

# Mechanisms of immune-related adverse events during the treatment of cancer with immune checkpoint inhibitors

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## Abstract

Immune checkpoint inhibitors are novel biologic agents to treat cancer by inhibiting the regulatory interactions that limit T cell cytotoxicity to tumours. Current agents target either CTLA-4 or the PD-1/PD-L1 axis. Because checkpoints may also regulate autoreactivity, immune checkpoint inhibitor therapy is complicated by side effects known as immune-related adverse events (irAEs). The aim of this article is to review the mechanisms of these events. irAEs can involve different tissues and include arthritis and other rheumatic manifestations. The frequency of irAEs is related to the checkpoint inhibited, with the combination of agents more toxic. Because of their severity, irAEs can limit therapy and require immunosuppressive treatment. The mechanisms leading to irAEs are likely similar to those promoting anti-tumour responses and involve expansion of the T cell repertoire; furthermore, immune checkpoint inhibitors can affect B cell responses and induce autoantibody production. Better understanding of the mechanisms of irAEs will be important to improve patient outcome as well as quality of life during treatment.

**Key words:** checkpoint, co-stimulation, CTLA-4, PD-1, PD-L1, arthritis, autoreactivity, T cell, B cell, autoantibodies

### Rheumatology key messages

- Checkpoint inhibition can promote anti-cancer responses by blocking regulatory interactions limiting T cell cytotoxicity.
- Checkpoint inhibition can lead to immune-related adverse events that limit cancer treatment.
- Immune-related adverse events result from changes in patterns of T and B cell expression.

## Introduction

Immune checkpoint inhibitors (ICIs) are biologic agents that represent a revolutionary approach to treating cancer [1–3]. Rather than killing cancer cells directly, these agents reset the checks and balances that regulate T cell cytotoxicity against tumours. This approach exploits the capacity of the immune system to defend against malignancy by T cells targeting tumour neoantigens [4]. These cells may fail to prevent or eradicate cancer, however, because of regulatory interactions known as checkpoints. With checkpoints blocked by ICIs, a cytotoxic

T cell response can emerge and provide powerful anti-tumour activity.

While ICIs have improved the survival of patients with previously untreatable cancers, the benefits have come at the cost of serious side effects known as immune-related adverse events (irAEs) [5–8]; these side effects can limit cancer therapy and necessitate treatment. Clinically diverse, irAEs affect the skin, gastrointestinal tract, lung, heart and endocrine system. irAEs can also cause arthritis and related rheumatic disease (Table 1) [9–16].

In many respects, irAEs are not unexpected since the checkpoints that inhibit the response to tumour antigens would likely also inhibit autoreactivity. As such, irAEs would appear an almost inevitable consequence of ICIs [17, 18]. In view of the frequency and severity of irAEs, delineating their mechanisms is important in developing strategies for prevention and treatment. While a damaging consequence of immunotherapy, irAEs nevertheless

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**TABLE 1** Immune-related adverse events according to specialty

Gastroenterology	Dermatology	Endocrinology	Rheumatology
Colitis Hepatitis Pancreatitis	Vitiligo Skin rash <sup>a</sup> Alopecia	Thyroiditis Adrenal insufficiency Hypophysitis Type I diabetes Pituitary disorders	Inflammatory arthritis Sicca syndrome Polymyalgia rheumatica Myositis/myocarditis Uveitis

<sup>a</sup>Skin rash includes psoriasis and psoriaform rashes.

**TABLE 2** FDA approved immune checkpoint inhibitors

Immune checkpoint inhibitor	Year of FDA approval	Mechanism of action	Indications
Ipilimumab	2011	Anti-CTLA-4	Melanoma, CRC, MCC, RCC
Pembrolizumab	2014	Anti-PD-1	Melanoma, HCC, NSCLC, PMBCL, cervical cancer, gastric/gastroesophageal carcinoma, solid tumour, urothelial carcinoma, Hodgkin lymphoma, HNSCC
Nivolumab	2014	Anti-PD-1	Melanoma, NSCLC, SCLC, CRC, RCC, HCC, urothelial carcinoma, HNSCC, Hodgkin lymphoma
Atezolizumab	2016	Anti-PD-L1	NSCLC, urothelial carcinoma
Durvalumab	2017	Anti-PD-L1	NSCLC, urothelial carcinoma
Avelumab	2017	Anti-PD-L1	Urothelial carcinoma, MCC
Cemiplimab	2018	Anti-PD-1	CSCC

