Pathology of immune-mediated tissue lesions following treatment with immune checkpoint inhibitors

Hajir Ibraheim 1,2,*, Esperanza Perucha3,* and Nick Powell1,2

Abstract
Immune check point inhibitor (CPI) therapy has revolutionized treatment paradigms for several cancers, but at the cost of triggering a diverse spectrum of immune-mediated injury to non-cancer tissues. The complex biology of these toxicities remains incompletely understood, partly because tissue acquisition from affected areas can be challenging to retrieve, thus hindering development of targeted therapy. Here, we review the literature describing pathology of immune-mediated tissue lesions including gastrointestinal, skin, rheumatic, pulmonary, cardiac, renal and hepatic lesions and highlight key immunological insights.

Key words: irAE pathology, CPI enterocolitis, CPI skin toxicity, CPI renal injury, CPI rheumatic toxicity, CPI hepatitis, CPI pulmonary toxicity, CPI cardiac toxicity

Introduction
Whilst immune check point inhibitors (CPIs) induce durable anti-cancer responses in a subset of patients, this comes at the cost of incurring immune mediated toxicities (immune related adverse events [irAEs]). These affect virtually any organ system and can cause significant morbidity, mortality and impaired quality of life. Accordingly, there is a pressing need to understand the etiopathology of irAEs in order to avoid interruption to CPI therapy and offer a targeted approach to their management. Advances in this emerging field are hampered by a paucity of data examining immunopathological aspects of disease. This is partly related to inherent challenges in acquiring tissue from affected areas. In this review, we offer pathological insights into irAEs that have associated histological data (summarized in Table 1).

Autopsy studies permit sampling of tissue that would otherwise be easily accessible (e.g. thyroid, brain), and have been described in this context. Koelzer et al. performed an autopsy study of a young patient treated with anti-CTLA-4 and anti-PD-1 therapy, who developed clinically apparent CPI pneumonitis. Intriguingly, two distinct lung pathologies were found, as well as aseptic meningoencephalitis and myocarditis, suggesting that clinically or radiographically apparent irAE may only represent a small proportion of irAEs that actually occur [1].

Gastrointestinal toxicities
CPI-induced enterocolitis is one of the most common reasons for CPI discontinuation and treatment-related death. The relative ease of tissue sampling of the GI tract via endoscopy offers valuable insights into the immunopathological aspects of this toxicity, as well as in advancing mechanistic insights into CPI therapy.

1Division of Digestive Diseases, Faculty of Medicine, Imperial College London, 2Gastroenterology Unit, Royal Marsden Hospital, London, UK and 3Centre for Inflammation Biology and Cancer Immunology, Centre for Rheumatic Diseases, King’s College London
Submitted 21 May 2019; accepted 5 September 2019
*Hajir Ibraheim and Esperanza Perucha contributed equally to this paper.
Correspondence to: Nick Powell, Division of Digestive Diseases, Imperial College London, 10th Floor QEQM building, St Mary’s Hospital, Praed Street, London W2 1NY, UK. E-mail: npowell@ic.ac.uk

© The Author(s) 2019. Published by Oxford University Press on behalf of the British Society for Rheumatology.
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com
### Table 1 Summary of histopathological and immunological findings in various CPI-induced toxicities

| Reference | Toxicity | CPI regimen/s | Histological findings | Immunological findings and platform used |
|-----------|----------|---------------|-----------------------|-----------------------------------------|
| [2, 6, 9–16, 19, 20, 24] | GI-enterocolitis | Anti-PD-1 monotherapy, anti-CTLA-4 monotherapy and combination regimen | Combined findings: Inflammatory infiltrate in the lamina propria composed of lymphocytes, neutrophils, eosinophils and plasma cells; neutrophilic crypt abscess formation; increased apoptotic activity within the crypt epithelium; crypt epithelial atrophy and crypt dropout. Chronic inflammatory changes including crypt distortion, basal plasmacytosis and paneth cell metaplasia. Granulomas (but uncommon). Lymphocytic colitis and collagenous colitis also described. | Immunohistochemistry and flow cytometry |
| Anti-PD-1: Features of acute colitis; chronic colitis (basal lymphoplasmacytosis and crypt architectural irregularity, paneth cell metaplasia); crypt abscesses; apoptosis; inflammatory infiltrate in the lamina propria composed of lymphocytes, neutrophils, eosinophils and plasma cells. Lymphocytic colitis and collagenous colitis. | Predominance of CD8⁺ cells |
| Anti-CTLA-4: Features of acute colitis; chronic colitis (basal lymphoplasmacytosis and crypt architectural irregularity, paneth cell metaplasia); neutrophilic inflammation only; lymphocytic inflammation only; combined neutrophilic and lymphocytic inflammation; intra-epithelial neutrophilic lymphocytes; cryptitis; crypt abscesses; apoptosis; inflammatory infiltrate in the lamina propria composed of lymphocytes, neutrophils, eosinophils and plasma cells; granulomas. Lymphocytic colitis. | Predominance of CD4⁺ cells with high TNF-α expression. Significantly increased expression of the major Th-1 and Th-17 pro-inflammatory cytokines IFN-γ and IL-17A. No decrease in FoxP3⁺ T regulatory cells. |
| Combination regimen: Lymphocytic colitis. | n/a |
| [28–39, 41, 49, 50] | Skin | Anti-PD-1/PD-L1 monotherapy, anti-CTLA-4 monotherapy and combination regimen | Combined findings: wide range depending on skin lesion but includes: (i) Inflammatory subtype- perivascular lymphocytic infiltrate with eosinophils in the superficial dermis (ranging from scattered to florid), overlying epidermal spongiosis and patchy necrotic keratinocytes are seen. Lichenoid dermatosis shows hyperkeratosis and hypergranulosis. (ii) Immunobullous (only with anti-PD-1/PD-L1) subepidermal cleft and linear deposition of IgG and C3 at the blister roof at the dermoeidermal junction, and a band-like pattern at the dermoeidermal junction. (iii) Alterations of epidermal keratinocytes. (iv) Alterations of epidermal melanocytes. (v) Other, for example, cutaneous interstitial granulomatous dermatitis, with granulomatous infiltrates of interstitial lymphocytes and histiocytes accompanied by | Immunohistochemistry |
| Anti-PD-1 or anti-CTLA-4 monotherapy (combined results, with majority on anti-CTLA-4): | |
| (i) Clear predominance of CD4⁺ over CD8⁺, only rarely they represented approximately equal proportions (n=2, where one patient exposed to both regimens, and another anti-CTLA-4 treated). (ii) Presence of FoxP3⁺ T regulatory cells (not quantified). |

