Journal for ImmunoTherapy of Cancer

Early rise in brain damage markers and high ICOS expression in CD4+ and CD8+ T cells during checkpoint inhibitor-induced encephalomyelitis

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To cite: Bjursten S, Pandita A, Zhao Z, *et al*. Early rise in brain damage markers and high ICOS expression in CD4+ and CD8+ T cells during checkpoint inhibitor-induced encephalomyelitis. *Journal for ImmunoTherapy of Cancer* 2021;**9**:e002732. doi:10.1136/jitc-2021-002732

► Additional online supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jitc-2021-002732).

Accepted 10 June 2021

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ABSTRACT

We report a case of rapid eradication of melanoma brain metastases and simultaneous near-fatal encephalomyelitis following double immune checkpoint blockade. Brain damage marker S-100B and C reactive protein increased before symptoms or signs of encephalomyelitis and peaked when the patient fell into a coma. At that point, additional brain damage markers and peripheral T cell phenotype was analyzed. The analyses were repeated four times during the patient's recovery. Axonal damage marker neurofilament light polypeptide (NFL) and astrocytic damage marker glial fibrillar acidic protein (GFAP) were very high in blood and cerebrospinal fluid and gradually normalized after immunosuppression and intensive care. The costimulatory receptor inducible T cell costimulatory receptor (ICOS) was expressed on a high proportion of CD4+ and CD8+T cells as encephalomyelitis symptoms peaked and then gradually decreased in parallel with clinical improvement. Both single and double immune checkpoint inhibitor-treated melanoma patients with other serious immune-related adverse events (irAE) (n=9) also expressed ICOS on a significantly higher proportion of CD4+ and CD8+T cells compared with controls without irAE (n=12). In conclusion, our results suggest a potential role for ICOS on CD4+ and CD8+T cells in mediating encephalomyelitis and other serious irAE. In addition, brain damage markers in blood could facilitate early diagnosis of encephalitis.

Immune checkpoint blockade increases survival in patients with metastatic malignant melanoma, renal cell carcinoma, and lung cancer.[1–4] Double immune checkpoint blockade activates T cells by blocking the inhibitory receptors programmed cell death 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) simultaneously, thereby promoting T cells to kill cancer cells. However, it increases the risk of autoimmune reactions in healthy tissue, immune-related adverse events (irAE).[5] Any organ system can be affected by irAE and among the most feared is engagement of the brain, encephalitis.[6–8] With the growing use of immune checkpoint blockade in cancer patients, encephalitis cases are expected to increase. A clinical challenge is to quickly and accurately diagnose encephalitis, which is difficult to distinguish from conditions such as progression of brain metastasis or infection that give rise similar symptoms. Also, little is known about the T cell mediated mechanisms promoting encephalitis and other serious irAE.

ENCEPHALOMYELITIS CASE AND CONTROLS

Encephalomyelitis case

A 67-year-old man with metastases of melanoma in the brain, adrenal glands, lung, subcutis, and lymph nodes started double immune checkpoint blockade; PD1-inhibitor nivolumab (1 mg/kg) and the CTLA-4 inhibitor ipilimumab (3 mg/kg) given four times at 3-week intervals (figure 1A). After two treatments, MRI scans (MRI) showed regression of the brain metastases (figure 1B). The day after the fourth treatment, he developed a fever (39.1°C), elevation of C reactive protein (CRP) (45 mg/L), and dizziness. He was admitted to the oncology ward at Sahlgrenska University Hospital and was given IV antibiotics. After 2 days, he developed paraparesis in his lower limbs, decreased consciousness, and respiratory failure. His CRP level increased to 130 mg/L.

He was admitted to the intensive care unit (ICU), intubated, and put on mechanical ventilation. On ICU day 1, MRI showed complete regression of his brain metastases but new diffuse lesions in the brain, brainstem, and cerebellum with a bilateral and asymmetric pattern (figure 1B), indicating acute disseminated encephalomyelitis.[9] Analyses of cerebrospinal fluid (CSF) suggested

autoimmune encephalitis (online supplemental table S1). Immunosuppression with methylprednisolone (1 g daily intravenously) was started. The next day he was alert during sedation stops but required respiratory support, reflecting brain stem damage. MRI on ICU day 3 revealed unchanged brain lesions and lesions in the spinal cord. Cyclophosphamide was administered (1.5 g intravenously as a single dose), but his condition did not improve. On ICU day 6, mycophenolate mofetil (1 g two times per day intravenously) was started to inhibit T and B cell proliferation.[10] The next day, he had improved and could be extubated and transferred back to the oncology ward. He had flaccid paraparesis in his lower limbs with minor residual sensation and urine and fecal incontinence. High-dose cortisone and mycophenolate mofetil were continued, and intravenous immunoglobulins were given (35 g daily for 5 days). MRI 10 and 17 days after ICU admission revealed gradual decrease in lesions in the brain and spinal cord (figure 1B).

After 6 weeks, the patient was discharged from hospital care. Now, he had regained most sensory functions, and he could sit up and operate a wheelchair. After 10 weeks, the inflammatory lesions had resolved on MRI. The immunosuppressant treatment was tapered and permanently discontinued after 14 months. More than 2 years after the first dose of double immune checkpoint blockade, the patient no longer suffers from fecal incontinence and he can walk more than 100 steps with assistive devices. By becoming more independent, the patient's quality of life has improved significantly and he remains tumor-free.

Control patients with or without irAE

In addition to the encephalomyelitis patient, we also analyzed T cell characteristics in checkpoint inhibitor-treated patients with (n=9) or without other irAE (n=12) (table 1). The irAE were moderate to severe and occurred during double (n=2, including the encephalomyelitis patient) or single (PD-1) inhibition (n=8). Similarly, samples were obtained from patients without irAE during double (n=2) or single (PD-1) checkpoint inhibition (n=10). In addition, we performed repeated analysis of cytokines and soluble checkpoint proteins in CSF from the encephalomyelitis patient. As controls for this analysis, we used CSF from patients with autoimmune systemic lupus erythematosus without encephalitis (n=4).

METHODS

T cell characteristics, brain damage markers, and soluble checkpoint proteins were analyzed as described in online supplemental appendix.

RESULTS

Incidence of encephalitis

The number of reported cases of encephalitis following both single and double immune checkpoint blockade is increasing and the fatality rate is high (WHO global database VigiBase, online supplemental figure S2).

