma (n=21), kidney (n=11), gastric (n=3), colon (n=3) and bladder (n=1) cancer.

Only 7 of 102 patients had a history of a previous immune-mediated disease: psoriasis (Ps) (n=2), psoriatic arthritis (PsA) (n=1), systemic lupus erythematosus (SLE) (n=1), spondyloarthritis (SpA) (n=1), rheumatoid arthritis (RA) (n=1) and skin lupus (n=1).

Table I. Main general features, clinical manifestations, treatment and follow-up of 102 and immune-related adverse events in patients undergoing Immune Checkpoint Blockade Therapy.

	Anti PD-1 (Nivolumab, Pembrolizumab)	Anti PD-L1 (Atezolizumab)	Anti CTLA-4 (Ipilimumab)	Total
Number of patients	87	10	5	102
Age, mean (SD) years	60.6 (9.9)	62.9 (5.38)	66.6 (11.0)	60.2 (9.75)
Sex, men/women, n/n	52/35 9/1	3/2 64/38		
Underlying solid tumour				
Lung	54	9	0	63
Melanoma	16	0	5	21
Kidney	11	0	0	11
Colon	3	0	0	3
Gastric	3	0	0	3
Bladder	0	1	0	1
Previous diagnosis of irAEs, n (%)	6 (6.9%)	0 (0%)	1 (20%)	7
Psoriasis, n	2	0	0	2
Psoriatic arthritis, n	1	0	0	1
Systemic lupus erythematosus, n	0	0	1	1
Spondyloarthritis, n	1	0	0	1
Rheumatoid arthritis, n	1	0	0	1
Skin lupus, n	1	0	0	1
Patients with irAEs, n (%)	76 (87.4%)	9 (90.0%)	2 (40.0%)	87 (85.3%)
Gastrointestinal, n (%)	31 (35.6%)	7 (70.0%)	1 (20.0%)	39 (38.2%)
Thyroid, n (%)	16 (18.4%)	2 (20.0%)	0 (0%)	18 (17.6%)
Musculoskeletal, n (%)	12 (11.76%)	2 (20.0%)	0 (0%)	14 (13.7%)
Cutaneous, n (%)	11 (12.6%)	1 (10.0%)	1 (20.0%)	13 (12.7%)
LFT alterations, n (%)	6 (6.9%)	2 (20.0%)	0 (0%)	8 (7.8%)
Nephritis, n (%)	4 (4.6%)	2 (20.0%)	0 (0%)	6 (5.8%)
Vasculitis, n (%)	1 (1.1%)	0 (0%)	0 (0%)	1 (1.0%)
Severe irAEs (grade 3 and 4)	9 (10.3%)	3 (30.0%)	1 (20%)	13 (12.7%)
Treatment of irAEs				
Glucocorticoid treatment, n (%)	16 (18.4%)	2 (20.0%)	0 (0%)	18 (17.6%)
ICBT withdrawal, n (%)	34 (39.1%)	5 (50.0%)	2 (40.0%)	41 (40.2%)
- Definitive	8 (9.2%)	2 (20.0%)	1 (20.0%)	11 (10.8%)
- Temporarily	26 (29.9%)	3 (30.0%)	1 (20.0%)	30 (29.4%)

CTLA-4: cytotoxic T-lymphocyte antigen 4; ICBT: immune checkpoint blockade therapy; irAEs: immune-related adverse events; LFT: liver function tests; PD-1: programmed death cell protein 1; PD-L1: programmed cell death ligand; SD: standard deviation.

Incidence, clinical features, and autoantibodies of irAEs

After a mean follow-up of 14.4±7.7 months since the ICBT onset, we observed 99 autoimmune AEs in 87 patients (85.3%).

Eleven patients experienced several irAEs. However, the mean age of these patients (60.5 years) was similar to that of the complete cohort. Of note, 7 of them (64%) had arterial hypertension, 5 (45%) hyperlipidaemia, 3 (27%) diabetes mellitus, 2 (18%) chronic obstructive pulmonary disease and 1 (9%) ischaemic heart disease.