(continued)
**Table 1 Continued**

| Reference      | Toxicity        | CPI regimen/s                                      | Histological findings                                                                 | Immunological findings and platform used |
|----------------|-----------------|----------------------------------------------------|----------------------------------------------------------------------------------------|------------------------------------------|
| [54, 60]       | Rheumatic       | Anti-PD-1 monotherapy, anti-CTLA-4 monotherapy and combination regimen | - Combined findings: inflammatory arthritis – synovial fluid shows high neutrophil counts.  
- Giant cell arteritis – inflammatory infiltrate of the adventitia and muscularis layers in the temporal artery.  
- Myositis - multifocal necrotic myofibers, sarcocennal MHC-I, and endomyosal inflammation.  
- Sicca syndrome - diffuse lymphocyte aggregates and acinar injury. | Immunohistochemistry (sicca syndrome and myositis), TCR repertoire analysis (myositis only) |

Anti-PD-1:  
Anti-CTLA-4:  
Combination regimen:

- Slight predominance of CD4+ over CD8+ T cells.  
- Positive PD-1 and PD-L1 expression.

Sicca syndrome:  
Anti-PD-1 and combination regimen treated patients:

- Abundance of CD4+, CD8+ and CD69+ cells (with absence of CD20+ B cells).  
- TCR analysis of skeletal muscle lymphocytes shows clonal expansion.  

(continued)
| Reference       | Toxicity | CPI regimen/s                                                                 | Histological findings                                                                 | Immunological findings and platform used                        |
|-----------------|----------|-------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|------------------------------------------------------------------|
| [71, 72]        | Renal    | Anti PD-1 monotherapy, anti-CTLA-4 monotherapy and combination regimen         | Combined findings: Tubulointerstitial inflammation is most common finding.            | Combined findings: Anti-PD-1 monotherapy, anti-CTLA-4 monotherapy and combination regimen: Predominantly a CD4⁺ infiltrate. |
|                 |          |                                                                               | Acute interstitial nephritis characterized by diffuse interstitial inflammation and focal severe tubulitis, with a lymphocytic infiltration, and some eosinophils and plasma cells. Granulomatous features also described. 1 patient with thrombotic microangiopathy. |                                                                  |
|                 |          |                                                                               | Combined findings: Combined findings: Tubulointerstitial inflammation is most common finding. |                                                                  |
|                 |          |                                                                               | Combined findings: Acute interstitial nephritis characterized by diffuse interstitial inflammation and focal severe tubulitis, with a lymphocytic infiltration, and some eosinophils and plasma cells. Granulomatous features also described. 1 patient with thrombotic microangiopathy. |                                                                  |
|                 |          |                                                                               | Combined findings: Combined findings: Acute interstitial nephritis characterized by diffuse interstitial inflammation and focal severe tubulitis, with a lymphocytic infiltration, and some eosinophils and plasma cells. Granulomatous features also described. 1 patient with thrombotic microangiopathy. |                                                                  |
| [77, 78]        | Hepatic  | Anti-CTLA-4 or anti-PD-1/PD-L1                                                | Combined findings: main subtypes are an acute hepatitis pattern with panlobular hepatitis, perivenular infiltrate with endothelialitis or biliary pattern with bile ductular proliferation, mild mixed portal inflammation with little lobular necroinflammation. | Combined findings: main subtypes are an acute hepatitis pattern with panlobular hepatitis, perivenular infiltrate with endothelialitis or biliary pattern with bile ductular proliferation, mild mixed portal inflammation with little lobular necroinflammation. |
|                 |          |                                                                               | Anti-PD-1/L1: no granulomatous inflammation, and infrequent occurrence of central vein endotheliitis. | Combined findings: main subtypes are an acute hepatitis pattern with panlobular hepatitis, perivenular infiltrate with endothelialitis or biliary pattern with bile ductular proliferation, mild mixed portal inflammation with little lobular necroinflammation. |
|                 |          |                                                                               | Anti-CTLA-4: granulomatous inflammation, associated with severe lobular necrotic and inflammatory activity and central vein endotheliitis. | Combined findings: main subtypes are an acute hepatitis pattern with panlobular hepatitis, perivenular infiltrate with endothelialitis or biliary pattern with bile ductular proliferation, mild mixed portal inflammation with little lobular necroinflammation. |
|                 |          |                                                                               | Predominance of CD8⁺ cells in portal tracts and lobules. | Combined findings: main subtypes are an acute hepatitis pattern with panlobular hepatitis, perivenular infiltrate with endothelialitis or biliary pattern with bile ductular proliferation, mild mixed portal inflammation with little lobular necroinflammation. |
| [80, 82-84]     | Pulmonary| Anti-PD-1/PD-L1                                                              | Range of findings including cellular interstitial pneumonitis, organizing pneumonia, diffuse alveolar damage and non-caseating granulomas. | Range of findings including cellular interstitial pneumonitis, organizing pneumonia, diffuse alveolar damage and non-caseating granulomas. |
|                 |          |                                                                               | Immunohistochemistry and mass cytometry (for sarcoid lung lesions): Sarcoid lung lesions | Immunohistochemistry and mass cytometry (for sarcoid lung lesions): Sarcoid lung lesions |
|                 |          |                                                                               | Increased CD4⁺: CD8⁺ ratio in bronchoalveolar lavage. | Increased CD4⁺: CD8⁺ ratio in bronchoalveolar lavage. |
|                 |          |                                                                               | Mass cytometry showed abnormally high circulating Th-1/17 cells in the two patients that developed pulmonary sarcoid, prior to commencing CPI. | Mass cytometry showed abnormally high circulating Th-1/17 cells in the two patients that developed pulmonary sarcoid, prior to commencing CPI. |
| [64, 85]        | Cardiac  | Anti-PD-1 monotherapy, anti-CTLA-4 monotherapy and combination regimen         | Combined findings: patchy to florid T-cell-predominant lymphocytic infiltrate with absence of granulomas or giant cells. | Combined findings: patchy to florid T-cell-predominant lymphocytic infiltrate with absence of granulomas or giant cells. |
|                 |          |                                                                               | Anti-PD-1/L1: Anti-CTLA-4: | Combined findings: patchy to florid T-cell-predominant lymphocytic infiltrate with absence of granulomas or giant cells. |
|                 |          |                                                                               | Anti-PD-1/L1: | Combined findings: patchy to florid T-cell-predominant lymphocytic infiltrate with absence of granulomas or giant cells. |
|                 |          |                                                                               | Anti-CTLA-4: | Combined findings: patchy to florid T-cell-predominant lymphocytic infiltrate with absence of granulomas or giant cells. |
|                 |          |                                                                               | n/a | Combined findings: patchy to florid T-cell-predominant lymphocytic infiltrate with absence of granulomas or giant cells. |
|                 |          |                                                                               | Immunohistochemistry, TCR repertoire analysis, whole transcriptome sequencing | Immunohistochemistry, TCR repertoire analysis, whole transcriptome sequencing |
|                 |          |                                                                               | n/a | Combined findings: patchy to florid T-cell-predominant lymphocytic infiltrate with absence of granulomas or giant cells. |
Inflammation has been described along the entire gastrointestinal (GI) tract, from the oesophagus to the colon, with a predilection for the colon—particularly the left side [2-7]. Notably, this may be influenced by sampling bias, as the left side of the colon is more accessible via flexible sigmoidoscopy, whereas right-sided colonic biopsies can only be sampled during colonoscopy (which is more time consuming, costly and requires oral bowel preparation, and hence has additional logistical challenges). Endoscopic findings broadly resemble aspects of inflammatory bowel disease (IBD) including oedema, loss of vascular pattern (in lower GI tract), erythema, erosions, ulcers and mucosal friability, including frank luminal bleeding [2-7]. Necrotising gastritis has also been described [7]. Continuous, confluent inflammation starting from the distal colon and mimicking ulcerative colitis (UC) is typical, but diffuse patchy lesions with normal-looking intervening colonic mucosa, reminiscent of Crohn’s disease (CD) is also seen [3, 6].