Brain damage markers and inflammation

Retrospective analysis of blood tests unexpectedly revealed a distinct pattern that was not evident during the patient's stay. The brain damage marker S-100B and the inflammatory marker CRP covaried strikingly over time (figure 1C), and levels of both were elevated after two treatments with ipilimumab/nivolumab. At that point, the patient had no symptoms, and MRI showed regression of the brain metastases and no signs of encephalitis. After the fourth and final treatment, S-100B and CRP peaked, indicating combined inflammation and brain damage. The patient rapidly deteriorated, and MRI showed acute disseminated encephalomyelitis. These results suggest that brain damage markers in blood may indicate encephalitis before the appearance of typical signs on MRI or clinical neurological symptoms.

Extensive analysis of blood and CSF confirmed brain damage and inflammation (online supplemental figure S3, tables S1 and S4) but showed no signs of infection or autoantibodies (online supplemental tables S1 and S4). The brain damage markers neurofilament light polypeptide (NFL) and glial fibrillar acidic protein (GFAP) were extremely high in both CSF and plasma as symptoms peaked and gradually normalized during immunosuppression (online supplemental figure S3, tables S1 and S3). Tau and S-100B levels were moderately increased (online supplemental table S3). Collectively, these findings suggest severe axonal damage (NFL) and astrocyte injury (GFAP).[11] Interferon-γ, tumor necrosis factor-α, and interleukin-6 levels were increased in CSF when symptoms peaked and normalized during recovery (online supplemental figure S3). The checkpoint proteins PD1, PD-L1, and Tim-3 showed a similar pattern (online supplemental figure S4). CTLA-4 and LAG-3 were not elevated.

T cell characteristics

Flow cytometry of peripheral T cells was done on ICU day 2 and repeated four times. The analysis included T cell subtypes, activation markers, costimulatory receptors, inhibitory immune checkpoints, transcription factors, and attack enzymes (online supplemental table S2). In addition to our encephalomyelitis patient, T cell phenotype was also analyzed in patients with other irAE as well as in patients without irAE (table 1). The most striking observation in the encephalomyelitis patient was high expression of inducible T cell costimulatory receptor (ICOS) on all subtypes of CD4 +T helper cells (figure 2A) (online supplemental figure S5) and on CD8+ cytotoxic T cells. ICOS expression on T cells gradually normalized during immunosuppression and in parallel with clinical improvement (figure 2B–D) (online supplemental figure S5B,C). Similarly, high ICOS on CD4+ and CD8+T cells was detected also in patients with other irAE (figure 2A) and decreased when irAEs had resolved (figure 2B). One