CSCC: cutaneous squamous cell carcinoma; CTLA-4: cytotoxic T-lymphocyte antigen 4; CRC: colorectal cancer; FDA: Food and Drug Administration; HCC: hepatocellular carcinoma; HNSCC: head and neck squamous cell carcinoma; MCC: Merkel cell carcinoma; NSCLC: non-small cell lung cancer; PD-1: programmed cell death protein-1; PD-L1: programmed cell death ligand-1; PMBCL: primary mediastinal large B cell lymphoma; RCC: renal cell carcinoma; SCLC: small cell lung cancer.

provide a setting for elucidating the mechanisms that govern autoreactivity.

### Checkpoint inhibitors

Currently, seven immune checkpoint blocking agents have received approval by the Food and Drug Administration to treat cancer. These agents target cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), also known as CD152; programmed cell death protein-1 (PD-1); and programmed cell death ligand-1 (PD-L1) (Table 2). As shown in many clinical trials, irAEs commonly complicate ICI therapy and clinically present with autoimmune or autoinflammatory manifestations. In general, anti-CTLA-4 agents lead to more frequent irAEs than do anti-PD-1/anti-PD-L1 agents. Combined checkpoint blockade (CCB) is associated with an increased frequency of irAEs along with higher levels of C-reactive protein [19].

In addition to currently recognized irAEs, the spectrum of these side effects may change as ICIs are used to treat different malignancies and patient populations. Possible influences include age, sex, comorbidities like pre-existent autoimmune disease, prior anti-cancer treatment and the composition of the microbiome [20–22]. Interestingly, older patients may be at particular risk for side effects

because of age-related changes in the immune system [23]. While irAEs can be considered ‘off-target’ since they are distinct from the anti-tumour effect, some studies indicate that the occurrence of irAEs may be associated with improved tumour response [24]. Table 3 indicates factors that may influence the development of irAEs.

### The mechanisms of ICIs

ICIs act on the basic mechanisms regulating the T cell response to antigen. As is now well recognized, T cell activation requires two signals: TCR recognition of antigen and co-stimulation. For the first signal, antigen recognition occurs in the context of MHC molecules on antigen presenting cells (APCs). Co-stimulation occurs between membrane-bound molecules on T cells and APCs, with the interaction of CD28 molecules on T cells with CD80/86 molecules on APCs a key event in co-stimulation (Fig. 1) [25, 26].

Following activation of T cells, the expression of CTLA-4 is induced. CTLA-4 is expressed on both activated T cells and on a subset of CD25<sup>+</sup>CD4<sup>+</sup> T cells called T-regulatory (T-reg) cells [26]. A member of the immunoglobulin supergene family, CTLA-4 is ~30% homologous with CD28;

CTLA-4 binds CD80/86 with higher affinity and avidity than CD28. The binding of CTLA-4 by CD80/86 decreases T cell-mediated immune responses by reducing IL-2 and IL-2 receptor expression [27]. Another mechanism by which CTLA-4 can regulate immunity is via its effects on T regulatory (T-reg) cells [28].