Histologically, there is a wide spectrum of disease, which does not appear to correlate with the type of CPI agent used or whether patients are on immunosuppressive therapy prior to biopsy [2, 8]. The most common findings include an inflammatory infiltrate in the lamina propria, composed of lymphocytes, neutrophils, eosinophils and plasma cells [2, 6, 9-11]. Neutrophilic infiltration of the intra-epithelial compartment, and neutrophilic crypt abscess formation are also common [2, 6, 9-11] (Fig. 1). Increased apoptotic activity within the crypt epithelium, reminiscent of graft vs host disease is a finding in up to around half of cases. Crypt epithelial atrophy and crypt dropout is also reported [11]. Granulomas, resembling those seen in CD are very infrequent [12, 13]. Occasionally, features of chronic inflammation including crypt distortion, basal plasmacytosis and Paneth cell metaplasia, which can mimic IBD, have been reported, although the prominent apoptosis and crypt atrophy or dropout seen in CPI-enterocolitis would be unusual in IBD [11, 12, 14]. To date, the temporal relationship between emergence of GI toxicity and chronicity on biopsy is unclear.

A microscopic colitis-like pattern of disease is being increasingly described [7, 15, 16]. Classical microscopic colitis encompasses lymphocytic colitis and collagenous colitis, both of which exhibit a normal endoscopic appearance and are differentiated by histology. There is some evidence that compared with classical microscopic colitis, CPI-microscopic colitis induces a more aggressive disease course requiring more intensive immunosuppression and a greater need for hospitalization [15].

Additional findings in the upper GI tract include lymphocytic gastritis (<30 intraepithelial lymphocytes per 100 epithelial cells). In the duodenum, as well as chronic inflammation with a neutrophil, lymphocyte and plasma cell infiltrate, villus blunting and atrophy have also been described [7, 17].

It is worth highlighting that insights into pathology of lesions mainly stem from mucosal biopsies. Because
colectomy is a rare event, data examining pathology across the colonic walls is sparse. In one case of anti-PD-1 perforating colitis, multiple ulcerations, transmural inflammation and necrosis were described [18]. In four colectomy specimens from patients with anti-CTLA-4 enterocolitis, all showed extensive acute severe colitis with abrupt transition between ulcerations and normal mucosae [12].

There are only a few studies that have characterized histological and immunological features in parallel, but predictably an abundance of CD3+ T cells (and not B cells) are commonly reported [19, 20]. In one study of nine ipilimumab-treated patients with CPI enterocolitis, colonic mucosal expression of the major T helper-1 (Th-1) and Th-17 pro-inflammatory cytokines IFN-γ and IL-17A, were significantly upregulated (>10-fold and >5-fold, respectively). IL-17 has a critical role in regulating colonic neutrophil recruitment [21], which may account for the neutrophilic infiltrate frequently seen in this disease. Patients with CPI-enterocolitis also have increased levels of IL-17A in blood [22] suggesting the IL-17 axis, like in IBD [23], may be a key driver of inflammation.

Given that CTLA-4 is constitutively expressed by FoxP3+ T regulatory (Treg) cells, it was initially postulated that CPI toxicity is mediated by loss of this subset. However, several studies have shown the converse to be true: immunohistochemistry and flow cytometry analysis suggest mucosal Treg cells are not depleted and are often increased [8, 9, 20, 24] along with the regulatory cytokine IL-10 [19]. Further studies are needed to determine whether CPI therapy induces changes to Tregs on a functional level, as has been demonstrated in murine models [25].