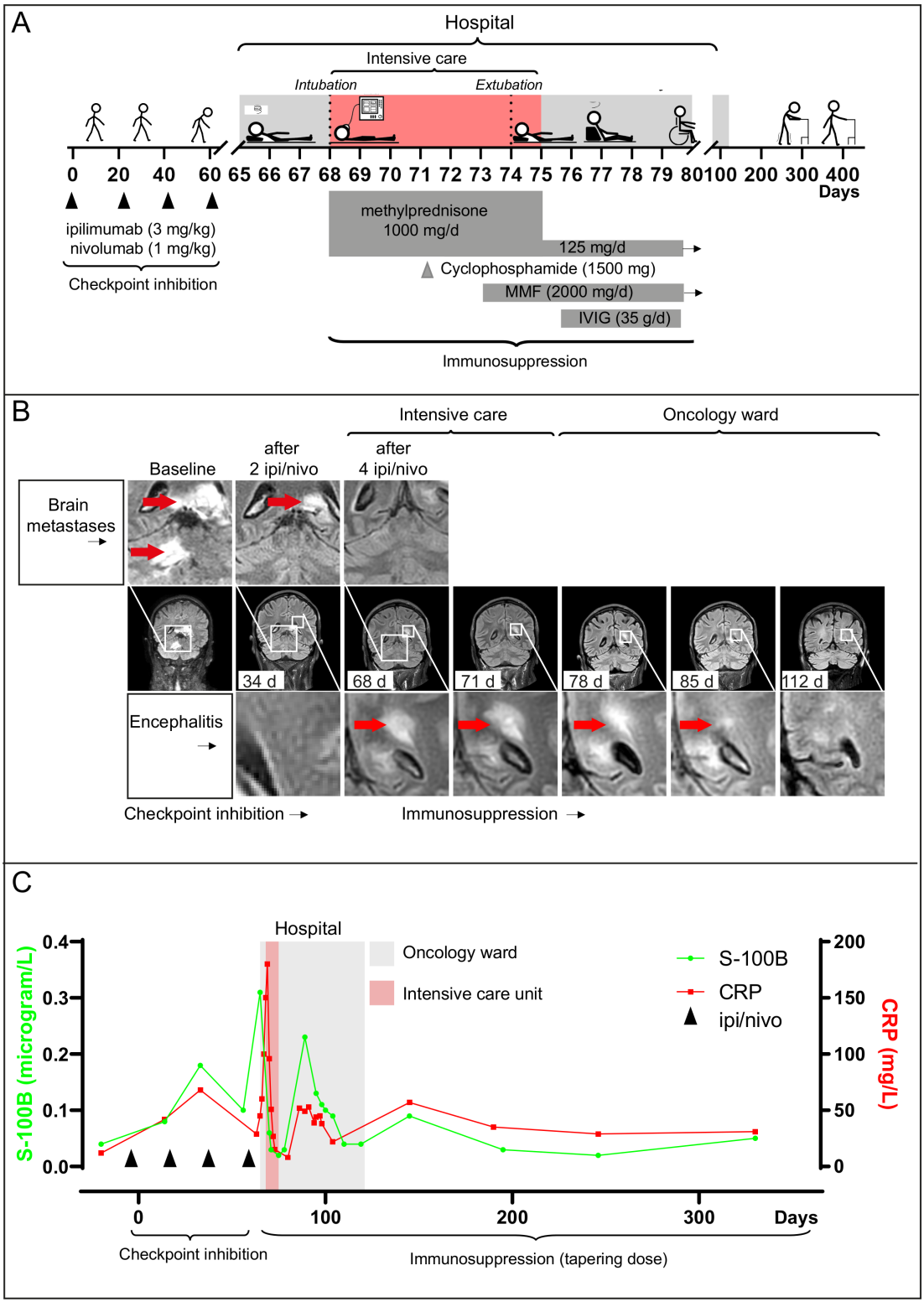

Figure 1 Regression of encephalomyelitis induced by double immune checkpoint blockade. (A) shows a clinical overview. (B) shows regression of brain metastases and progression of encephalitis on MRI scans. At baseline, the patient had a 25 mm metastasis in the left portion of the splenium corpus callosum and an 18 mm metastasis in the right cerebellar hemisphere (red arrows). After two treatments (34 days), both metastases had partially regressed. After four treatments (68 days), regression was complete but new diffuse lesions were seen in the posterior horns of the lateral ventricles indicating encephalitis (red arrows). MRI scans were unchanged at day 71 and showed gradual decrease of the lesions in the brain at 78 and 85 days and complete resolution at 112 days. (C) shows serum levels of S-100B and C reactive protein (CRP), starting at baseline. The highest measurements coincided with the most severe symptoms of encephalitis. However, the first peak in S-100B and CRP levels occurred before the patient had any symptoms or radiological findings of encephalitis, suggesting a potential biomarker for early detection. IPI, ipilimumab; IVIG, intravenous immunoglobulin; MMF, mycophenolate mofetil; nivo, nivolumab.

Table 1 Patient characteristics

Age	Sex	BRAF	Tumor stage*	Treatment	Time to irAE/ test†	Affected organ(s)	Grade (CTCAE 5)‡	Treatment of irAE
68	M	V600K	IV	Ipi+Nivo	2 months	Brain and spinal cord§	4	CORT, MMF, IVIG, CP
52	F	Wt	IV	Ipi+Nivo	2 months	Liver	4	CORT, MMF
72	F	Not known	IV	Pembro	3 months	Blood (neutropenia)	4	CORT, filgrastim
72	M	V600	IV	Pembro	13 months	Colon	3	CORT, infliximab
55	F	V600	IV	Nivo	14 months	Joints and muscles	3	CORT, MTX
38	F	Wt	IV	Nivo¶	5 months	Joints and muscles	3	CORT
83	F	V600E	IV	Nivo	1 month	Colon	3	CORT, infliximab
53	M	V600K	IV	Nivo	11 months	Lungs	2	CORT
80	F	Wt	IV	Nivo	10 months	Lungs	2	CORT
74	M	V600K	IV	Nivo±Relatlimab	2 months	Joints and muscles	2	CORT
71	M	Wt	IV	Ipi+Nivo	9 months	No irAE	0	none
80	M	Wt	IV	Ipi+Nivo	2 months	No irAE	0	none
80	M	V600K	IV	Pembro	5 months	No irAE	0	none
77	F	Wt	IV	Pembro	4 months	No irAE	0	none
82	F	Wt	IV	Pembro	3 months	No irAE	0	none
76	M	V600E	IV	Pembro	3 months	No irAE	0	none
67	M	V600	IV	Pembro	2 months	No irAE	0	none
68	M	V600E	IV	Nivo	4 months	No irAE	0	none
62	F	V600E	IV	Nivo	3 months	No irAE	0	none
57	M	V600E	IV	Nivo	2 months	No irAE	0	none
68	M	Wt	III	Nivo	1 month	No irAE	0	none
93	M	Wt	III	Nivo	1 month	No irAE	0	none

*Stage III: locally advanced disease. Stage IV: metastatic disease.
†Time from treatment start until adverse event (and sample) or time from treatment start until sample in controls without irAE.
‡CTCAE are a set of criteria for the standardized classification of adverse effects of cancer drugs. The scale ranges from grade 0 to grade 5. Grade 0 is no adverse event. Grade one adverse events have no or mild symptoms with or without laboratory abnormalities whereas grade five events are lethal.
§Encephalomyelitis patient.
¶irAE during Nivolumab monotherapy. Ipi/Nivo treatment previously without any side effects.
CORT, corticosteroids; CP, cyclophosphamide; CTCAE, Common Terminology Criteria for Adverse Events; Ipi, ipilimumab; irAE, immune related adverse event; IVIG, intravenous immunoglobuline; MMF, mycophenolate mofetil; MTX, methotrexate; Nivo, nivolumab; Pembro, pembrolizumab; wt, wild type.

patient developed grade 4 hepatitis during treatment with double checkpoint inhibition. In this patient, one sample was analyzed before irAE, when liver enzymes were normal. Interestingly ICOS on CD4+ and CD8+T cells increased in parallel with liver enzymes and decreased again after immunosuppression, and subsequent normalization of liver enzymes (figure 3). Collectively, our data indicate that ICOS may promote development of irAE. High ICOS expression could not be explained by longer duration of checkpoint inhibition because the difference was significant also if patients with late irAE (>6 months, 4 patients) were excluded from the analysis (irAE vs non-irAE; ICOS on CD8+ T cells, p<0.010; ICOS on CD4 +T cells, p<0.027). In addition, the difference in ICOS expression was significant also when only patients treated with single PD-1 inhibition was compared (irAE vs non-irAE; ICOS on CD8 +T cells, p<0.0085; ICOS on CD4+T cells, p<0.0062). This indicates that the difference in ICOS was not specific for anti-CTLA-4 treatment. Full data on T cell characteristics is shown in online supplemental figure S6. In addition to ICOS, TIM-3 (an inhibitory checkpoint protein and activation marker) was significantly higher on