**TABLE 3** Potential influences on irAE development

Malignancy-related factors	Underlying host factors
Cancer type	Age
ICI treatment	Genetic predisposition to auto-immunity
Molecular target <sup>a</sup>	Pre-existing autoimmune disease
Monotherapy	CCB
CCB	Microbiome
Sequence of therapy <sup>b</sup>	
Possible Influences	
Duration of therapy	
Prior chemotherapy	

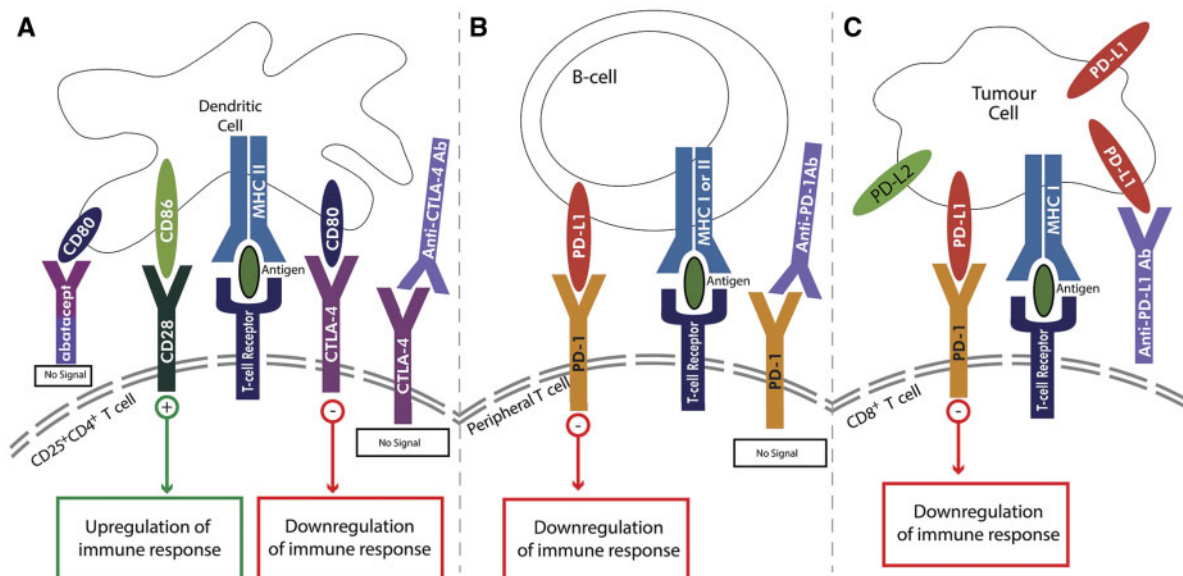
<sup>a</sup>Anti-CTLA-4 agents vs anti-PD-1/anti-PD-L1 agents. <sup>b</sup>Use of anti-CTLA-4 therapy followed by anti-PD-1/anti-PD-L1 therapy or vice versa. CCB: combined checkpoint blockade; ICI: immune checkpoint inhibitor; irAE: immune-related adverse event.

While anti-CTLA-4 antibodies are termed checkpoint inhibitors, these agents may have other actions that may manifest in certain locales (i.e. tumour microenvironment) and involve other immune cell types [29–31]. Thus, treatment with anti-CTLA-4 can eliminate T-reg cells in a tumour microenvironment via Fc-receptor-mediated interactions. The relationship between a local reduction of T-reg cells and the emergence of irAEs is not clear since this mechanism seems most relevant for an established site of inflammation.

While the PD-1–PD-L1 axis also regulates T cells, the outcome is distinct from that of CTLA-4. PD-1 is a member of the immunoglobulin supergene family, with activation of peripheral T cells and B cells inducing its expression. The main action of PD-1 appears to be the maintenance of peripheral tolerance [32]. PD-1 interacts with two ligands in the peripheral tissues: PD-L1 and PD-L2. PD-L1 is expressed on resting B cells, T cells, macrophages and dendritic cells [33]. PD-L2 is uncommonly expressed on resting immune cells, but its production can be induced by pro-inflammatory cytokines [33].