Interestingly, there is a suggestion that anti-CTLA-4 and anti-PD-1-induced enterocolitis have distinct immunological characteristics. In a study investigating the mucosal immunological profile (using immunohistochemistry and flow cytometry) of 17 anti-CTLA-4 and five anti-PD-1 induced enterocolitis patients, colonic mucosal T-helper CD4+ cells with high expression of the proinflammatory cytokine TNFα were enriched in the former, whilst cytotoxic CD8+ T cells were enriched in the latter [26]. Further work is needed to develop this line of enquiry in order to deliver a personalized medicine approach and answer questions about the efficacy of anti-TNF therapy between CPI regimens.

Skin toxicities

Dermatologic irAEs exhibit a myriad of clinical manifestations, but most commonly involve pruritic, and/or maculopapular eruptions. Biopsies are easily acquired from the skin, explaining the relatively higher number of publications reporting on their pathological features. Curry et al. categorises this toxicity into four groups, according to their histological patterns: inflammatory, immunobullous, alterations of epidermal keratinocytes and alterations of epidermal melanocytes [27].

The inflammatory subtype, encompassing dermal hypersensitivity reactions manifesting as maculopapular eruptions, are the most commonly occurring cutaneous irAE. Histologically, inflammatory lesions display a perivascular lymphocytic infiltrate with eosinophils in the superficial dermis (ranging from scattered to florid), overlying epidermal spongiosis and patchy necrotic keratinocytes [28–30]. Typically, these are managed with
emollients, topical steroids, and anti-histamines, and do not always necessitate discontinuation of CPI therapy. Also within this category are the increasingly described lichenoid dermatides, which are associated with anti-PD-1/PD-L1 therapy [31–33]. Skin biopsies show a dense, band-like lymphocytic infiltrate, hyperkeratosis, hypergranulosis, saw-tooth ridge pattern, and dyskeratosis [33]. In one case series of three anti-PD-1 treated patients, immunohistochemical staining showed a predominance of the CD4+ component compared with CD8+ and only 10% of T cells staining positive for PD-1 [32].

More severe mucocutaneous skin reactions such as Stevens-Johnson syndrome or toxic epidermal necrolysis (TEN) have been described but are rare [30, 34–36]. In one case of nivolumab-induced TEN, from initial biopsy of a morbilliform pruritic skin eruption to TEN presentation, there was an increase in the number of CD8+ lymphocytes within the dermal–epidermal junction and an increase of PD-L1 expression in both lymphocytes and keratinocytes [34].

Autoimmune blistering eruptions associated with immune checkpoint blockade were initially thought to be rare but are being increasingly reported [37–41]. Bullous pemphigoid (BP), a disease characterized by formation of sub-epidermal blisters as a result of auto-antibodies against hemidesmosomal antigens, has been described in this context. Interestingly, it has only been reported with the anti-PD-1 or anti-PD-L1 regimens. Histology and direct immunofluorescence show the pathognomonic features seen in both classic and drug-induced BP, of subepidermal clef and linear deposition of IgG and C3 at the blister roof of the dermorepidermal junction [37–39]. In one patient where serum ELISA was used as part of the diagnostic work up, auto-antibodies to BP180 (typically seen in classic BP) were also found [39]. Patients tend to respond to corticosteroid therapy but compared with patients with other drug-induced BP, they appear to have a prolonged course, possibly reflecting continued in vivo immune activation.

Lesions associated with altered keratinocytes are uncommon and include Grover’s disease (biopsies show acantholytic dyskeratosis) and prurigo nodularis (histology data not available) [27, 42, 43]. On the other hand, lesions associated with altered melanocytes are more commonly reported, including vitiligo, tumoral melanosis (pigmented lesions that clinically resemble melanoma, but histologically show dense aggregates of melanin-laden benign macrophages and no malignant cells) and regression of melanocytic naevi [28, 30, 44–48].

Additionally, there is the emergence of a new CPI-dermatologic toxicity which does not fit into the categories above: cutaneous interstitial granulomatous dermatitis. This manifests as non-pruritic, often annular plaques involving the inner aspects of the arms, thighs and intertriginous areas. Histologically, granulomatous infiltrates of interstitial lymphocytes and histiocytes are accompanied by fragmentation of collagen and elastic fibres and overlying vascular interface dermatitis [41, 49, 50].

There are at least several case reports of anti-CTLA-4 treated and anti-PD-1 treated patients developing pulmonary sarcoidosis with cutaneous involvement. Dermatologic lesions are either papular or nodular and have been described on the forearms and face with biopsy confirming non-caseating granulomas [51–53].

Characterizing the immunological properties of skin lesions may inform a more targeted therapeutic approach, but data is lacking, especially high-resolution immunophenotyping. In one study, immunohistochemistry of 10 biopsies from nine melanoma patients who developed skin irAEs from either anti-CTLA-4 or anti-PD-1 monotherapy were assessed. Predictably, lymphocytes were mainly CD3+ T cells (isolated CD20+ B cells were also present), with a predominance of CD4+ cells over CD8+ cells. CD4+ cells tended to surround the vascular plexus, while CD8+ cells were more loosely scattered in the dermis, sometimes affecting the epidermis (exocytosis). FoxP3+ Treg cells, representing 1–10% of the total lymphocytes were located in the dermis with a perivascular distribution [50].

Rheumatic toxicities

Inflammatory arthritis

Inflammatory arthritis can affect both small and large joints and be oligo or polyarticular. Radiographic and ultrasound findings show joint effusions, synovial thickening and proliferation, and positive colour power doppler on ultrasound in keeping with active inflammation [54–56]. Consistent with this inflammatory phenotype, synovial fluid analyses show increased white cell counts with high neutrophil infiltration, reminiscent of findings in RA [54]. Interestingly, some CPI treated patients undergo sero-conversion, becoming positive for anti-cyclic citrullinated peptide (anti-CCP) antibodies and/or rheumatoid factor [57].

