CD8 T-cell-mediated cerebellitis directed against Purkinje cell antigen after ipilimumab for small cell lung cancer

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Central nervous system complications have been associated with the use of immune checkpoint inhibitors for treating malignancy, including meningoencephalitis, encephalitis, cerebellitis and hypophysitis [1]. Paraneoplastic autoimmune central nervous system syndromes also occur without immune checkpoint inhibitor treatment, which correlate with the endogenous antitumour response [1]. Histological detail is now required to decipher the antibody and/or cytotoxic mechanisms in these cases of immune checkpoint inhibitor associated central nervous system complications and compare these to recognised paraneoplastic syndromes, such as paraneoplastic cerebellar degeneration (PCD). Reports of immune-mediated cerebellar syndromes following immune checkpoint inhibitors have been published (see Table S1). One study reported brain biopsy results revealing a non-specific mature T-cell perivascular infiltrate [2], but none provided clear evidence of a pathological mechanism. Here, we report cerebellitis following CTLA-4 inhibitor monotherapy (ipilimumab) in combination with carboplatin and etoposide and provide histological evidence for a CD8 T-cell-driven response against a Purkinje cell antigen.

A 63-year-old man presented with superior vena cava obstruction secondary to a tumour in the right lung apex. Histology from an endobronchial ultrasound-guided biopsy revealed small cell lung cancer. Baseline anti-neuronal antibodies including anti-Hu, Ri, CRMP5, Ma1, Ma2, amphiphysin, voltage-gated calcium channel (VGCC) and voltage-gated potassium channel complex were absent, except for weakly positive anti-Yo antibodies. After stenting, he entered a single-arm Phase II trial of ipilimumab with carboplatin and etoposide chemotherapy for extensive small cell lung cancer [3]. Serial body computed tomography scans 10–24 weeks after presentation showed...
a partial and almost complete response to treatment. He developed an asymptomatic anti-VGCC antibody response (522 pM) in Week 12 after ipilimumab. Early adverse events, considered to be ipilimumab-related, were an acneiform skin rash (Grade 1, Common Terminology Criteria for Adverse Events Version 4.0) and diarrhoea (Grade 1); both settled spontaneously. A CT scan in Week 36 showed tumour recurrence in the right upper lobe and hilar lymph nodes, which were positive on positron emission tomography.

During Week 37, he developed nausea, vomiting, slurred speech and incoordination. On examination, he had a gross cerebellar syndrome with a scanning dysarthria and nystagmus, and he could not walk due to axial and limb ataxia. A mild left abducent nerve palsy was present. There were no symptoms or signs of Lambert–Eaton syndrome. Brain and spinal cord magnetic resonance imaging with gadolinium contrast was normal. Cerebrospinal fluid was acellular with normal glucose but a raised protein (1070 mg/L). Isoelectric focusing showed matched oligoclonal bands in cerebrospinal fluid and serum. Anti-neuronal antibody screen was comparable with previous with weakly positive anti-Yo, anti-VGCC (665 pM) and negative antibodies against Hu, Ri, CRMP5, Ma1, Ma2, amphiphysin, gangliosides, glutamic acid decarboxylase and voltage-gated potassium channel complex. Despite treatment with intravenous methylprednisolone (1 g daily for 4 days) and infliximab (5 mg/kg), he continued to rapidly deteriorate and died of an aspiration pneumonia in Week 39.

Post-mortem macroscopic examination of the lungs confirmed the right upper lobe bronchopneumonia and bulky lymph nodes in the right hilum. Histological examination confirmed right upper lobe bronchopneumonia. Hilar lymph nodes were infiltrated with small cell lung cancer cells (Figure 1a), staining strongly for CD56 (neural cell adhesion molecule; Figure 1b), scantly for synaptophysin and negative for chromogranin A, suggesting neuronal differentiation. A high mitotic index (45% Ki-67 positive cells) was observed (Figure 1c). Macroscopic appearance of the brain was unremarkable. Histology of the cerebral cortex, hippocampus and corpus striatum was normal, whereas the brainstem showed a mild lymphocytic perivascular infiltrate in the midbrain and medulla. The main abnormalities were in the cerebellum, where marked patchy Purkinje cell loss was observed and confirmed using calbindin immunohistochemistry (Figures 1d and 2a,b). In the cerebellar meninges and white matter, a diffuse T-cell infiltrate was present. In the cerebellar cortex, there was patchy infiltration by CD3-positive T cells spatially corresponding to areas of Purkinje cell loss; this spatial concordance was striking (Figure 1d). Microglia expressed CD68 and HLA-DR and had a typical activated microglial morphology; their presence in the molecular layer suggests that these cells were busy with phagocytosis of the Purkinje cell dendrite tree. CD8 T-cell infiltration was identified in areas of Purkinje cell loss (Figure 1e), with occasional clear evidence of CD8 polarisation towards the Purkinje cells (Figure 1f), a surprising finding because cytotoxic CD8 T-cells kill their target within minutes [4]. Infiltrating T cells were positive for TIA-1, which is constitutively expressed by cytotoxic CD8 T cells [5] (Figure 2c) but negative for granzyme B and perforin, probably because the patient was treated with high-dose steroids [6,7]. Multiple instances of contact between CD8 T cells and Purkinje cell bodies and processes were observed by confocal microscopy of sections double-stained for CD8 and calbindin (Figure 2d–f). B-cells were sparse (6/mm³), compared with CD4 and CD8 T cells (250/mm³ and 243/mm³, respectively). The CD4:CD8 ratio was 1.03 in the cerebellum and 0.83 in the Purkinje cell layer. Glial fibrillary acidic protein immunohistochemistry revealed an isomorphic gliosis in the molecular layer as a result of activation of Bergmann astrocytes, particularly intense in areas of Purkinje cell loss. Neurofilament staining was reduced in areas where Purkinje cells were absent. Occasional Purkinje axonal swellings (‘torpedoes’) were observed, reflecting damage to Purkinje cells.