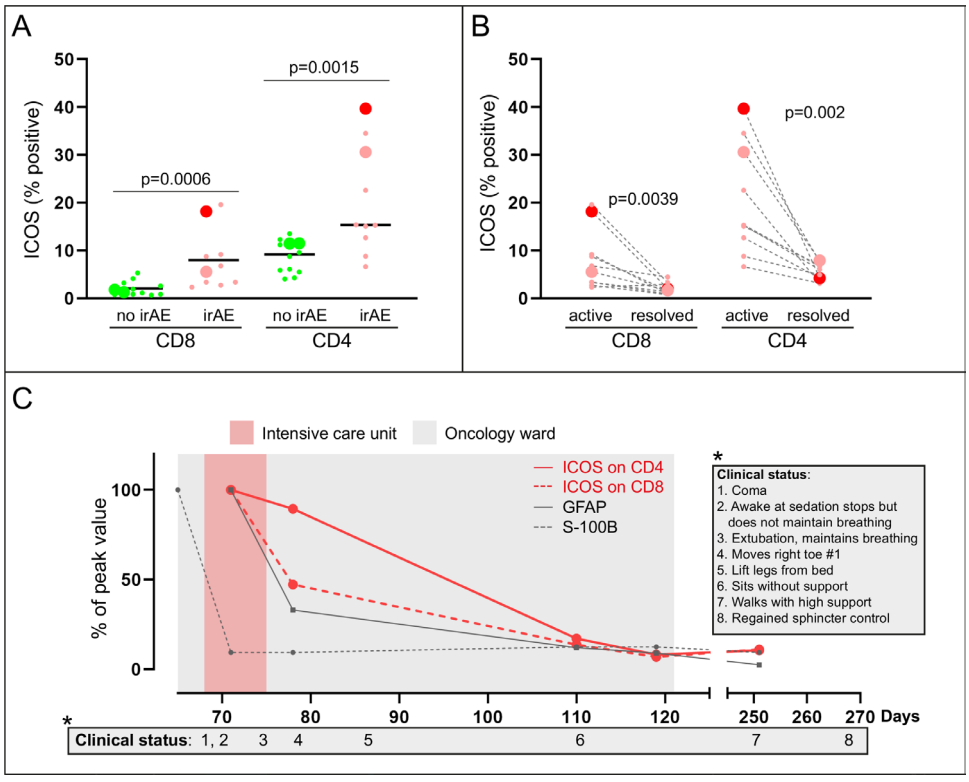

Figure 2 High proportion of ICOS-expressing CD4+ and CD8+T cells during active encephalomyelitis. Panel (A) shows higher proportion of T cells expressing the costimulatory receptor ICOS at the peak of symptoms in checkpoint inhibitor-treated patients with immune-related adverse events (irAE; pink dots and the big red dot which indicates the encephalitis patient) than in patients without irAE (no irAE; green dots) (large dots—double inhibition; small dots—single PD-1-inhibition) (Mann-Whitney U test). The encephalitis patient had the highest proportion of ICOS positive CD4+T cells and the second highest proportion of ICOS-expression on CD8+ cells. (B) shows that immunosuppression decreased the proportion of ICOS expressing CD8+ and CD4+T cells in the encephalitis patient as well as in patients with other irAE (Wilcoxon matched-pairs signed rank test). (C) shows that the proportion of ICOS expressing CD8 (dotted red line) and CD4 (solid red line) T cells decreased in parallel with clinical improvement (box) and with decrease in brain damage marker GFAP in blood. GFAP, glial fibrillar acidic protein; ICOS, inducible T cell costimulatory receptor; PD-1, programmed cell death 1.

CD8 +T cells in patients with irAE and normalized when irAE were resolved. Also, PD-1 expression on CD4+ T cells was significantly higher in patients with irAE. The patients with irAE had similar proportion of immunosuppressive regulatory T cells as checkpoint inhibitor-treated controls (online supplemental figure S7). There was no difference in total number of CD8+ cytotoxic T cells, CD4+T helper cells, B cells, or natural killer cells, between our patient and checkpoint inhibitor-treated controls (online supplemental figure S8).

DISCUSSION AND CONCLUSION

In this study, we demonstrate a specific T cell phenotype in a patient with encephalomyelitis as well as in patients with other severe irAE. The most striking feature is high expression of costimulatory receptor ICOS on CD4+ and CD8+T cells. In addition, our study shows that brain damage markers in blood can help in early diagnosis of encephalitis.

irAE are a diverse set of checkpoint inhibitor-induced autoimmune reactions but little is known about the mechanisms promoting irAE.[5] Here, we identify high ICOS expression, on both CD4+ and CD8+ cells, during encephalomyelitis and other serious irAE. ICOS decreased when the irAE resolved suggesting that ICOS may promote irAE. In agreement, ICOS has been linked to the development of different autoimmune diseases.[12] The association between T cell expression of ICOS and the clinical course of irAE is clear but it is important to clarify if the ICOS molecule promotes irAE. If this is the case, targeting ICOS with antagonists may constitute a therapeutic approach to dampen severe irAE; such as the near-fatal encephalomyelitis described here. However, it is possible that such an intervention, as well as the immunosuppressive treatments used in our patients, may increase the risk of tumor progression or recurrence because ICOS has also been identified as a mediator of response.[13 14]

Double immune checkpoint blockade is often more effective than PD-1 inhibition alone, as targeting CTLA-4 also activates CD4 +T cells.[15] In mice, the absence of CTLA-4 promotes the expansion of ICOS-positive CD4+ effector T cells, which are important in mediating the response to CTLA-4 inhibition.[14–17] Interestingly, high ICOS expression on CD4 +cells also promotes

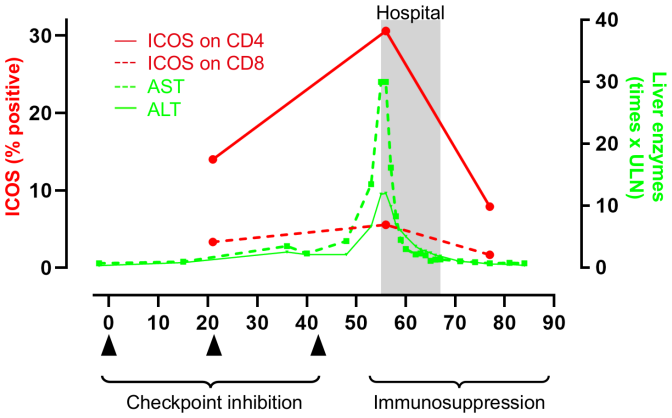

Figure 3 High proportion of ICOS-expressing CD4+T cells during severe checkpoint inhibitor-induced hepatitis The figure shows covariation of liver enzymes—aspartate transaminase (AST; solid green line) and alanine aminotransferase (ALT; dotted green line)—and ICOS expression on CD4+T cells (solid red line) before, at the peak of, and after severe checkpoint inhibitor–induced hepatitis (grade 4). ICOS on CD8+T cells (dotted red line) showed a similar, but less pronounced, covariation with liver enzymes. The black triangles indicate time points for double checkpoint inhibition with ipilimumab and nivolumab. ICOS, inducible T cell costimulatory receptor.

the development of neuromyelitis optica spectrum disorder,[18] an autoimmune demyelinating disease of the central nervous system. The high levels of ICOS expression on CD4+ effector cells in our patient could help explain both the efficient eradication of tumor cells and the collateral damage to normal brain cells. Consistent with our data, activated CD4+ memory cells accumulated in inflamed brain tissue from a patient who died from checkpoint inhibitor-induced encephalitis.[7] At autopsy, no signs of remaining melanoma brain metastases were found. In combination with previous clinical and experimental data, the findings in our case support a role for ICOS expression on CD4+ cells in mediating an aggressive immune reaction.