Signalling via both CTLA-4 and PD-1 converges on Akt, although the pathways and consequences of antibody inhibition are distinct [34]. Akt is a serine threonine kinase that plays a key role in the regulation of processes such as metabolism, apoptosis and proliferation. For T cells, ligation of CD28 leads to activation of phosphatidylinositol

**FIG. 1** Two-step signalling process for activation of naïve T cells



Antigen presenting cells (APCs) such as dendritic cells (DCs) or B cells present antigen to T cells via MHC class I or II molecules (signal 1). The co-stimulatory signal occurs with binding of CD80/86 on an APC (A) to the CD28 receptor on the CD25<sup>+</sup>CD4<sup>+</sup> T cell resulting in upregulation of immune responses (signal 2). Alternatively, a co-inhibitory signal can occur with binding of the cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) receptor on the CD25<sup>+</sup>CD4<sup>+</sup> T cell to CD80/86 (B) or binding of PD-1 on the peripheral T cell to PD-L1 or PD-L2 on an APC (B); both pathways result in downregulation of immune responses. Tumour cells can evade immune system recognition via upregulation of PD-L1 or PD-L2 on the tumour cell surface (C) to bind with CD8<sup>+</sup> T cells resulting in downregulation of immune response. DC: dendritic cell; MHC: major histocompatibility complex.

3-kinase (PI3K) whose products bind to Akt, promoting its phosphorylation. Whereas PD-1 signalling can antagonize PI3K directly, the effects of CTLA-4 occur via the phosphatase called PP2A. As such, anti-CTLA-4 and anti-PD-1 act differently suggesting that combination therapy may lead to more global effects that are not observed with either therapy alone; this situation could lead to increased effectiveness against cancer as well as increased incidence of irAEs.

Together, these findings indicate that the actions of anti-CTLA-4 and anti-PD-1/PD-L1 differ in terms of the stage of T cell activation, downstream pathway affected and localization of action. These differences have been reflected in terminology [35]. Anti-CTLA-4 and anti-PD-1/PD-L1 antibodies have recently been termed 'immune enhancers' and 'immune normalizers', respectively. The latter terminology is consistent with the idea that anti-PD-1 ICIs 'normalize' T cell immunity in the tumour microenvironment [35]. Consistent with differences in their mechanism, PD-1 ICIs have greater activity and less toxicity compared with anti-CTLA-4 ICIs in melanoma patients [36–38].

### CTLA-4 and PD-1 in autoimmune disease

Immune homeostasis can prevent autoimmune disease and the CTLA-4 and PD-1 pathways are vital for this balance. Evidence for this role derives from studies on knockout mice that lack these checkpoint molecules [39, 40]. Thus, mice lacking CTLA-4 develop an aggressive immune-mediated condition characterized by the extensive infiltration of activated lymphocytes in lymph nodes, spleen and thymus. Relevant to irAEs, infiltration of lymphocytes occurs in the heart, lung, liver and pancreas but, interestingly, not in the kidney. Antibody levels in the knockout mice are also strikingly elevated.

The lack of kidney involvement in the knockout mice is interesting because of the high levels of immunoglobulin in these mice. Often, increases in immunoglobulin production in genetically manipulated mice are commonly associated with anti-DNA and other antinuclear antibodies that characterize SLE, a prototype autoimmune disease with glomerulonephritis. In studies thus far, development of either antinuclear antibodies or lupus-like illness has not been a prominent feature of irAEs, perhaps suggesting a distinct pattern of B cell expression that occurs after CTLA-4 inhibition in humans and mice.

While germline knockout of CTLA-4 can lead to autoimmunity, the role of CTLA-4 is complex and may include cell autonomous and non-autonomous effects operating at different steps in tolerance. Studies by Klocke *et al.* on mice undergoing conditional deletion of CTLA-4 shed light on these mechanisms [41]. With conditional deletion in adult mice, CTLA-4 deficiency leads to lymphoproliferation, pneumonitis, gastritis and insulinitis among other manifestations. In contrast to congenital deficiency, acquired deficiency in adult mice is not fatal.

With adult deficiency, disease can be transferred by T cells, although abnormalities among B cells also develop including hyperglobulinaemia as well as antibodies to insulin, gastric antigen and the Ro52 protein. Despite these

serological abnormalities, the levels of anti-DNA, an important lupus marker, are less prominent. Autoantibody findings, therefore, appear more selective than might be anticipated with a global breakdown in tolerance.

