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PD-L1 expressing granulomatous reaction as an on-target mechanism of steroid-refractory immune hepatotoxicity.

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Abstract

Immune-related hepatitis is an important toxicity from immune-checkpoint inhibitor (ICPI) therapy, affecting up to 20% of patients on dual CTLA-4/PD-1 inhibitors. The mechanisms underlying this type of drug-induced liver injury (DILI) are poorly understood. We report the case of a patient with ICPI-related hepatitis where the presence of a diffuse granulomatous, PD-L1-positive infiltrate on liver biopsy correlated with a poor response to corticosteroids. Our findings suggest a potential role for activation of the PD-1 pathway within the histiocitic infiltrate as a mechanism of toxicity; further study should attempt to target macrophages in this patient group characterised by steroid-refractoriness.

Keywords: Immune checkpoint inhibitors, hepatotoxicity, hepatitis, DILI, toxicity.

Introduction

Immune-checkpoint inhibitors (ICPI) have revolutionised the treatment landscape of a wide range of malignancies including metastatic melanoma, where combination immunotherapy affords unprecedented 3-year survival rates of 58% in treatment-naïve patients[1]. Currently approved ICPIs can reconstitute the host anti-tumour immune response by blocking tumoural mechanisms of immune evasion, such as the programmed cell death 1 (PD-1) or the cytotoxic T-lymphocyte antigen 4 (CTLA-4) pathway[2].

Inhibition of these immune-tolerogenic pathways acts at different levels of the immune synapsis; where PD-1 inhibitors such as nivolumab re-invigorate the immune-exhausted effector T-cell responses at the periphery, anti-CTLA-4 antibodies including ipilimumab act at the priming phase of the cancer immunity cycle[2].

Combination ICPI therapy exerts synergistic anti-tumour effects, and dual administration of nivolumab/ipilimumab confers a significantly improved rate of objective responses when compared to ipilimumab alone[3].

Immunotoxicity is a major challenge in patients treated with ICPIs, where a wide range of immune-related adverse events (irAE) have been described and clinical presentation frequently mimics autoimmune pathology. Prevalence and intensity of immunotoxicity significantly increases with dual checkpoint blockade, where the probability of Grade 3-4 adverse events can be as high as 59[3]. Hepatitis is a recognised form of immune-related drug-induced liver injury (DILI) that affects approximately 10% of patients on single-agent CTLA-4 blockade and up to 18% of patients treated with dual CTLA-4/PD-1 antibody therapy[4]. The pathogenesis of DILI is influenced by a number factors relating to the offending agent and the host, and in the context of ICPI treatment, prediction of DILI remains clinically elusive[5].

The mainstay of treatment of ICPI-induced DILI is represented by discontinuation of immunotherapy and immediate initiation of high-dose corticosteroids. However, responses are highly heterogeneous, with the toxicities of some patients resolving without corticosteroids and some patients requiring escalation to second-line immune suppressive therapy. Here, we investigated the mechanisms underlying ICPI-mediated hepatotoxicity using multiplex immunohistochemistry on a diagnostic liver biopsy sample of a patient with immune-mediated hepatotoxicity describing a PD-L1^{+} histiocytic reaction to be an immunopathologic correlate of steroid-refractory ICPI-related DILI.

Clinical case

We report the case of a 56-year old man who was first diagnosed with BRAF V600E-mutant melanoma of unknown primary site in September 2016, having presented with a painless left axillary lump. Following axillary lymphoadenectomy, he relapsed in March 2017 with multifocal liver metastases, at which point he was commenced on induction ipilimumab (3 mg/kg) and nivolumab (1 mg/kg) immunotherapy. After 2 cycles he returned to clinic compaining of general malaise, arthro-myalgias and mildly raised ALT at 110 IU/L for which he was started on dexamethasone 8 mg OD.

He presented to the Emergency Department 1 week later with fevers (39.5°C), right upper quadrant tenderness and nausea. Initial investigations revealed worsening liver laboratory tests (ALT 147, AST 120 IU/ml, ALP 144 IU/ml, Albumin 39 g/L, bilirubin 3 umol/L) with ongoing evidence of grade 2 transaminitis but no signs of acute liver failure such as jaundice, ascites or coagulopathy. Full blood count with differentials was within normal range (WCC 6.6 with Eosinophils 0.1, Hb 132, PLT 287). C-reactive protein was elevated at 26 ng/ml and a full septic screen inclusive of blood, urine, throat swab cultures and Chest X-Ray did not reveal a source of infection. Hepatitis serology including Virus B, C and Cytomegalovirus viraemia were negative and a TB elispot test was non-reactive. Serology screen including antibodies against Bartonella, Coxiella species, and anti Herpes Simplex virus IgM were negative, alongside auto-antibody screen inclusive of ANA, ANCA. Restaging MRI showed disease stability within the liver but no radiographic findings to account for the transaminitis. A doppler ultrasound ruled out Budd-Chiari Syndrome, and spleen volume was within normal limits.