Table I summarises the main general features, the previous diseases, and the irAEs, according to the type of ICBT. Most patients (97 of 102) were treated with anti-PD-1/anti-PD-L1 drugs, and only 5 patients were on anti-CTLA-4 antibodies.

The only significant factor associated to irAEs development was the use of ipilimumab *versus* nivolumab, with Hazard ratio 3.56 (95% confidence interval 1.07–11.8); 4.37 (1.29–14.9) when adjusted for age, gender and immunotherapy drug, although we have to keep present that the size of this sample was small (only 5 patients received treatment with ipilimumab). Complete results of the analyisis are ahown in Table II.

Figure 1 shows the risk of having an irAE during the follow-up time with each one of the 4 ICBT agents studied.

Table II. Factors associated with immunologic adverse effects; hazard ratios obtained via Cox regression.

Factor	Hazard ratio (95% CI)	*p*	Adjusted hazard ratio (95% CI)*	*p*
Age (per year)	0.98 (0.96-1.00)	0.05	0.97 (0.95-1.00)	0.02
Gender (ref.: woman)	1.35 (0.86-2.10)	0.19	1.50 (0.93-2.42)	0.09
Arterial hypertension	0.76 (0.47-1.22)	0.26		
Dyslipidaemia	0.77 (0.46-1.28)	0.32		
Diabetes	1.19 (0.59-2.40)	0.63		
Tumour localisation (ref.: melanoma)				
Lung	0.90 (0.51-1.57)	0.71		
Kidney	0.68 (0.31-1.50)	0.35		
Stomach	0.82 (0.24-2.82)	0.75		
Histology (ref.: adenocarcinoma)				
Epidermoid	1.34 (0.72-2.49)	0.35		
Clear cell	0.86 (0.42-1.82)	0.72		
Tumour stage (ref.: I or II)				
III	1.48 (0.74-2.98)	0.27		
IV	1.52 (0.80-2.92)	0.20		
Missing	1.86 (0.41-8.46)	0.42		
Immunotherapy drug (ref.: nivolumab)**				
ipilimumab	3.56 (1.07-11.8)	0.04	4.37 (1.29-14.9)	0.02
pembrolizumab	1.29 (0.78-2.12)	0.32	1.32 (0.80-2.17)	0.28
atezolizumab	1.57 (0.76-3.23)	0.23	1.33 (0.62-2.85)	0.46

*Hazard ratios adjusted for age, gender and immunotherapy drug.

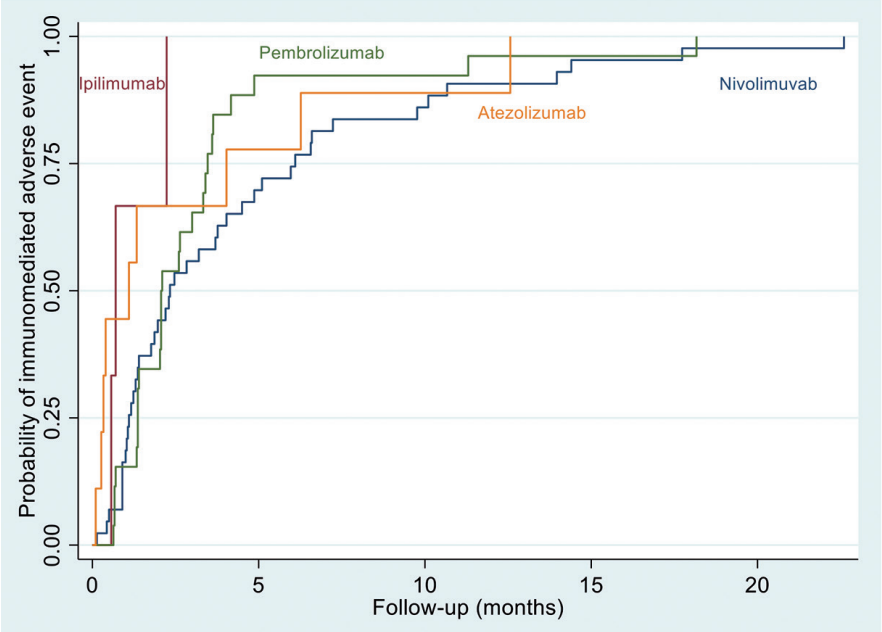

Fig. 1. Probability of immune-related adverse event related to the time of follow-up with nivolumab, pembrolizumab, atezolizumab and ipilumab.