Unlike the situation with germline-deficient mice that develop rapid fatality, with CTLA-4 deletion during adulthood, there is adequate time to test experimental induction of autoimmunity. As studies by Klocke *et al.* showed, collagen-induced arthritis is more severe in mice with adult CTLA-4 deficiency than in wild-type mice [41]. Levels of induced antibodies to collagen are also increased. In contrast, mice with adult CTLA-4 deficiency are protected from the experimental allergic encephalomyelitis induced by myelin oligodendrocyte glycoprotein (MOG) peptide immunization; with a MOG protein-induced model, however, experimental allergic encephalomyelitis was delayed but not prevented.

In another approach to elucidate irAEs, Lute *et al.* investigated the relationship between autoimmunity and the anti-tumour effect in human CTLA-4 knock-in mice by comparing the activity of three monoclonal antibodies differing in CTLA-4 binding [42]. These studies showed that the antibodies varied in their anti-tumour activity as well as the development of autoimmunity although these activities were not linked. Interestingly, in this model system, animals with tumours treated with anti-CTLA-4 showed robust anti-DNA responses although the magnitude of these responses varied among the antibodies. These findings suggest that the presence of a tumour may influence the development of autoimmunity because of tumour-associated immune disturbances or release of self-antigen by a tumour undergoing cytotoxicity.

The development of abatacept, a CTLA-4-Fc fusion protein that competes with CD28 for CD80/86 binding, also attests to the role of CTLA-4 in the pathogenesis of RA [43]. While this therapy is effective in the treatment of rheumatoid arthritis, it has not been used to treat irAE arthritis, likely because of concerns on its effect on anti-tumour responses [44].

### The role of PD-1/PD-L1

The link between autoimmunity and PD-1 was first demonstrated in studies of PD-1-deficient murine models [45]. Depending on the strain, mice lacking PD-1 develop a lupus-like disease marked by glomerulonephritis and renal deposition of IgG3 and C3. In addition, in these studies, the majority of PD-1-deficient mice also developed inflammatory arthritis as shown by histology [45]. In an extension of this model, Nishimura *et al.* crossed the PD-1 knockout mice with mice of the B6-*lpr/lpr* strain, which develop lymphoproliferation because of genetic deficiency of Fas [45]. In these studies, autoimmunity in the cross of B6-*lpr/lpr* by PD-1<sup>-/-</sup> mice was accelerated in comparison with that observed in B6-*lpr/lpr* mice, displaying greater lymphadenopathy as well as immune complex glomerulonephritis.

The serological findings in these mice are interesting in terms of the mechanisms for irAEs. Thus, the B6-PD-1<sup>-/-</sup> mice do not display antibodies to either anti-DNA or RF.

Furthermore, the B6-PD-1<sup>-/-</sup> by *lpr/lpr* mice showed an increase in IgG2a anti-DNA but not IgG3 anti-DNA. As in the case of the CTLA-4 knockout, the PD-1 knockout mice did not lead to serological findings of SLE. In other studies, Nishimura *et al.* demonstrated autoimmune dilated cardiomyopathy with severe heart failure in PD-1 knockout mice [46].

While both CTLA-4 and PD-1 knockout mice develop immune-mediated conditions, the disease is quite different. Whereas CTLA-4 knockout mice die rapidly, disease in mice lacking PD-1 is gradual, with disease developing after a year. With inhibition of either checkpoint, it appears that background genes can influence the development of autoimmunity.

Evidence for the role of PD-1 in arthritis also comes from studies of patients with RA demonstrating high levels of PD-1 in synovial fluid. Liu *et al.* showed positive correlation of serum levels of soluble PD-1 with DAS28 scores, a measure of RA disease activity [47]. In other studies, Guo *et al.* showed increased PD-1 expression in synovial tissue of RA patients compared with osteoarthritis patients or normal controls. There was also statistically significant elevation in soluble PD-1 in the serum of ACPA positive RA patients compared with seronegative RA patients [48].