The current case shows that brain damage biomarkers in blood can help to diagnose encephalitis. Our patient had increased levels of the brain damage marker S-100B and CRP after two treatments, when he was asymptomatic and MRI showed no signs of encephalitis. S-100B and CRP peaked after the fourth and final treatment, when his encephalitis rapidly progressed. S-100B was analyzed because it is a melanoma marker. However, it was negative before treatment, and therefore the elevated level reflected treatment-induced brain damage and not progression of melanoma. Additional biomarkers were analyzed in the ICU and repeatedly during recovery. Most notably, the axonal damage marker NFL and the marker of astrocytic injury GFAP were extremely high in both blood and CSF and normalized during improvement. To facilitate diagnosis of encephalitis, we suggest that a set of brain damage markers in blood be included in laboratory panels taken during double-checkpoint inhibition. Our patient developed very severe encephalomyelitis and it needs to be investigated if brain damage markers in blood also indicate less severe cases of encephalitis.

Checkpoint inhibitor-induced encephalitis is a diagnostic challenge. Given our patient's serious cancer diagnosis, oncologists and intensivists discussed whether he should be admitted to the ICU. The decision to do so was based on the argument that the clinical and radiologic findings were consistent with causes other than cancer progression, such as infection or neurotoxicity. At admission, the patient was unconscious and had central respiratory depression. He would have died without ICU treatment.

In conclusion, this study suggests a potential role for ICOS on CD4+ and CD8+T cells in mediating encephalitis and other serious irAE. In addition, our case suggests that brain damage markers in blood should be analyzed to facilitate early diagnosis of encephalitis.

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Acknowledgements We thank Elisabeth Kapocs for coordinating patient samples, the Department of clinical immunology at Sahlgrenska University Hospital for performing flow cytometry, and Kerstin Andersson for flow cytometry gating. Ellen Nohle is acknowledged for discussing early drafts of the manuscript. We thank Stephen Ordway for editing the manuscript.

Contributors Acquisition of data: ML, SB, AP, ZZ, CJ, TU, HJ, HZ, AR. Analysis and interpretation of data: All authors. Writing the manuscript: SB and ML. Review, and/or revision of the manuscript: All authors.

Funding Supported by funding from the Swedish state under the agreement between the Swedish government and the county councils, the ALF-agreement (ALFGBG-828121 and 870 351 to ML; ALFGBG-717541 to AR; ALFGBG-933754 to SB), Jubileumsklinikens cancerfond (to ML) and Stiftelsen Assar Gabrielssons Fond (to SB).

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

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SUPPLEMENTARY APPENDIX

CONTENTS

METHODS

Ethical considerations – The encephalitis patient and control patients received oral and written information about the purpose of blood samples and cerebrospinal fluid taken for research. All patients signed an informed consent form. The first research samples from the encephalitis patient was obtained when he was deeply unconscious in a respirator. The sample was taken after discussion with the regional ethics committee. Following their instruction, the patient was informed after recovery and at that point he approved analysis of samples for research purposes. If he had declined, the samples would have been destroyed. The research protocols were approved by the Regional Ethics Review Board in Gothenburg (numbers 151-16 and 433-11). The encephalitis patient gave an additional separate consent to publish this case.

Flow cytometry – Peripheral blood mononuclear cells (PBMCs) were separated from heparinized whole blood, stained with fluorochrome-conjugated antibodies (Table S2) and analyzed in a FACSLyric flow cytometer. CD4 and CD8 T-cell subsets were defined by gating with FlowJo software. Total counts of T-cells, B-cells, and natural killer cells (NK cells) were determined using TruCount. PBMCs from checkpoint inhibitor treated melanoma patients with (n=9) or without (n=12) immune related adverse events were used as controls. Checkpoint proteins in CSF were analyzed with LEGENDplex bead-based multiplex assay panels; data was acquired on a FACSVerse flow cytometer, and concentrations were calculated with FCAP Array software. CSF from patients with systemic lupus erythematosus without autoimmune manifestations in the brain were used as controls (n=4).

Brain damage markers – The S-100B concentrations in CSF and serum were measured by immunoassay on the cobas Elecsys platform. CSF concentrations of neurofilament light polypeptide (NFL) and glial fibrillary acidic protein (GFAP) were measured with in-house enzyme-linked immunosorbent assays as described.[1,2] CSF tau concentration was measured with a Lumipulse immunoassay. Plasma concentrations of NFL, GFAP, and tau were measured with ultrasensitive single-molecule array technology and commercially available kits. CSF and serum concentrations of albumin and IgG were measured by nephelometry on the cobas Elecsys platform. Oligoclonal IgG bands in serum and CSF were visualized by isoelectric focusing in a polyacrylamide gel and silver staining as described.[3]

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SUPPLEMENTARY FIGURES

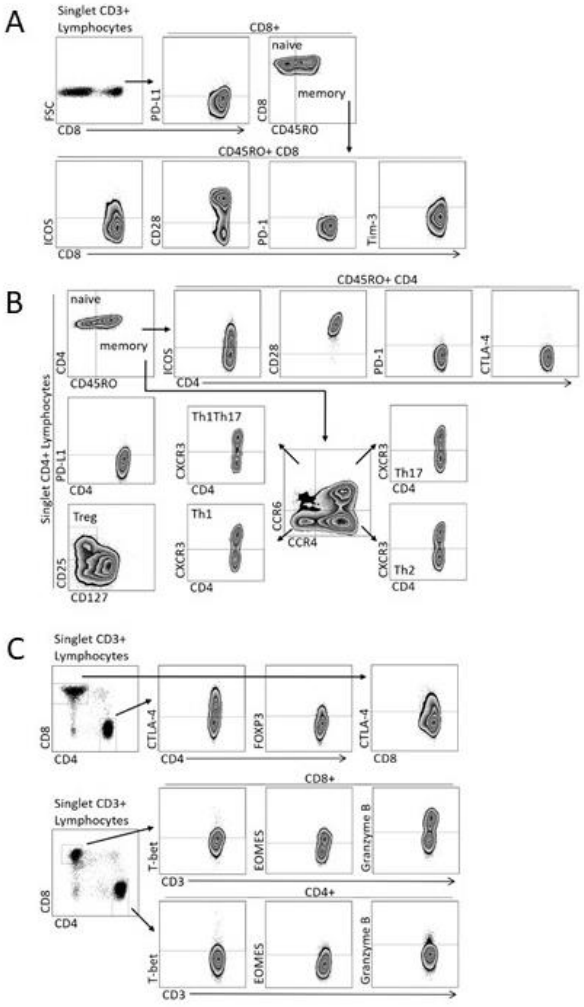


Figure S1. Gating strategy for CD8+ and CD4+ lymphocytes and their sub-populations in blood