The most frequent irAEs were gastrointestinal (n=39; 38.2%): diarrhoea (n=26/39; 66.7%), colitis (n=9/39; 23.1%), and mucositis (n=4/39; 10.2%). Thyroidopathy was the second most frequent irAE (n=18; 17.6%), including either hypo (n=14/18; 77.8%) or hyper thyroid dysfunction (n=4/18; 22.2%). Musculoskeletal side effects were observed in 14 patients (14.7%): inflammatory arthralgia (n=8/14; 57.1%), arthritis (n=6/14; 42.9%) and myositis (n=2/14; 14.3%). Among the patients who suffered from arthralgia/arthritis, the most frequent pattern was oligoarticular (40%), followed by polyarticular (30%) and monoarticular pattern (30%). The most commonly affected joints were the knees (n=4), followed by wrist (n=3), hand (n=3), ankle (n=2), shoulder (n=2) and foot (n=1). Apart from the articular disease, 2 patients suffered from myositis in the lower limbs, with weakness of legs and arthralgia in their knees. Cutaneous manifestations appeared in 13 patients: rash (n=7/13; 53.8%), vitiligo (n=2/13; 15.4%), erythema nodosum (n=2/13; 15.4%), psoriasis (n=1/13; 7.7%) and alopecia (n=1/13; 7.7%). Liver function test (LFT) alterations were observed in 8 patients (7.8%) showing cholestasis (n=4) or hepatocellular (n=4) patterns. Seven patients (6.9%) exhibited sicca syndrome features (6 patients had dry eyes and 1 dry eyes and dry mouth). The Schirmer test was positive in 6 of them. However, none of them fulfilled classification criteria for Sjögren's syndrome. Also, 6 patients (5.8%) suffered from nephritis, although kidney biopsy was not performed in any case. The only case of vasculitis was an asymptomatic aortitis diagnosed by PET/CT scan.

As mentioned above, there were 7 patients with a previous diagnosis of immune-mediated disease. Nonetheless, in only 2 of them the underlying disease worsened during the treatment (a flare of skin psoriasis and psoriatic monoarthritis, respectively). Two patients, one with RA and another with SLE, had non-specific colitis. Therefore, 4 out of 7 patients with an underlying immune-mediated disease experienced irAEs (57.2%). Of note, none of the patients from our series had haematological, neurological, ophthalmological or cardiac AEs.

Regarding immunological tests, 3 patients had positive autoantibodies before starting ICBT: a patient with a previous diagnosis of SLE (antinuclear antibodies [ANA] and anti-DNA antibodies), another with RA (rheumatoid factor [RF] and anti-citrullinated peptide antibodies [ACPA]), and a patient with cutaneous lupus (positive ANA). None of them had a raise in the autoantibodies level or a relapse of the underlying disease following ICBT. The remaining 4 patients had negative autoantibodies results.