A liver biopsy showed moderate portal, periportal and lobular inflammation with evidence of multiple non-caseating epitheloid granulomata, with no evidence of fibrosis or ductular damage, consistent with previous descriptions of immune-mediated drug-induced liver injury

(4). The inflammatory infiltrate was strongly PD-L1-positive and PD-L2-negative, with strong T-cell enrichment and little evidence of B-cell or NK cell involvement (Fig. 1 B-F). No fibrin ring granulomata were seen.

As the patient's liver ALT levels continued to worsen to grade 4 (peak 915 IU/L), following hepatology consultation, he was started on pulsed methylprednisolone (up to 2 mg/kg OD) and subsequently escalated to mofetil-mycofenolate (MMF, 1 g BD). The systemic inflammatory reaction gradually subsided, however ALT normalisation was not reached until 6 weeks after presentation.

Written informed consent was taken from the patient prior to publication of this case report.

Discussion

ICPI-related DILI is a poorly understood irAE with varied clinical presentations and histopathological correlates[6]. The limited evidence currently available is derived from small number of case series of patients treated either with single agent CTLA-4 or dual agent CLTA-4/PD-1 blockade.

Typically, clinico-pathological features of ICPI-related DILI are variable, and the formation of granulomas is a rare histopathological correlate, not reported in initial studies [7,8]. More recent evidence from a study of 16 patients with DILI secondary to ICPIs described for the first time a pattern of granulomatous hepatitis associated with lobular inflammation and necrosis as a typical feature of CTLA-4-associated DILI, which contrasted with the non-granulomatous inflammatory appearances seen in patients receiving single-agent anti PD-1 therapy[4].

Convicing evidence of association between ICPI exposure and granulomatous hepatitis has emerged in the studies by Kleiner, Johncilla and Everett, and is reviewed in Table 1 [4, 9-11]. Interestingly, the presence of fever is not universally present as a prodromic symptom of this peculiar type of DILI, and immunopathologic features remain poorly characterised, more so with regards to the molecular pathways underlying this organ-specific type of auto-immunity. Liver laboratory test values may improve spontaneously following discontinuation of ICPIs or with low-dose corticosteroids, and it has been suggested that it may be possible to stratify patients according to a combination of the degree of derangement of serum bilirubin and INR, alongside the severity of histological appearance [4]. However, at present there exists no validated clinical or histopathological predictor to differentiate patients who will follow a benign course from those with steroid-refractory disease for whom escalation to immunosuppressants is recommended by clinical guidelines[12].

The potential negative impact of steroids on long-term outcomes for patients receiving ICPI reinforces the need for a comprehensive immunopathologic characterisation of ICPI-related DILI in an attempt to improve patient stratification and optimise management[13].

In our case, liver toxicity presented atypically. The systemic outset with fever and constitutional symptoms dominated the clinical picture and pre-dated the ALT rise, whilst the protean manifestation and the protracted time to ALT clearance were recognised challenges in the management of our patient.

Despite discontinuation of ICPIs and administration of high-dose corticosteroids, liver laboratory test values continued to worsen requiring escalation to mofetil-mycophenolate (MMF).

In this patient, dual checkpoint inhibition led to diffuse granulomatous infiltration of the liver parenchyma. Notably, a focal PD-L1-positive and PD-L2-negative, predominantly histiocitic, infiltrate was seen, a finding that was not documented in previous studies. These data, whilst based on a single case observation, may provide useful insight into the pathogenesis of this atypical form of liver injury, confirming, for the first time, ICPI-related DILI as an "on-target" adverse effect of immunotherapy.

Mechanistic evidence suggests dexamethasone to suppress CD28-stimulated differentitation of naïve T lymphocytes, with blockade of CTLA-4, but not PD-1, significantly reducing the immune-suppressive effects of corticosteroids[14]. A PD-L1-expressing granuloma is an important hypothesis-generating finding: it suggests the importance of dual CTLA-4/PD-1 blockade of the tissue resident macrophages that drive immune pathology, whilst activation of the PD-1 pathway within the infiltrate implies its role as a potential mediator of steroid-refractoriness.

Conclusion

We have described a case of corticosteroid-refractory hepatotoxicity secondary to immune checkpoint inhibitors where biopsy revealed a granulomatous infiltrate that was shown to express PD-L1. This gives insight into the mechanisms underlying this on-target toxicity; further characterisation of its immunobiology may improve our ability to manage this challenging side-effect.