### Mechanisms of T cell reactivity in irAEs

Since the antigen specificity of T cells mediating irAEs is unknown, studies of the mechanisms of these side effects have assessed general features of the T cell repertoire as a clue to the aetiology. Studies by Robert *et al.* demonstrated that treatment with tremelimumab (an anti-CTLA-4 monoclonal antibody) can increase the number of unique productively rearranged TCR V- $\beta$  sequences in the blood of patients with metastatic melanoma [49]. Using measures called richness and the Shannon diversity index, importantly, this study showed that patients with and without irAEs differed in terms of the total number of productive TCR V  $\beta$  sequences in the complementarity determining region (CDR3). While these differences were noted in terms of the frequency of irAEs, responders and non-responders in terms of the anti-tumour response showed similar patterns of sequence expression.

While this study did not show the expansion of particular clones in the peripheral blood, Robert *et al.* posited that TCR expansion may be considered a pharmacodynamic effect of ICIs that reflects the overall immune activation. Since the emergence of irAEs and the increase in clonal diversity may be associated, these findings suggest that irAEs may result from a mobilization of large numbers of T cells, some of which are autoreactive. The lack of correlation of TCR diversity with treatment effect may further suggest that autoreactive and anti-tumour cells represent distinct populations.

A study by Oh and colleagues reached a similar conclusion [50]. This study involved patients with metastatic castrate-resistant prostate cancer treated with ipilimumab in association with GM-CSF. In this study, treatment with ipilimumab led to a greater diversification of the T cell

repertoire in those patients who developed irAEs in comparison to those without this complication. This diversification was notable in the number of clonotypes that were increased along with the presence of newly detected clones.

As demonstrated in this study, changes in T cell populations occurred early after treatment, suggesting a relationship with the subsequent development of irAEs. Nevertheless, toxicity in general occurs later in the course of therapy. Given the time lag, it is possible that pathogenic T cells gradually emerge from the larger number of clonotypes that occur after checkpoint blockade. Interestingly, the time of onset of inflammatory arthritis (IA) ranges from 7 weeks to 24 months, which is later than that of other non-rheumatic irAEs. In patients with melanoma who develop IA, the average time of onset is 6–24 months, suggesting that the onset of IA may be affected by the nature of the T cell population in the periphery as well as tumour microenvironment in different types of cancers [44, 51, 52].

### Mechanisms of B cell reactivity in irAEs

While the goal of ICI therapy is to increase cytotoxic T cells, these agents can also affect B cells either directly or indirectly. The data on B cell changes after ICI therapy are limited, although a study by Das *et al.* demonstrated that patients with advanced melanoma receiving CCB had a significant decrease in the number of circulating B cells after treatment [53]. This effect was not observed in patients treated with anti-CTLA-4 or anti-PD-1 monotherapy. These investigators also demonstrated an increase in the number of plasmablasts and plasma levels of CXCL13, a marker of germinal centre activation in humans, with CCB in comparison to monotherapy. Furthermore, B cells following CCB showed increased clonality in terms of the expression of immunoglobulin genes [53].

In the Das *et al.* study, the early changes in B cells correlated with the higher rates of grade 3 or higher irAEs 6 months after CCB. Together, these findings suggest that B cells may be important contributors of autoimmunity following CCB. In this regard, a subset of CD21<sup>lo</sup> memory B cells appeared to be particularly affected by CCB as shown by restricted expression of PD-1 on the CD21<sup>lo</sup> subset, increased B cells with a CD21<sup>lo</sup> genomic profile and increased IFN- $\gamma$  signalling in CD21<sup>lo</sup> B cells after CCB [53]. This B cell subset was previously described as a functionally and phenotypically distinct population on the path to long-lived plasma cells [54]. Patients who are haploinsufficient for CTLA-4 exhibit similar B cell findings to those observed following CCB [55].

In contrast to studies on T cells, evidence of B cell autoreactivity is clearer for certain conditions. Thus, patients with thyroid abnormalities (i.e., thyroiditis and hypothyroidism) display autoantibodies to thyroidal antigen [56, 57]. Similarly, patients who develop diabetes on ICIs show antibodies to islet cell antigens as well as glutamic acid decarboxylase-65 [58, 59]. Although autoantibodies

may be present in patients who develop rheumatic irAEs, most patients are seronegative [10, 14, 16, 51, 60, 61].