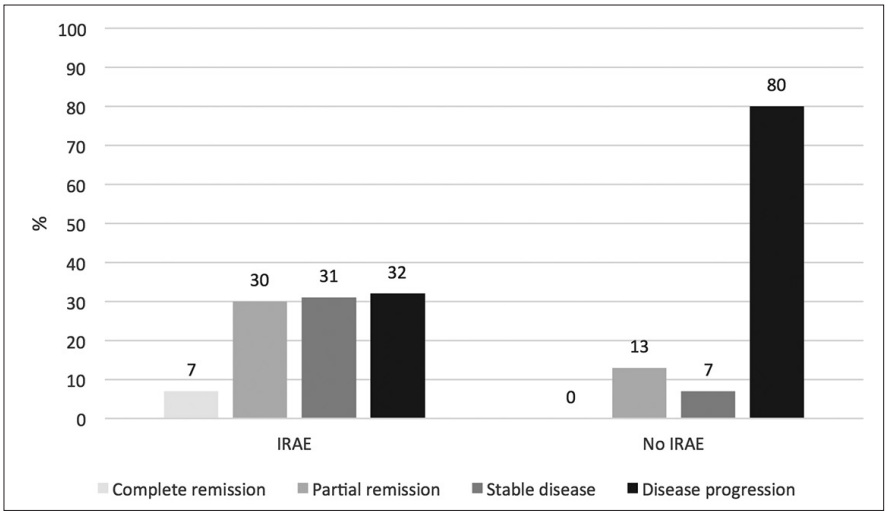

Fig. 2. Anti-tumour therapeutic response in patients stratified according to the presence of immune-related adverse events or not.

Table III. Factors associated with tumour progression; odds ratios obtained via logistic regression.

Factor	Odds ratio (95% CI)	*p*	Adjusted odds ratio (95% CI)*	*p*
Age (per year)	0.97 (0.93–1.02)	0.26	0.96 (0.91-1.01)	0.16
Gender (ref.: woman)	1.96 (0.74-5.18)	0.17	2.47 (0.86-7.04)	0.09
Arterial hypertension	0.42 (0.15–1.22)	0.11		
Dyslipidaemia	0.60 (0.19–1.89	0.39		
Diabetes	1.75 (0.43-7.14)	0.44		
Tumour localisation (ref.: melanoma)				
Lung	1.67 (0.51-5.49)	0.40		
Kidney	0.65 (0.10-4.18)	0.65		
Stomach	1.30 (0.10-17.7)	0.84		
Histology (ref.: adenocarcinoma)				
Epidermoid	3.90 (1.10-13.8)	0.04		
Clear cell	0.74 (0.13-4.20)	0.74		
Tumour stage (ref.: I or II)				
III	1.20 (0.28-5.15)	0.81		
IV	1.00 (0.26-3.90)	1.00		
Missing	2.25 (0.23-1.44)	0.49		
Immunotherapy drug (ref.: nivolumab)**				
ipilimumab	8.00 (0.82-78.5)	0.07	10.98 (1.02-118.2)	0.05
pembrolizumab	0.89 (0.31-2.54)	0.83	0.90 (0.29-2.74)	0.85

*Odds ratios adjusted for age, gender and immunotherapy drug.
**Atelozizumab could not be analysed as no patient treated with atelozizumab presented tumour progression.

The patient who developed aortitis had negative immunological tests before starting ICBT. However, high levels of both IgG anti-beta-2 glycoprotein and IgG anticardiolipin antibodies were found at the time of diagnosis of aortitis. In this case, no specific therapy was started, and a new PET/CT scan performed 6 months later did not show vascular inflammation signs.

Immunological laboratory tests including RF, ACPA and ANA were done in 8 of the 14 patients with musculoskeletal irAEs, being negative in all of them.

Among the 18 patients with thyroid disorders, 14 patients developed hypothyroidism and 4 hyperthyroidism. Only 2 patients with thyroid disturbances (11.1%) had positive anti-thyroid antibodies after the ICBT onset (one with hyperthyroidism and another with hypothyroidism). The first patient had positive anti-thyroglobulin antibodies, and the second one positive results for anti-thyroglobulin and anti-thyroid peroxidase antibodies. Both patients achieved normal levels of thyroid hormones after receiving thiamazole and levothyroxine, respectively.

Efficacy of ICBT in patients who developed irAEs

Good oncological response to the ICBT (complete or partial remission, or stable disease) was achieved in 59 of 87 patients (67.8%) who developed some kind of irAE while on treatment. By contrast, in the remaining 28 (32.2%) cases, cancer progression was observed. Among the 15 patients who did not suffer irAEs, only 3 (20%) had a controlled disease and 12 (80%) suffered progression of the disease. The therapeutic response in patients stratified according to the presence of immune-related adverse events or not is shown in Figure 2. A logistic regression analysis was done in order to identify different factors associated with tumour progression, but only a histology of epidermoid *versus* adenocarcinoma resulted to increase the risk of tumour progression. Table III shows the results of this analysis.