Future Perspective

The use of ICPIs is becoming more widespread for a variety of tumour types. As such, clinicians may see an increasing incidence of this hepatotoxicity in the future. Further characterisation

of the immunobiology of ICPI-related DILI, with a focus on macrophage function, should be prioritised in future studies.

Whilst this represents a key step to understanding the mechanism of liver immunotoxicity, it may also serve as a diagnostic tool to aid clinicians in dissecting the clinical and biological heterogeneity of this patient population and help to rationalise treatment strategies on the basis of severity. Importantly, our case suggests that selective inhibition of pathways regulating the activation of tissue-resident macrophages may be a therapeutic target within the challenging patient group characterised by refractoriness to steroids.

Given that 10-20% of patients on single or dual agent ICPI are affected by ICPI-related DILI, more effective stratification and treatment of these patients is urgently required.

Summary Points

Immune checkpoint inhibition

- Immune checkpoint inhibitors are a highly effective therapy across a number of malignancies.
- ICPIs target either the CTLA-4 or PD-1 axis, and in metastatic melanoma simultaneous dual-agent therapy against both pathways confers significant added benefit.
- Immune-related adverse events are common with this form treatment and their clinico-pathology can appear to be similar to auto-immune pathology.

ICPI-related DILI

- Hepatotoxicity secondary to ICPIs is a common adverse event and has a varied clinical presentation.

- A small number of studies have analysed liver biopsies of affected patients and have shown varied histo-pathology: a pattern of granulomatous hepatitis is demonstrated within certain patients.
- Recommended therapy involves immunosuppression with high-dose corticosteroids; responses are heterogenous, in certain patients the hepatitis fails to resolve and frequently escalation to mofetil-mycophenolate (MMF) is required.
- There is little evidence to stratify which patients will require, and respond to, immunosuppression and this represents a significant barrier to anti-cancer treatment within this subset of patients on ICPI therapy.
- The immunobiology of ICPI-related DILI is poorly understood.

Our case

- We describe a case of dual-agent ICPI-related DILI where a prodrome of fever and constitutional symptoms were followed by an ALT rise which did not resolve following treatment with high-dose corticosteroids and required escalation to MMF.
- PD-L1 positive diffuse granulomatous infiltrate was seen on liver biopsy, demonstrating that this ICPI-related DILI represents an 'on-target' immune-related adverse event.
- This suggests a pathological role for the PD-1 pathway in mediating this steroid-refractory ICPI-related DILI.

NO DISCLOSURES:

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

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

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

INFORMED CONSENT DISCLOSURE:

The authors state that they have obtained verbal and written informed consent from the patient/patients for the inclusion of their medical and treatment history within this case report.

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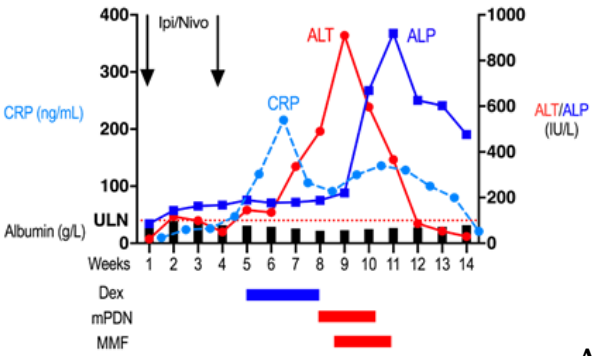
Figure Legends.

Figure 1. A. Changes in alanine transaminase (ALT, IU/L red line) above upper limit of normality (ULN, IU/L red dotted line), alkaline phosphatase (ALP, IU/L dark blue line), C-reactive protein (CRP, ng/ml light blue line) and albumin (Alb g/L, black columns) following treatment with ipilimumab/nivolumab (black arrows) dexamethasone (Dex), methyl-prednisolone (mPDN), and mofetil-mycophenolate (MMF). **B-F.** Liver biopsy specimen

showing evidence of non-necrotizing granulomata (**B**) with strong PD-L1-positive (**C**), PD-L2-negative (**D**) infiltrate. Multi-colour immunohistochemistry in panel **E** shows CD4 (brown chromogen) and CD8 (red chromogen) T-cell enrichment, with occasional presence of $CD4^{+}/FOXP3^{+}$ regulatory T-cells, and a paucity of B-cell (panel **F**, blue chromogen) and NK cell infiltration (brown chromogen).

Table 1. Table overviewing the clinical findings of reported cases of granulomatous hepatotoxicity from the studies of Kleiner, Johncilla and Everett (9-11). In particular, we outline whether fever was present in the prodrome, the value at which ALT peaked, the ICPI used, and whether the patient required treatment with corticosteroids, and if so, whether they responded sufficiently or if escalation to MMF was necessitated. N/a's represent cases for which the relevant information was not inclued in the named study.