Panel A. Singlet CD3+ lymphocytes were gated for CD8+ T cells and then gated for PD-L1+ cells. CD8+ T cells were divided into CD45RO- (naïve) and CD45RO+ (memory) cells, and memory cells were gated according to the expression of ICOS, CD28, PD-1 and Tim-3. To set the gates for the CD8+ T-cell subtypes we used Fluorescence Minus One (FMO) except for PD-L1, where an isotype control was used.Panel B. Singlet CD4+ lymphocytes were gated for PD-L1 and for CD25 and CD127. CD4+ T cells were divided into CD45RO- (naïve) and CD45RO+ (memory) cells, and memory cells were gated according to the expression of ICOS, CD28, PD-1 and CTLA-4. Memory cells were also gated into Th1, Th2, Th17 and Th1Th17 based on the expression of CCR4, CCR6 and CXCR3. To set the gates for the CD8+ T-cell subtypes we used FMO except for PD-L1, where an isotype control was used. Panel C. Singlet CD3+ were first gated into CD8+ and CD4+ T cells, followed by gating for the intracellular molecules CTLA-4, FOXP3, T-bet, EOMES and Granzyme B. Isotype controls were used for T-bet, EOMES and Granzyme B, whereas FMO was used for CTLA-4. The cut off for FOXP3 positivity in CD4+ cells was determined based on FOXP3 expression in CD25neg gated CD4+ cells.

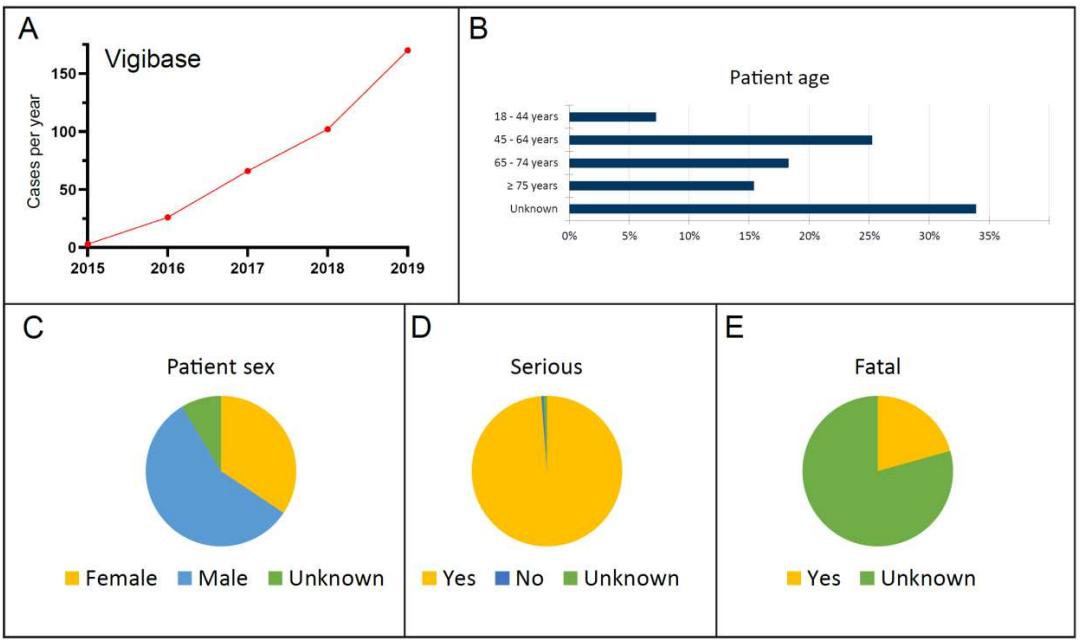


Figure S2. Results of search of the WHO Global Database VigiBase for reported cases of encephalitis during single- or double-checkpoint Inhibition.

Panel A shows annual number of reported cases from 2015 through 2019. Panel B to D shows the ages of the cases reported in panel (B), the sex of the patients (C), the severity of their condition (D) and the outcome (E).

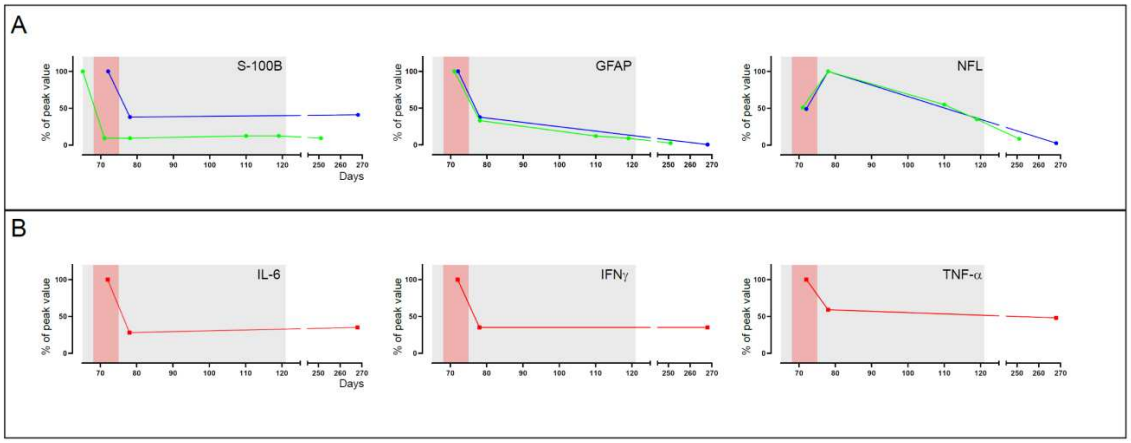


Figure S3. Brain damage markers and cytokines in cerebrospinal fluid during active encephalomyelitis

Panel A shows relative concentrations (% of highest value) of brain damage markers in blood (green line) and cerebrospinal fluid (CSF) (blue line) during encephalomyelitis and recovery. Panel B shows relative concentrations of cytokines in CSF.