Vasculitis, polymyalgia rheumatica and giant cell arteritis (GCA)

The most common vasculitis associated with CPI therapy are the large vessel vasculitides (including GCA, and aortitis) and vasculitis affecting the nervous system [58]. Of interest, vasculitis is most commonly associated with anti-PD-1 treatment. The PD-1/PD-L1 axis has recently been reported to be deficient in GCA, with low expression reported in affected tissue. Moreover, T-cell infiltration is increased by CPI treatment in a humanized mouse model of granulomatous vasculitis, with increased expression of inflammatory markers [59]. In a case report of CPI-induced GCA, inflammatory infiltrate of the adventitia and muscular layers in the temporal artery was observed, together with narrowing of the arterial lumen, consistent with intima proliferation and active arteritis [60].

Myositis and myopathy

CPI myositis has been described in several case reports and case series across a range of CPI regimens [61–63] and his associated with myocarditis [61, 63, 64]. In one study, muscle biopsy in all six patients with CPI-myositis...
(anti-PD-1 monotherapy and combination regimen CPI) showed multifocal necrotic myofibers, sarcolemmal MHC-I, and endomyosal inflammation consisting mainly of CD68+ cells (a marker for monocytes/macrophages) expressing PD-L1, and CD8+ cells expressing PD-1 [62]. An abundance of CD4+, CD8+ and CD68+ cells (with absence of CD20+ B cells) was also noted in an autopsy study of two patients [64]. Kimura et al. [61] report a severe case of CPI-induced myocarditis, myoarthritis and myasthenia gravis following one dose of anti-PD-1, with biopsy revealing prominent CD4+ and CD68+ T cells in muscle fibres.

Intriguingly, TCR repertoire analysis performed in the latter two studies both showed clonal expansion in skeletal muscle, suggesting an active antigen-driven adaptive immune response [61, 64].

CPI-induced dermatomyositis appears to be rare [65, 66], displaying classic features of a photosensitive cutaneous rash and elevated serum creatine kinase. In one case, features of inflammation were seen on muscle biopsy [65], whilst in another, biopsies only showed muscle atrophy [66]. The authors suggested this may be because the biopsy was taken after corticosteroid treatment, although in the majority of irAEs biopsies taken whilst patients are on immunosuppressive therapy still yields positive results.

In a cohort study of 1293 patients who received any CPI agent, 10 had myopathy, with five of these undergoing biopsy. Muscle fibre necrosis was reported in two, and a non-specific myopathic process in the other three patients [67].

Other rheumatic manifestations

CPI-induced sicca syndrome has been described in a few cases [54, 68, 69]. One case series (n = 4), included imaging findings of hypoechoic lesions of the glands with lymphocytic aggregates, resembling those reported in Sjögren syndrome. However, in contrast to classical Sjögren syndrome, none of these patients were positive for Ro or La serum antibodies [54]. Another key difference relates to the predominance of T-cell infiltrates found in CPI-induced sicca compared [69] with B-cell infiltrates in Sjogrens [70]. Taken altogether, this suggests that salivary gland destruction in CPI mediated toxicity is mediated by a distinct pathological mechanism.

Renal toxicities

Other pathological findings include granulomatous formations with multinucleated giant cells, lupus nephropathy, thrombotic microangiopathy, nphrotic syndrome focal segmental glomerulosclerosis, minimal-change disease, membranous nephropathy, pauci-immune glomerulonephritis and IgA nephropathy [72]. Renal biopsy of sicca-associated interstitial nephritis demonstrates a T-cell rich infiltrate, suggestive of autoimmune interstitial nephritis, and eosinophils, which are suggestive of a hypersensitivity-like reaction [54].

Administering CPI in renal transplant patients and indeed other recipients of solid organ transplants has been a major concern, with limited data in this subgroup given they are frequently excluded from clinical trials. Similar transplant rejection rates have been reported in both anti-PD-1 and anti-CTLA-4 treated patients [73, 74]. Where available, biopsy findings were consistent with acute... with acute rejection, with a mixture of cellular and antibody mediated rejection.

Hepatic toxicities

Immune-mediated liver injury most frequently manifests as asymptomatic elevations in liver function tests, particularly the transaminases, although fulminant hepatitis and death has been reported [75]. Hepatitis occurs in ~5% of patients on CPI monotherapy [11] and up to 30% on combination therapy [76]. Histologically, this may manifest as a predominant injury to hepatocytes (acute hepatitis pattern) or to bile ducts (biliary pattern) [77]. In the former, findings can include panlobular hepatitis, perivenular infiltrate with endothelitis [77, 78]. In the latter, histological features include bile ductular proliferation and mild mixed portal inflammation with little lobular necroinflammation [77, 78].

Although liver biopsy is infrequently performed it can be useful in discriminating between CPI-induced liver injury, primary autoimmune hepatitis or drug induced liver injury—the diagnosis of which influences management. Eosinophilic infiltration and plasmacytosis seems to occur less frequently in CPI liver injury, with significantly fewer CD20+ or CD4+ lymphocytes [79].

There appear to be differences in the histological patterns between anti-CTLA-4 and anti-PD-1/PD-L1 therapy. In a case series of 16 patients who underwent liver biopsy for CPI hepatitis, anti-CTLA-4 treated patients (n = 7) had granulomatous inflammation, associated with severe lobular necrotic and inflammatory activity and central vein endothelitis. Anti-PD-1/PD-L1 treated patients (n = 9) did not have granulomatous inflammation, and central vein endothelitis was infrequent. Immunostaining suggested that lymphocytes in the portal tracts and lobules were mainly represented by CD8+ T cells in anti-CTLA-4 treated patients, whilst in the other group CD4+ and CD8+ infiltrates were equally represented in the portal tract, with CD8+ cells dominating lobular infiltrates [78].

Pulmonary toxicities

Lung biopsy data in patients with CPI pneumonitis are scarce, but where available show a range of findings,
showed abundant CD4+ and CD8+ T cells, as well as...[80].