Management and outcomes of irAEs

Specific treatment for irAEs was given to 27 of the 87 patients. Glucocorticoids were the most frequently used agents, either as systemic (n=18), intra-articular (n=2), or topic (n=1) administration. In this regard, systemic glucocorticoids were prescribed to 18 patients for a mean of 7.6 weeks, with a good response in 12 of them. The irAEs that required the use of systemic glucocorticoids were gastrointestinal (n=6), arthritis (n=5), skin reactions (n=3), aortitis (n=1), nephritis (n=1), thyroiditis (n=1) and LFT alterations (n=1). One patient with *de novo* psoriasis received topic glucocorticoids during follow-up attaining complete remission. Two patients with arthritis also required a single dose of intra-articular glucocorticoid injection with clinical resolution. A good oncological response was observed in 86.7% of the patients who received systemic glucocorticoids and

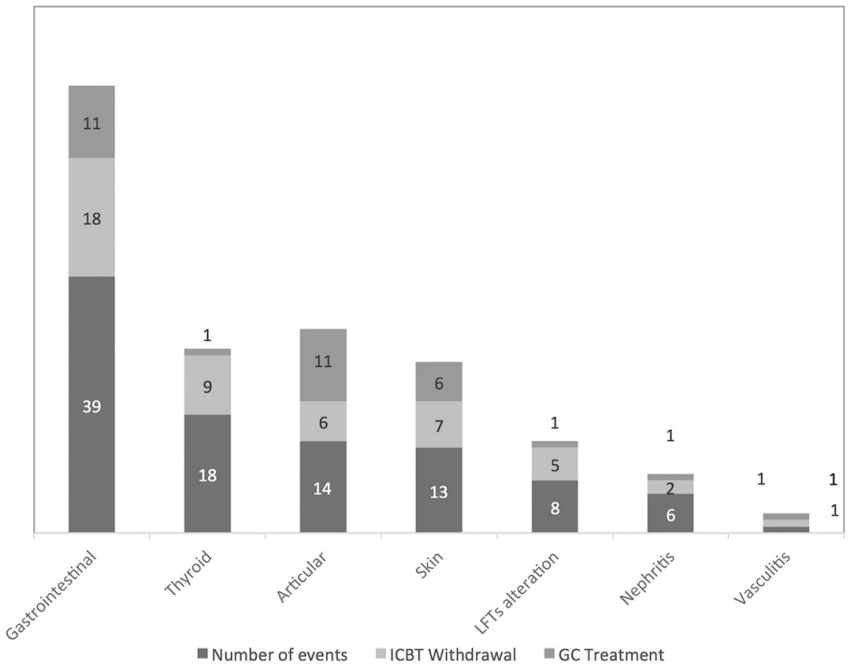

Fig. 3. Types of irAE. Each bar represent the number of patients who experienced a specific irAE as well the number of patients who required temporally or permanent discontinuation of the ICBT and glucocorticoid therapy.
ICBT: immune checkpoint blockade therapy; GC: glucocorticoids; LFT: liver function tests.

in 56.6% of those who were not treated with systemic glucocorticoids. However, this difference may be influenced by the degree of severity of the toxicity, as only patients with grades 3 and 4 of toxicity received systemic glucocorticoids. Severe colitis (grades 3 and 4 of ASCO) was observed in 4 of 39 (10.3%) patients who developed gastrointestinal AEs (either diarrhoea, colitis or mucositis). All of them required the withdrawal of ICBT and the administration of systemic glucocorticoids with complete resolution of symptoms in all the cases. None of the mild gastrointestinal events required either specific treatment or ICBT withdrawal.

In 6 of the 14 patients with articular irAEs (arthralgia/arthritis), the ICBT was temporally discontinued but it was reintroduced once the AE was resolved (mean time of 33±18.9 days of retrintroduction after removal). Six patients who suffered musculoskeletal manifestations were treated with NSAIDs and 5 with oral prednisone (2 patients with arthritis also required intra-articular corticosteroids).

Thyroid dysfunction required treatment in 10 cases, either with levothyroxine or thiamazole. In all cases the treatment was permanent, achieving normal serum TSH and free T4 levels in 8 patients (80%) after a 6-month follow-up. One patient also needed systemic glucocorticoid for 8 weeks.