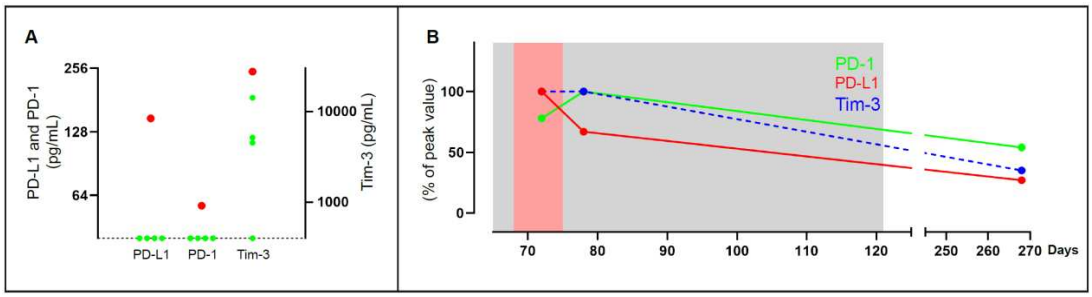


Figure S4. High levels of soluble checkpoint proteins PD-1, PD-L1 and Tim-3 in cerebrospinal fluid during active encephalomyelitis

Panel A shows cerebrospinal fluid (CSF) concentrations of the soluble checkpoint proteins PD-1, PD-L1, and Tim-3 at the peak of symptoms in the encephalomyelitis patient (big red dot) than in CSF from patients with autoimmune systemic lupus erythematosus without encephalitis (green dots). The base line on the y-axes indicates detection level (PD-1 and PD-L1 – 40 pg/mL; Tim-3 – 400 pg/mL). Panel B shows that immunosuppression decreased PD-1, PD-L1 and Tim-3 concentrations in CSF in the encephalitis patient.

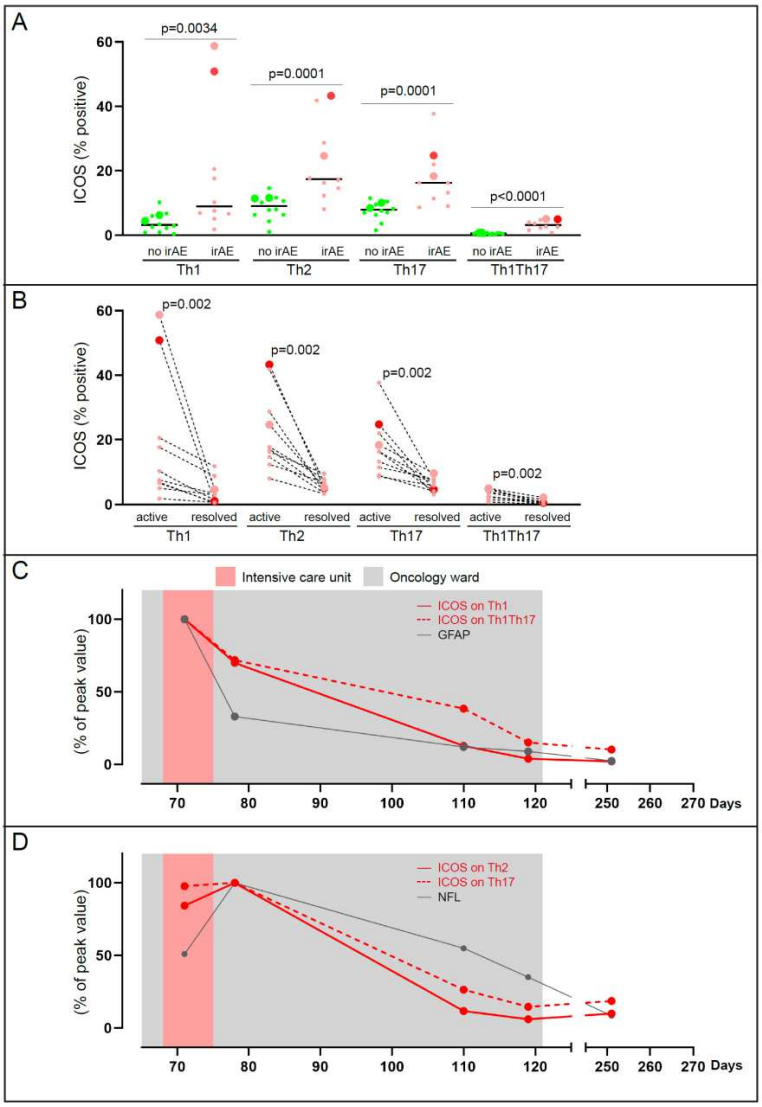


Figure S5. High proportion of ICOS-expressing Th1, Th2, Th17, and Th1Th17 cells during active encephalomyelitis.

Panel A shows higher proportion of CD4+ cell subtypes (Th1, Th2, Th17, and Th1Th17) that express the co-stimulatory receptor ICOS at the peak of symptoms in the encephalomyelitis patient (big red dot) than in checkpoint inhibitor-treated patients with other immune related adverse events (pink dots) or in patients without immune-related adverse events (green dots) (Mann Whitney test). Big dots indicate double inhibition and small dots single inhibition. Panel B shows that immunosuppression decreased the proportion of CD4+ T cell subtypes that express ICOS in the encephalitis patient as well as in patients with other immune-related adverse events (Wilcoxon matched-pairs signed rank test). Panel C shows that the proportion of ICOS-expressing Th1 and Th1Th17 cells decreased in parallel with clinical improvement and with decrease in glial injury marker GFAP in blood. Panel D shows that the proportion of ICOS-expressing Th2 and Th17 cells increased initially during immunosuppression and then decreased. A similar dynamic was seen in axonal damage marker NFL in blood.