Sarcoid-like lung lesions have been described with bronchoalveolar lavage showing an increased CD4+:CD8+ ratio, and bronchial biopsies revealing non-caseating epithelioid granulomas [1, 81, 82]. One study performed immunophenotyping (mass cytometry) on peripheral blood mononuclear cells prior to the first dose of 15 anti-PD-1 treated melanoma patients, of whom two developed sarcoidosis. These were compared with age- and sex-matched healthy controls (n = 15). Analysis demonstrated abnormally high numbers of circulating Th-1/17 T-cells (a subpopulation of human CD4+ T cells that co-produce IFN-γ and IL-17) in five of 15 melanoma patients, including both patients who developed sarcoidosis post CPI [83]. These findings support data that implicates Th-1/17 cells in the pathogenesis of classic sarcoidosis [84].

Cardiac toxicities
Cardiac irAEs are an uncommon but potentially fatal outcome of CPI therapy. In one study where 11 patients with CPI-myocarditis had a cardiac biopsy or autopsy, histological findings included a patchy to florid T-cell-predominant lymphocytic infiltrate, with absence of granulomas or giant cells [85]. Composition of the lymphocyte compartment was not described. Johnson et al. offer further histological and immunological insights, through their autopsy study on two combination-regimen treated (anti-CTLA-4 and anti-PD-1) patients who developed fatal myocarditis [64].

Myocardial and skeletal T-cell infiltrates in both patients showed abundant CD4+ and CD8+ T cells, as well as CD68+ cells. An absence of CD20+ B cells was confirmed...[80].

Cardiac irAEs are an uncommon but potentially fatal outcome of CPI therapy. In one study where 11 patients with CPI myocarditis...[80]. To our knowledge, the cardiac toxicities were not described. Johnson et al. offer further histological and immunological insights, through their autopsy study on two combination-regimen treated (anti-CTLA-4 and anti-PD-1) patients who developed fatal myocarditis [64].

Myocardial and skeletal T-cell infiltrates in both patients showed abundant CD4+ and CD8+ T cells, as well as CD68+ cells. An absence of CD20+ B cells was confirmed...[80].

Acknowledgements
Authors N.P. and H.I. acknowledge that their affiliation has now changed. The previous affiliation was: Centre for Inflammation Biology and Cancer Immunology, King’s College London, London, UK.

Disclosure statement: N.P. has received advisory fees from AbbVie, Allergan, Debiopharm International, Ferring and Vifor Pharma and lectures fees from Allergan, Falk, Janssen, Tillotts and Takeda. The other authors have declared no conflicts of interest.

Funding: This paper was published as part of a supplement funded by an educational grant from BMS.

References
1. Koelzer VH, Rothschild SI, Zihler D et al. Systemic inflammation in a melanoma patient treated with immune checkpoint inhibitors—an autopsy study. J Immunother Cancer 2016;4:13.
2. Poppen MHG, Rozeman EA, van Wijpe S et al. Immune checkpoint inhibition-related colitis: symptoms, endoscopic features, histology and response to management. ESMO Open 2018;3:e000278.
3. Abu-Sbeih H, Ali FS, Luo WY et al. Importance of endoscopic and histological evaluation in the management of immune checkpoint inhibitor-induced colitis. J Immunother Cancer 2016;4:95.
4. Marthey L, Mateus C, Nachury M et al. Ipilimumab colitis: a GETAID multicentric study. J Crohns Colitis 2014;8:S146-S.
5. Gonzalez RS, Salaria SN, Bohannon CD et al. PD-1 inhibitor gastroenterocolitis: case series and appraisal of ‘immunomodulatory gastroenterocolitis’. Histopathology 2017;70:558–67.
6. Verschuren EC, van den Eertwegh AJ, Wonders J et al. Clinical, endoscopic, and histologic characteristics of ipilimumab-associated colitis. Clin Gastroenterol Hepatol 2016;14:836–42.
7 Collins M, Michot JM, Danlos FX et al. Inflammatory gastrointestinal diseases associated with PD-1 blockade antibodies. Ann Oncol 2017;28:2860–5.

8 Adler BL, Pezouh MK, Kim A et al. Histopathological and immunophenotypic features of ipilimumab-associated colitis compared to ulcerative colitis. J Int Med 2018;283:568–77.

9 Gonzalez RS, Salaria SN, Bohannon CD et al. PD-1 inhibitor gastroenterocolitis: case series and appraisal of ‘immunomodulatory gastroenterocolitis’. Histopathology 2017;70:558–67.

10 Wang Y, Abu-Sbeih H, Mao E et al. Endoscopic and histologic features of immune checkpoint inhibitor-related colitis. Inflamm Bowel Dis 2018;24:1695.

11 Karamchandani DM, Chetty R. Immune checkpoint inhibitor-induced gastrointestinal and hepatic injury; pathologists’ perspective. J Clin Pathol 2018;71:665–71.

12 Marthey L, Mateus C, Mussini C et al. Cancer immunotherapy with anti-CTLA-4 monoclonal antibodies induces an inflammatory bowel disease. J Crohns Colitis 2016;10:395–401.

13 Beck KE, Blansfield JA, Tran KQ et al. Enterocolitis in patients with cancer after antibody blockade of cytotoxic T-lymphocyte-associated antigen 4. J Clin Oncol 2006;24:2283–9.

14 Chen JH, Pezouh MK, Lauwers GY, Masria R. Histopathologic features of colitis due to immunotherapy with anti-PD-1 antibodies. Am J Surg Pathol 2017;41:2283–9.

15 Choi K, Abu-Sbeih H, Samdani R et al. Can immune checkpoint inhibitors induce microscopic colitis or a brand new entity? Inflamm Bowel Dis 2019;35:443.

16 Ibraheim H, Spain L, Samani A et al. PWE-025 microscopic colonic inflammation in immune check point inhibitor-induced diarrhoea/colitis. BMJ Publishing Group, 2018;67:A90.