Withdrawal of ICBT was required in 41 patients due to irAEs, being temporally in 30 patients and permanent in 11. In 13 of these 41 patients, the irAE was resolved upon the discontinuation of the ICBT, without any other specific treatment. The distribution of patients according to the specific irAE manifestation is shown in Figure 3. This figure also describes the number of patients who required temporally or permanent discontinuation of the ICBT and glucocorticoid therapy for each irAE manifestation.

Discussion

In the present study, we describe the main irAEs that occurred in an unselected series of 102 consecutive oncologic patients with solid-organ tumours treated with ICBT as monotherapy. Regardless of the type of cancer and the duration of treatment, most patients experienced irAEs. Almost half of them required therapy, such as glucocorticoids, NSAIDs, levothyroxine or thiamazole. The frequency of irAEs in our series was similar to that previously reported (9). However, musculoskeletal manifestations were found more commonly than in former reports (10).

With respect to the irAE of each one of the ICBT assessed in the present study, The anti-PD-1 nivolumab used for metastatic melanoma was previously reported to yield irAE in 74–85% of the patients, being grades 3–4 in 12–20% of them (6, 9, 11). In patiens treated with nivolumab for advanced cisplatin refractory squamous non-small-cell lung cancer (NSCLC), the frequency of irAE was 58% being grades 3–4 in 7% (12). Similar frequency (69% and 10%, respectively) was reported following the use of nivolumab for metastatic cisplatin refractory non-squamous NSCLC cancer (13), and for tyrosine kinase inhibitor refractory metastatic renal cell carcinoma (79% and 19%, respectively (14). Regarding pembrolizumab, the other anti-PD-1 assessed in our study, the KEYNOTE-002 study, that compared the efficacy and side effects of pembrolizumab at doses of 2 and 10 mg/kg with investigator-choice chemotherapy for ipilimumab-refractory melanoma, irAEs grades 1–2 were found in 57–60% of patients, while grade 3–4 toxicity was observed in 14% (15). Similarly, in the KEYNOTE-006 study, comparing pembrolizumab (10 mg/kg either every 2 or 3 weeks) with ipilimumab, treatment-related toxicity due to pembrolizumab was observed in 73–80% of patients, being grade 3 or higher in 10–13.5% of them having (16). In keeping with these data, in our patients treated with anti-PD-1 nivolumab or pembrolizumab the frequency of irAE was 87.4%, being severe in 12.7%.

iAEs related to anti-CTLA-4 antibody ipilimumab at a dose of 3 mg/kg have been documented to occur in around 60–86% of patients (11, 17), mostly toxicity grade 1 and 2, although 10–27% of patients can develop grade 3–4 toxicity. These irAEs are dose-dependent and no grade 3–4 AEs were observed at a dose of 0.3 mg/kg, whereas toxicity increased up to 30% with a dose of 10 mg/kg (18). Only a few patients from our series were treated with atezolizumab (anti-PD-L1) or ipilimumab

(anti-CTLA-4). Therefore, this small number of cases does not allow us to raise conclusions on the frequency of iAEs in our population.

With regard to the each one of the irAE observed by the use of ICBT, in keeping with our findings, gastrointestinal complications were reported to be the most frequent irAEs, usually of low intensity, allowing conservative management without any specific treatment in most patients (1). Nevertheless, GI toxicity is the most severe irAE associated with anti-CTLA-4 therapy (19), being more common than with anti-PD-1/PD-L1 immunotherapy. Around one-third of the patients with anti-CTLA-4 therapy have diarrhea, while the frequency of colitis ranged from 8% to 22% (20). A number of interleukins (IL) are thought to play a role in the pathophysiology of immune-related colitis in patients treated with ICBT (21). High serum IL-17 levels have been found in patients with ipilimumab-induced colitis (22) and in some cases the use of anti-IL-17 therapy was found to improve irAEs response in patients on ICBT (23). In our study, the incidence of gastrointestinal AEs was 35.6% in patients that received anti-PD-1 antibodies, 70% in those with anti-PD-L1 and only 20% in patients treated with ipilimumab (anti-CTLA-4). Also, only 4 patients from our series suffered from grade 3-4 gastrointestinal AE that showed good response to oral glucocorticoids.