This is the first case demonstrating that CTLA-4 inhibitor monotherapy can trigger immune-mediated cerebellitis, adding to the existing reports of cerebellitis associated with immune checkpoint inhibitor therapy (Table S1). Unlike these cases, the illness was rapidly progressive and fatal and did not respond to immunosuppression. This poor treatment response, along with the clinical–radiological dissociation involving normal magnetic resonance imaging of the brain and cord, was highly reminiscent of PCD.

Small cell lung cancer is commonly associated with PCD [8], and in this context, ipilimumab treatment may have led to an accelerated form of PCD by triggering de novo cellular autoimmunity against an onconeural antigen [9], or exacerbating an underlying such response. This hypothesis is supported by findings from a transgenic mouse model of PCD, where a neo-self-antigen was expressed in Purkinje neurons and implanted breast tumour cells and CD8 T-mediated cerebellitis only occurred after CTLA-4 inhibition [10].

The typical histological findings in PCD include diffuse infiltrates of CD8 T cells and macrophages, and activated microglia, with no Purkinje cell-centred IgG or complement activation and few or absent CD4 T and B cells [11]. In our patient, we similarly observed a parenchymal CD8 T-cell cerebellar infiltrate with sparse B cells. This infiltrate spatially corresponded to areas of Purkinje cell loss and was associated with evidence of CD8-mediated cytotoxic attack on these cells. A Purkinje cell antigen was implicated, but it is unlikely to have been the P/Q VGCC, because CD8 T-cell infiltration was not observed in non-cerebellar areas known to express this VGCC isoform, such as the hippocampus and globus pallidus [12]. The onset of clinical symptoms of cerebellitis coincided with small cell lung cancer recurrence, and we postulate that the recurrent tumour started expressing an onconeural antigen shared with Purkinje cells, which was not expressed by the primary tumour. So-called antigenic drift is known to occur, especially during the emergence of new clones resistant to treatment [13].

An anti-VGCC antibody response was observed, but this was unlikely to be pathogenic because pronounced T-cell infiltration is not described in antibody-mediated encephalitides, there were no clinical features of Lambert–Eaton syndrome, and clinical symptoms manifested five months after the appearance of anti-VGCC antibodies, after which an unusual and extremely rapid pace of neurological progression was seen. Moreover, CD8 T-cell
infiltration was marked as shown by the CD4:CD8 T-cell ratio (1.03), lower than what is usually observed in cerebrospinal fluid (2.2–3.4) and blood (1.5–1.9) in controls, meningitis and multiple sclerosis [14], in keeping with preferential CD8 T-cell recruitment, especially in the Purkinje cell layer where the CD4:CD8 ratio was 0.8. In addition, multiple instances of CD8-Purkinje cell contact were observed. Hence, it is unlikely that anti-VGCC antibodies were the main and sole pathogenic driver, in keeping with the unresponsiveness of cerebellar dysfunction progression with plasma exchange in patients with PCD and anti-VGCC antibodies [15–17]. Also, when immunoglobulin G from a PCD patient with anti-VGCC antibodies was injected intrathecally, no Purkinje cell loss was observed despite short-lived ataxia [18]. The most parsimonious interpretation is that anti-VGCC antibodies do not cause Purkinje cell death and occur secondary to their damage in most cases.

Although a weak anti-Yo antibody response was present, the relative pathogenicity of these antibodies is under debate [19]. Their low titre in this case and the occurrence of a rapidly progressive cerebellitis in the absence of a rise in anti-Yo titre, as well as the direct histological demonstration of CD8-mediated attack, are highly suggestive of a T-cell-mediated mechanism. This is consistent with the fact that ipilimumab can stimulate de novo T-cell responses [9].

This case highlights the need for continued vigilance with ipilimumab and other immune checkpoint inhibitors, especially when anti-neuronal antibodies are present at baseline. We additionally provide a unique insight into the development of PCD-like neuropathology with ipilimumab. This may represent the early stage of PCD.
proper, the understanding of which is built on neuropathological study of advanced or end-stage disease. The pathogenic role of CD8 T-cells demonstrated here supports the use of immunosuppressive treatments aimed at T cells, such as tacrolimus [20].

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CONFLICT OF INTEREST
The authors report no conflict of interests.

ETHICAL STATEMENT
The ICE study had national Research Ethics Committee approval 10/H0502/95.

AUTHOR CONTRIBUTIONS
MH performed the literature review, performed cell quantification and wrote the manuscript under the supervision of IG; LN, CHO, EA and IG were part of the clinical team managing the patient; JARN and SJ performed post-mortem examination and histology; DJP processed samples and performed further analyses; JN performed immunohistochemistry; CHO was the chief investigator of the ICE study; all authors contributed to intellectual discussions and manuscript revision.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of this article.

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