17 Fazal MW, Spain L, Ibraheim H et al. Upper gastrointestinal inflammation in patients with immune-checkpoint inhibitor induced diarrhoea. Gut 2018;67:A66–A7.

18 Celli R, Kluger HM, Zhang X. Anti-PD-1 therapy-associated perforating colitis. Case Rep Gastrointest Med 2018;2018:1.

19 Baminis D, Delladetsima I, Perdiki M et al. Immunological characteristics of colitis associated with anti-CTLA-4 antibody therapy. Cancer Investig 2017;35:443–55.

20 Lord JD, Hackman RC, Moklebust A et al. Refractory colitis following anti-CTLA4 antibody therapy: analysis of mucosal FOXP3+ T cells. Digest Dis Sci 2010;55:1396–405.

21 Powell N, Walker AW, Stolarczyk E et al. The transcription factor T-bet regulates intestinal inflammation mediated by interleukin-7 receptor+ innate lymphoid cells. Immunity 2012;37:674–84.

22 Tarhini AA, Zahoor H, Lin Y et al. Baseline circulating IL-17 predicts toxicity while TGF-beta 1 and IL-10 are prognostic of relapse in ipilimumab neoadjuvant therapy of melanoma. J Immunother Cancer 2015;3:UNSP-39.

23 Globig A-M, Hennecke N, Martin B et al. Comprehensive intestinal T helper cell profiling reveals specific accumulation of IFN-γ+ IL-17+ coproducing CD4+ T cells in active inflammatory bowel disease. Inflamm Bowel Dis 2014;20:2321–9.

24 Oble DA, Mino-Kenudson M, Goldsmith J et al. α-CTLA-4 mAb-associated panenteritis: a histologic and immunohistochemical analysis. Am J Surg Pathol 2008;32:1130–7.

25 Read S, Greenwald R, Izcue A et al. Blockade of CTLA-4 on CD4+ CD25+ regulatory T cells abrogates their function in vivo. J Immunol 2006;177:4376–83.

26 Coutzac C, Adam J, Soulaure E et al. Colon immune-related adverse events: anti-CTLA-4 and anti-PD-1 blockade induce distinct immunopathological entities. J Crohns Colitis 2017;11:1238–46.

27 Curry JL, Tetzlaff MT, Nagarajan P et al. Diverse types of dermatologic toxicities from immune checkpoint blockade therapy. J Cutan Pathol 2017;44:158–76.

28 Sibaud V, Meyer N, Lamant L et al. Dermatologic complications of anti-PD-1/PD-L1 immune checkpoint antibodies. Curr Opin Oncol 2016;28:254–63.

29 Lacouture ME, Wolchok JD, Yospovich G et al. Ipilimumab in patients with cancer and the management of dermatologic adverse events. J Am Acad Dermatol 2014;71:161–9.

30 Goldinger SM, Stieger P, Meier B et al. Cytotoxic cutaneous adverse drug reactions during anti-PD-1 therapy. Clin Cancer Res 2016;22:4023–9.

31 Hwang SJE, Carlos G, Wakade D et al. Cutaneous adverse events (AEs) of anti-programmed cell death (PD)-1 therapy in patients with metastatic melanoma: a single-institution cohort. J Am Acad Dermatol 2016;74:455–61.e1.

32 Joseph RW, Cappel M, Goedjen B et al. Lichenoid dermatitis in three patients with metastatic melanoma treated with anti-PD-1 therapy. Cancer Immunol Res 2015;3:18–22.

33 Tetzlaff MT, Nagarajan P, Chon S et al. Lichenoid dermatologic toxicity from immune checkpoint blockade therapy: a detailed examination of the clinicopathologic features. Am J Dermatopathol 2017;39:121–9.

34 Vivar KL, Deschaine M, Messina J et al. Epidermal programmed cell death—ligand 1 expression in TEN associated with nivolumab therapy. J Cutan Pathol 2017;44:381–4.

35 Hofmann L, Forschner A, Loquai C et al. Colon immune-phenotypic features of ipilimumab-associated perforating colitis. J Cutan Pathol 2017;44:381–4.

36 Saw S, Lee HY, Ng QS. Pembrolizumab-induced Stevens–Johnson syndrome in non-melanoma patients. Eur J Cancer 2016;60:190–209.

37 Carlos G, Anforth R, Chou S, Clements A, Fernandez-Penas P. A case of bullous pemphigoid in a patient with metastatic melanoma treated with pembrolizumab. Melanoma Res 2015;25:265–8.

38 Jour G, Glitza IC, Ellis RM et al. Autoimmune dermatologic toxicities from immune checkpoint blockade with anti–PD-1 antibody therapy: a report on bullous skin eruptions. J Cutan Pathol 2016;43:688–96.
50 Perret RE, Josselin N, Knol AC. Cutaneous autoimmune effects in the setting of therapeutic immune checkpoint inhibition for metastatic melanoma. J Cutan Pathol 2016;43:787–91.

47 Woodbeck R, Metelitsa AI, Naert KA. Granulomatous toxicity from immune checkpoint blockade therapy with an anti-CTLA-4 but not PD-1. J Immunother Cancer 2016;4:55.

49 Abdel-Rahman O, ElHalawani H, Fouad M. Risk of cutaneous toxicity in patients treated with immune checkpoint inhibitors: a meta-analysis. Future Oncol 2015;11:2471–84.

51 Eckert A, Schoeffler A, Dalle S et al. Anti-CTLA4 monoclonal antibody induced sarcoïdosis in a metastatic melanoma patient. Dermatology 2009;218:69.

55 Mooradian MJ, Nasrallah M, Gainor JF, et al. Musculoskeletal rheumatic complications of immune checkpoint inhibitor therapy: a single center experience. Semin Arthritis Rheum 2019;Vol. 48:1127–32.

56 Kostine M, Rouxel L, Barnetche T et al. Rheumatic disorders associated with immune checkpoint inhibitors in patients with cancer—clinical aspects and relationship with tumour response: a single-centre prospective cohort study. Ann Rheum Dis 2018;77:393–8.