In our series, the second more frequent AE was thyroid disturbances (n=18; 17.6%). Previous studies have found that thyroid disease is more common upon a treatment that blocks the PD-1/PD-L1 axis (9, 14, 16). This fact was confirmed in our study since all the patients with thyroid alterations were receiving anti-PD-1/PD-L1 therapy. Noteworthy, patients from our series with thyroiditis were treated with either thiamazole or levothyroxine, depending on whether they suffered from hyper or hypothyroidism. Nevertheless, most patients with hyperthyroidism developed hypothyroidism during the follow-up. In all cases, the treatment for thyroiditis was permanent, with a good response in 80% of them.

Hypophysitis is a rare complication in patients treated with anti-CTLA-4 antibodies and very rare in those on anti-PD-1 and anti-PD-L1 therapy (24). This irAE was not observed in any of our patients. The mechanism of induction of hypophysitis after the treatment with anti-CTLA-4 remains unclear. Low levels of ectopic RNA and protein expression of CTLA-4 on thyrotropin and prolactin-secreting cells of the murine pituitary gland have been recently found, suggesting some association with this AE (25).

Previous studies have found higher incidence of arthralgia with combined ipilimumab/nivolumab immunotherapy (10.5%) than with ipilimumab or nivolumab in monotherapy (6.1 and 7.7%, respectively) (26). Severe arthralgia were described to occur in less than 1% of the patients (27). Permanent structural damage in the joints has uncommonly been reported (28). For mild cases (grade 1), the ESMO suggests the use of acetaminophen and/or NSAIDs as symptomatic therapy, while moderate symptoms (grade 2) may respond to 10–20 mg/day of prednisone-equivalent glucocorticoids. For severe cases (grades 3–4), consultation with a rheumatologist is advised, and it could be considered the use of high-dose glucocorticoids and TNF-α blocking agents (29). In our study, musculoskeletal irAEs were not severe, which is is agreement with previous results (28, 29). However, most of our patients with musculoskeletal irAEs required either immune checkpoint blockade withdrawal and/or glucocorticoid treatment. The incidence of these events in our study (13.7%) was slightly higher than in the pivotal studies (10, 30).

Inflammatory arthritis induced by ICBT may be a recurrence after discontinuation of immune ICBT. Regarding this, one of our patients developed musculoskeletal AE 2 months after removal of the checkpoint blocker, and another had a relapse of inflammatory arthritis after discontinuation of ICBT. In addition, there are reports of cases of relapse of colitis after ICBT cessation. Because of this, follow-up of these patients is advisable, at least during the first months after ICBT discontinuation, even when they are asymptomatic.

Sicca syndrome has been reported to be a relatively common adverse event of ICBT, most commonly related to PD-1 inhibitors (31). In keeping with these observations, the 7 patients from our series who developed sicca syndrome features were being treated with a PD-1 inhibitor (either nivolumab or pembrolizumab)

Cutaneous lesions are among the most frequent AEs observed in patients treated with either anti-CTLA-4 or anti-PD-1/PD-L1 (32, 33). Among them, vitiligo was associated with good clinical responses to anti-PD-1 antibodies in patients with melanoma (34). Sweet's syndrome or Stevens-Johnson's syndrome as well as toxic epidermolysis, pyoderma gangrenosum, and cutaneous sarcoidosis have been reported in patients on anti-CTLA-4 therapy (35). In our study, skin AEs were observed in 12.7% of the patients, without a remarkable difference between groups. They included rash, psoriasiform eruption and vitiligo. By contrast, we did not find any case of Sweet's syndrome, Stevens-Johnson´s syndrome, epidermolysis or pyoderma gangrenosum.

Hepatitis occurs in 5–10% of patients during therapy with ipilimumab, nivolumab, and pembrolizumab at the approved doses in monotherapy (11, 16). In our study, 8 patients (7.8%) had any type of alteration in LFTs during the treatment with ICBT. Two of them were severe (grade 3), and required treatment with glucocorticoids apart from ICBT withdrawal.