To delineate further autoantibody production following ICI, de Moel and colleagues screened pre-treatment and post-treatment samples of patients with advanced melanoma with a large panel of autoantigens [62]. The data indicated that, of 127 patients in the study, 20% were positive for any autoantibody prior to treatment while 29% were positive after treatment. Among patients who were antibody negative before treatment, 19.2% developed new autoantibodies, with anti-TPO (thyroperoxidase) and anti-TG (thyroglobulin) being the most common. The relationship between emergence of autoantibodies and irAEs was not significant. Data showed that 78.9% of patients who expressed any autoantibody had an irAE while only 57.5% who were autoantibody negative had an irAE.

Since ICIs can be given together or sequentially, de Moel *et al.* investigated the responses of patients who had developed an autoantibody during ipilimumab treatment and then received PD-1 blockade. These studies showed that 44.4% of patients who expressed anti-thyroid antibodies during therapy with anti-CTLA-4 and then received anti-PD-1 therapy had evidence of thyroid dysfunction. While these findings could suggest that anti-PD-1 can promote B cell autoimmunity initiated by anti-CTLA-4, irAEs can take time to develop.

### Pre-existing autoimmune disease and immune checkpoint therapy

Another setting to elucidate the mechanisms of irAEs concerns the response to ICIs in patients with pre-existing autoimmune disease. In these patients, ICIs can result in a flare in the underlying disease up to 50% of the time [63–65]. In a retrospective review of patients with pre-existing autoimmune disease, Abdel-Wahab *et al.* noted differences in the occurrence of irAEs in patients with active vs inactive autoimmune disease at the time of ICI initiation [65]. This same review noted that patients on immunosuppressive therapy at the start of ICI therapy had a lower incidence of irAEs. In this analysis, patients with irAEs were more likely to achieve partial or complete tumoral response compared with those without irAEs. In patients with pre-existing autoimmune disease, more disease flares were observed with anti-PD-1/PD-L1 agents compared with *de novo* irAEs reported with anti-CTLA-4 agents [65].

Menzies *et al.* showed an increase in the frequency of flares in patients with active pre-existing autoimmune disease compared with those with clinically inactive disease. Furthermore, this study showed that, while the risk of flare of pre-existing autoimmune disease was ~50%, the rate of *de novo* irAEs appeared similar to rates observed in clinical trials that excluded such patients [64]. For patients who experienced irAEs with anti-CTLA-4 ICI, then changed to anti-PD-1 ICI, recurrence of the same irAE was uncommon despite frequent irAEs [64]. Johnson *et al.* described a 50% combined risk of either flare of pre-existing autoimmune disease or development of a new irAE

[63]. Together, these findings suggest a predisposition to the development of irAEs, although the risk may depend on the ICI used.

### Case studies illustrating mechanisms

In a cohort at Duke University Medical Center, we have had two patients with autoantibodies consistent with rheumatoid arthritis and Sjogren's syndrome but who did not develop symptoms of disease until after starting ICI. The RA patient was a 53-year-old man with advanced melanoma who had a high titre ACPA but negative rheumatoid factor at the time of evaluation. While asymptomatic prior to therapy, his joint symptoms started after 2 months on pembrolizumab.

The patient with Sjogren's syndrome was a 70-year-old woman with non-small cell lung cancer who, at the time of evaluation, had a positive ANA of 1 : 2560 titre by immunofluorescence (homogeneous and speckled patterns), antibodies to SS-A (Ro60), inflammatory arthritis, and sicca symptoms (bilateral corneal erosions). She also did not develop symptoms until after starting CCB with ipilimumab and pembrolizumab. Her sicca symptoms started first, with inflammatory arthritis developing later. Both patients required long-term immunosuppression to control their symptoms, despite discontinuation of ICI (Table 4).