Ipi/Nivo
ALT
ALP
CRP
CRP (ng/mL)
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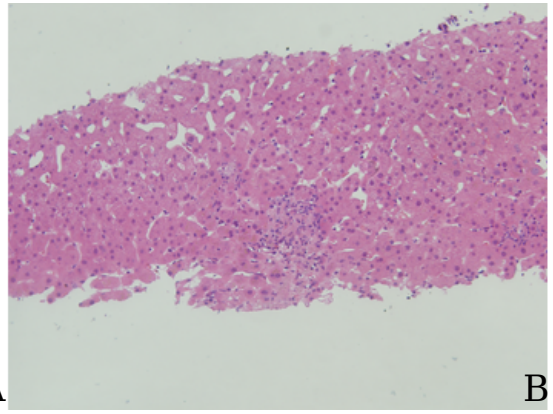
Albumin (g/L)
ULN
Weeks
Dex
mPDN
MMF

A



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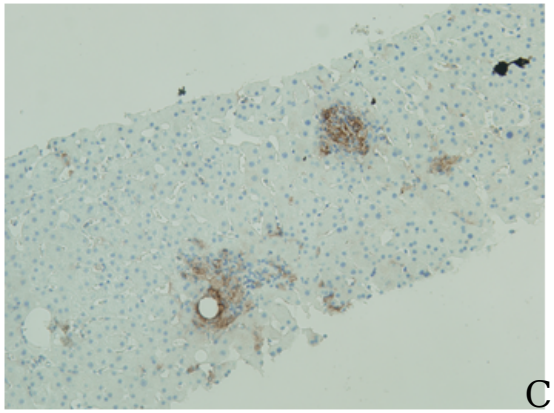

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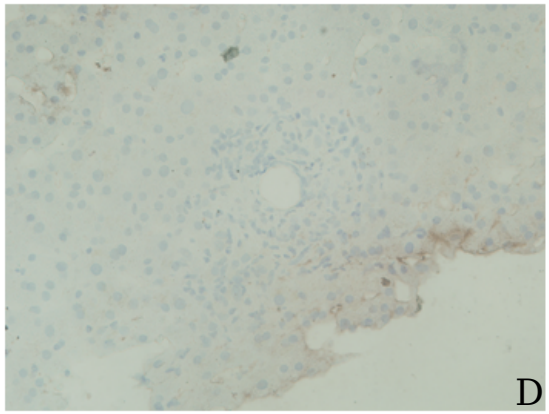

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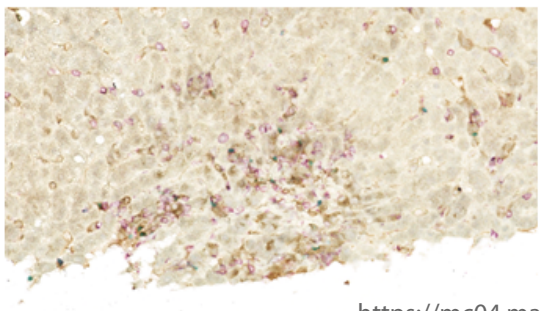

E

F

Case number	Reference	ICPI (Dual/Monotherapy)	Fever	ALT peak	Immunosuppressive therapy (corticosteroids/MMF)
1	De Martin*et al.*[4]	Dual	Yes	n/a	None
2	De Martin*et al.*[4]	Dual	Yes	n/a	Corticosteroids
3	De Martin*et al.*[4]	Single (CTLA-4)	Yes	n/a	MMF
4	De Martin*et al.*[4]	Single (CTLA-4)	Yes	n/a	Corticosteroids
5	De Martin*et al.*[4]	Single (CTLA-4)	Yes	n/a	Corticosteroids
6	De Martin*et al.*[4]	Single (PD-1)	No	n/a	None
7	De Martin*et al.*[4]	Single (PD-1)	No	n/a	None
8	Kleiner*et al.*[9]	Single (CTLA-4)	Yes	304	MMF
9	Johncilla*et al.*[10]	Single (CTLA-4)	n/a	3075	Corticosteroids – response unknown
10	Johncilla*et al.*[10]	Dual	n/a	189	Corticosteroids – response unknown
11	Johncilla*et al.*[10]	n/a	n/a	185	Corticosteroids – response unknown
12	Johncilla*et al.*[10]	n/a	n/a	384	Corticosteroids – response unknown
13	Everett*et al.*[11]	Dual	Yes	130	Corticosteroids
14	Everett*et al.*[11]	Dual	Yes	643	MMF
15	Black*et al.*	Dual	Yes	915	MMF