Renal dysfunction with ipilimumab and anti-PD-1 therapies is a rare AE, occurring in <1% of cases (36). The incidence is much higher with the combination of ipilimumab plus nivolumab; about 4.9%, 1.7% of cases with grade 3 to 4 toxicity (37). In our study, the incidence was slightly higher, with 6 patients showing raised levels of serum creatinine and urea after the onset of the ICBT, but this alteration was not severe in any case and it did not require specific treatment.

Immune checkpoint receptors, including CTLA-4 and PD-1, play a pivotal role in regulating the mechanisms of tolerance to self-antigens, through the down-regulation as well as the prevention of

abnormal activity against these antigens (38). Thus, the continuous release of antigens by tumour cells has been shown to upregulate the inhibitory immune pathways as a result of chronic stimulation (39). Once CTLA-4 and PD-1 bind to their ligands (CD80/86 and PD-L1/PD-L2, respectively), they negatively regulate intercellular interactions, even in the presence of tumour antigens (39). By blocking these interactions, checkpoint inhibitors lead to increased T cell proliferation and activity, followed by an anti-tumour response but also by potential autoimmune reactions (40). Nevertheless, the precise pathophysiology of these irAES remains unknown. Some potential mechanisms include increasing T-cell activity against antigens present in tumours and healthy tissue, increasing levels of preexisting autoantibodies, high levels of inflammatory cytokines, and enhanced complement-mediated inflammation due to direct binding of an antibody against CTLA-4 expressed in normal tissue (1).

An interesting matter of debate is the potential relationship between irAEs and the tumour response to ICBT. Several studies suggest that patients with irAEs have a better response to ICBT than those who do not develop such AEs (41, 42). However, other studies have not confirmed this point (43). Interestingly, in our series there was a better tumour response rate in patients who suffered from irAEs (70.1% of them reached a controlled disease) compared to those who did not develop any kind of irAE (20%), although no differences in type of tumour, therapy nor line of treatment were observed. On the other hand, since ICBT enhances the activity of the immune system against cancer itself, it could be thought that the treatment of irAEs with immunosuppressive drugs would lead to a lower response to the ICBT. However, some retrospective studies have shown similar outcomes between patients treated and not treated with immunosuppressive agents (25, 43). In our study, those who received glucocorticoids (the only immunosuppressive agent that we used to treat the irAEs) did not show a worse response rate compared to those who did not receive these agents (86.7% *vs*. 58.6%).

Another potential controversial issue is the presence of an autoimmune disease prior to the onset of ICBT. This could lead to AEs. Therefore, most studies of immunotherapy exclude patients with high risk for developing autoimmune events, such as those with autoimmune diseases, since the safety of ICBT in these cases remains unclear. However, some studies have pointed out that patients with autoimmune disorders can be safely treated with ICBT (44, 45). In our series, we treated 7 patients previously diagnosed with immune-mediated diseases. The incidence of irAEs in these patients was 57.2%, which was not higher than the overall incidence, while the rate of flares or worsening of the underlying disease was 28.6%. None of these events was considered severe, based on clinical data and ASCO/ESMO criteria.

With respect to presence of immunological tests, only 3 (3.4%) of 87 patients who developed irAEs from our series had positive immunological tests for autoantibodies when the AE occurred. This low incidence suggests that the mechanism through the irAEs takes place while on ICBT might be mainly caused by the activation of autoreactive T cells without the development of autoantibodies, as some studies have already indicated (46-48).

In conclusion, although results from our series are similar to those reported by other group, our study provides confirmatory data on the effect irAEs in a a large series of patients with different types of solid cancer treated with ICBT in monotherapy seen at a single tertiary-care hospital for a well-defined population of Northern Spain.The most common irAEs were gastrointestinal, followed by thyroid, musculoskeletal and cutaneous. Most of these irAEs had a good response to glucocorticoids and/or ICBT withdrawal. The group of patients who developed some irAEs had a better response to ICBT than the group who did not.

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