Consideration of the serological and clinical findings of these two patients raises questions about the mechanism for irAEs and the most informative terminology to describe their disease. In general, autoantibody production in RA, SLE and related autoimmune diseases pre-dates symptomatology by many years in a state known as pre-autoimmunity. For our patients, the initiation of ICI may have provided a trigger for the transition from pre-autoimmunity to autoimmunity. As such, the development of disease in pre-autoimmune individuals who are predisposed to disease may be mechanistically distinct from the development of an irAE in a patient without risk factors or pre-existent serological findings.

### Immunosuppression and tumour response

Since patients with pre-existent autoimmune or inflammatory disease may be receiving immunosuppressive therapy at the time of ICI therapy, their ability to respond to ICIs is an important question and relates to the mechanistic relationship of checkpoint inhibition and auto-reactivity. In a study by Menzies *et al.* patients with advanced melanoma and pre-existing autoimmune disease demonstrated a lower response rate to anti-PD-1 therapy if they were on immunosuppressive therapy at treatment onset than those not on immunosuppression [64]. It is not clear, however, whether the patients who were not on immunosuppression had previously been in remission or whether immunosuppressive therapies had been discontinued to facilitate ICI treatment.

In other studies by Kobayashi *et al.* and Raptopoulou *et al.* patients with rheumatological conditions like RA and Sjogren's were more likely to flare on anti-PD-1 therapy

TABLE 4 Patients with autoantibody positive rheumatic disease after immune checkpoint therapy

Patient age/sex	Cancer	Rheumatic diagnosis	Serologies	ICI (MOA)	ICI initiation, month/year	Onset of clinical symptoms (after first dose ICI)	DMARDs (current)	Tumour response	Next steps
53 yo M	Melanoma	RA	Neg RF, +ACPA	Pembrolizumab (anti-PD-1)	04/2017	2 months	Leflunomide + PDN	Progression	Change to ipilimumab (anti-CTLA-4)
70 yo F	NSCLC	Sjogren's syndrome	+ANA 1: 2560, +SSA	Pembrolizumab (anti-PD-1) + ipilimumab (anti-CTLA-4)	06/2016	6 months	Leflunomide + PDN	Stable disease	Surveillance CCB dc'd (03/2018)

ANA: anti-nuclear antibody; CCB: combined checkpoint blockade; CTLA-4: cytotoxic T-lymphocyte antigen-4; dc'd: discontinued; F: female; ICI: immune checkpoint inhibitor; M: male; MOA: mechanism of action; PD-1: programmed cell death protein-1; PDN: prednisone; SSA: SS antibody A; yo: year old.

compared with other autoimmune diseases; this effect may be related to the role of PD-1 positive T cells in rheumatological disease discussed above [66, 67].

Lee *et al.* reported that seven of eight patients with pre-existing RA on immunosuppressive therapy showed partial response or stable disease [68]. One explanation for a more robust treatment response concerns changes in immune function in seropositive RA; because of pre-existing defective T-reg suppressive function, activation of cancer-specific cytotoxic T cells may occur more readily [69].

### Treatment of rheumatic irAEs

Treatment of irAEs is often necessary to reduce symptoms and to allow ICI therapy to proceed. For ICI-associated IA, Naidoo *et al.* proposed an algorithm for evaluation and management [70]. This algorithm includes recommendations for NSAIDs, prednisone, and both non-biologic and biologic DMARDs. One case series by Kim *et al.* demonstrated clear and sustained response of ICI-associated IA to IL-6 inhibition [71]. Evidence thus far indicates that these recommended treatments do not impair the anti-tumour response suggesting differences in the pathways leading to irAEs and the anti-tumour response.

### Conclusion

The use of ICIs has led to a revolution in the treatment of cancer although improved outcomes have been associated with unique side effects known as immune-related adverse events. To advance this important new treatment modality, future studies will need to define the relationship between anti-tumour and anti-self reactivity, develop biomarkers for prediction, and assess new approaches for prevention and treatment, especially in patients with pre-existent autoimmune disease. In view of the number of checkpoints that operate in the immune system, the coming years will see very exciting mechanistic research to maximize anti-cancer responses while minimizing anti-self responses.

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