57 Belkhir R, Le Burel S, Dunogean L et al. Rheumatoid arthritis and polymyalgia rheumatica occurring after immune checkpoint inhibitor treatment. Ann Rheum Dis 2017;76:1747–50.

58 Daxini A, Cronin K, Sreih AG. Vasculitis associated with immune checkpoint inhibitors—a systematic review. Clin Rheumatol 2018;37:2579–84.

59 Zhang H, Watanabe R, Berry GJ et al. Immune checkpoint deficiency in medium and large vessel vasculitis. Proc Natl Acad Sci 2017;114:E970–E9.

60 Goldstein BL, Gedmitsas L, Todd DJ. Drug-associated polymyalgia rheumatica/giant cell arteritis occurring in two patients after treatment with pembrolizumab, an antagonist of CTLA-4. Arthritis Rheumatol 2014;66:768–9.

61 Kimura T, Fukushima S, Miyashita A et al. Myasthenic crisis and polymyositis induced by one dose of nivolumab. Cancer Sci 2016;107:1055–8.

62 Touat M, Maisonneuve T, Knauss S et al. Immune checkpoint inhibitor-related myositis and myocarditis in patients with cancer. Neurology 2018;91:e985–e94.

63 Anquetil C, Salem J-E, Lebrun-Vignes B et al. Immune checkpoint inhibitor-associated myositis: expanding the spectrum of cardiac complications of the immunotherapy revolution. Circulation 2018;138:743–5.

64 Johnson DB, Balko JM, Compton ML et al. Fulminant myocarditis with combination immune checkpoint blockade. N Engl J Med 2016;375:1749–55.

65 Liewluck T, Kao JC, Mauermann ML. PD-1 inhibitor-associated myopathies: emerging immune-mediated myopathies. J Immunother 2018;41:208–11.

66 Ali SS, Goddard AL, Luke JJ et al. Drug-associated dermatomyositis following ipilimumab therapy: a novel immune-mediated adverse event associated with cytotoxic T-lymphocyte antigen 4 blockade. JAMA Dermatol 2015;151:195–9.

67 Richter MD, Crowson C, Kottschade LA et al. Rheumatic syndromes associated with immune–checkpoint inhibitors: a single—center cohort of sixty-one patients. Arthritis Rheumatol 2019;71:468–75.

68 Smith MH, Bass AR. Arthritis after cancer immunotherapy: symptom duration and treatment response. Arthritis Care Res 2019;71:362–66.

69 Warner BM, Baer AN, Lipson EJ et al. Sicca syndrome associated with immune checkpoint inhibitor therapy. Oncologist 2019;24:1258.

70 Nocturne G, Mariette X. B cells in the pathogenesis of primary Sjogren syndrome. Nat Rev Rheumatol 2018;14:133–45.

71 Cortazar FB, Marrone KA, Troxell ML et al. Clinico-pathological features of acute kidney injury associated with immune checkpoint inhibitors. Kidney Int 2016;90:638–47.

72 Mamlouk O, Selamet U, Machado S et al. Nephrotoxicity of immune checkpoint inhibitors beyond tubulointerstitial
nephritis: single-center experience. J Immunother Cancer 2019;7:2.

73 Wanchoo R, Karam S, Uppal NN et al. Adverse renal effects of immune checkpoint inhibitors: a narrative review. Am J Nephrol 2017;45:160–9.

74 Abdel-Wahab N, Safa H, Abudayyeh A et al. Checkpoint inhibitor therapy for cancer in solid organ transplantation recipients: an institutional experience and a systematic review of the literature. J Immunother Cancer 2019;7:106.

75 O’day S, Maio M, Chiarion-Sileni V et al. Efficacy and safety of ipilimumab monotherapy in patients with pretreated advanced melanoma: a multicenter single-arm phase II study. Ann Oncol 2010;21:1712–7.

76 Belli C, Zuin M, Mazzarella L et al. Liver toxicity in the era of immune checkpoint inhibitors: a practical approach. Crit Rev Oncol Hematol 2018;132:125–9.

77 Kim KW, Ramaiya NH, Krajewski KM et al. Ipilimumab associated hepatitis: imaging and clinicopathologic findings. Invest New Drugs 2013;31:1071–7.

78 De Martin E, Michot J-M, Papouin B et al. Characterization of liver injury induced by cancer immunotherapy using immune checkpoint inhibitors. J Hepatol 2018;68:1181–90.

79 Zen Y, Yeh MM. Hepatotoxicity of immune checkpoint inhibitors: a histology study of seven cases in comparison with autoimmune hepatitis and idiosyncratic drug-induced liver injury. Mod Pathol 2018;31:965.

80 Chuzi S, Tavora F, Cruz M et al. Clinical features, diagnostic challenges, and management strategies in checkpoint inhibitor-related pneumonitis. Cancer Manag Res 2017;9:207.

81 Montaudie H, Pradelli J, Passeron T, Lacour JP, Leroy S. Pulmonary sarcoid–like granulomatosis induced by nivolumab. Br J Dermatol 2017;176:1060–3.

82 Cousin S, Toulmonde M, Kind M et al. Pulmonary sarcoidosis induced by the anti-PD1 monoclonal antibody pembrolizumab. Ann Oncol 2016;27:1178–9.

83 Lomax AJ, McGuire HM, McNeil C et al. Immunotherapy–induced sarcoidosis in patients with melanoma treated with PD–1 checkpoint inhibitors: case series and immunophenotypic analysis. Int J Rheum Dis 2017;20:1277–85.

84 Ramstein J, Broos CE, Simpson LJ et al. IFN-γ-producing T-helper 17.1 cells are increased in sarcoidosis and are more prevalent than T-helper type 1 cells. Am J Respir Crit Care Med 2016;193:1281–91.

85 Mahmood SS, Fradley MG, Cohen JV et al. Myocarditis in patients treated with immune checkpoint inhibitors. J Am Coll Cardiol 2018;71:1755–64.

vii28