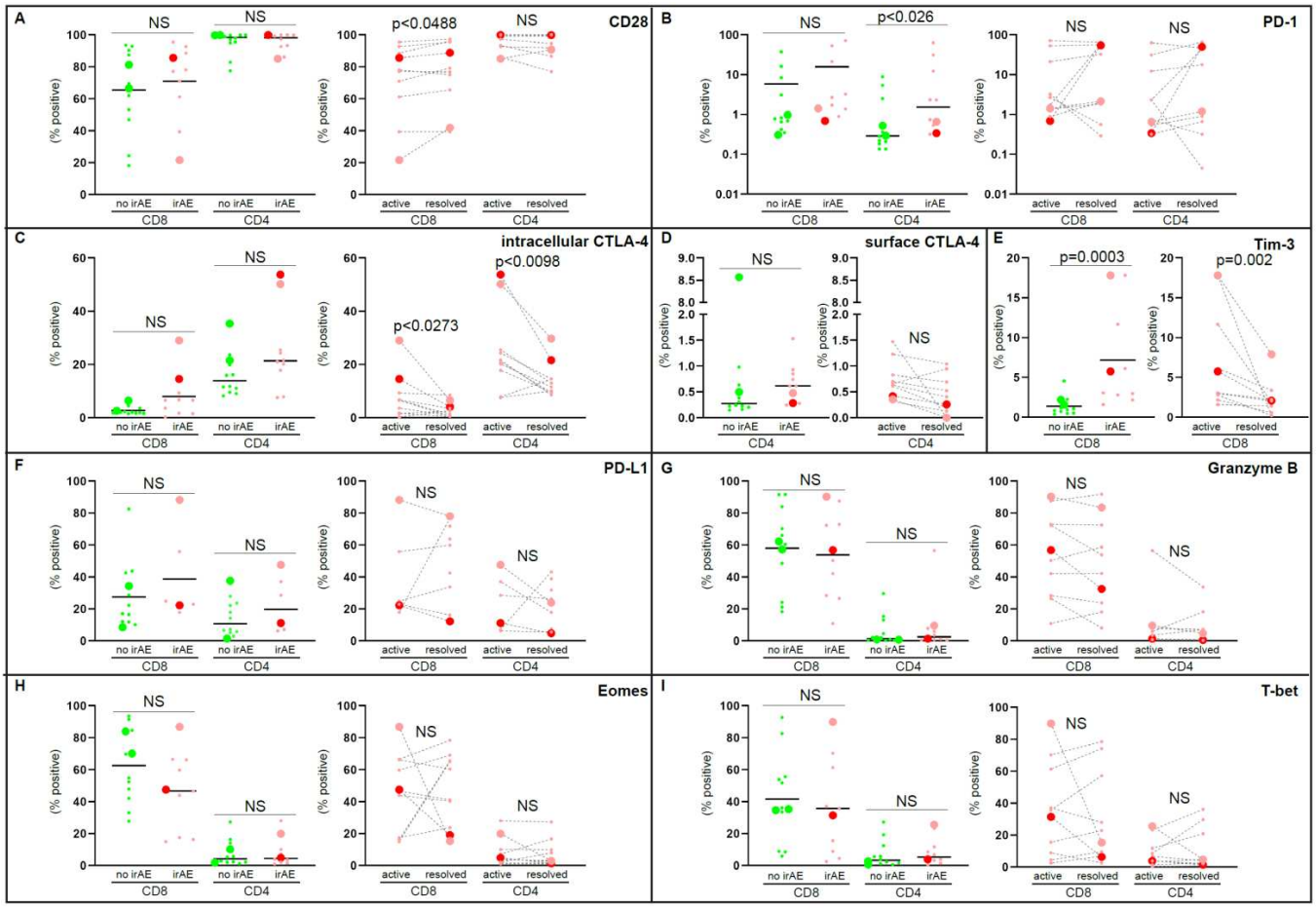


Figure S6. T cell characteristics during active encephalomyelitis and other irAEs

Panels A-I shows T cell characteristics at the peak of symptoms in the encephalitis patient (big red dot), patients with other immune related adverse events (irAE; pink dots) and patients without irAE (no irAE; green dots). Big dots indicate double inhibition and small dots single inhibition. Also shown is the effect of immunosuppression on proportion of T-cell subtypes in patients with irAE (active vs resolved). Proportion of CD8+ and CD4+ T cells that express co-stimulatory checkpoint protein CD28 (A); co-inhibitory checkpoints PD-1 (B), intracellular (C) and cell-surface CTLA-4 (D), and Tim-3 (E); PD-1 ligand PD-L1 (F); attack enzyme Granzyme B (G); activating transcription factors T-bet (H) and Eomes (I) are shown. The proportion of CD8+ and CD4+ T cells that express intracellular CTLA-4 decreased after immunosuppression (C). A higher proportion of CD8 cells expressed Tim-3 in patients with immune related adverse events and Tim-3 expression decreased after immunosuppression (E). P values; Mann Whitney test (no irAE vs. irAE) or Wilcoxon matched-pairs signed rank test (active vs. resolved); NS – non significant.

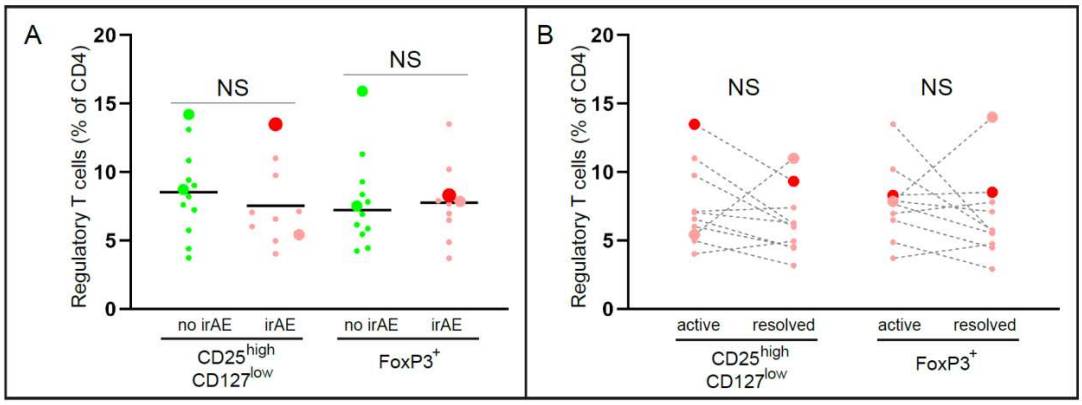


Figure S7. Proportion of regulatory T cells during active encephalomyelitis and other irAEs

Panel A shows no difference in two populations of regulatory T cells, $CD4^{+}CD25^{high}CD127^{low}$ and $CD4^{+}FoxP3^{+}$, in patients with (pink dots) or without immune related adverse events (green dots). Big dots indicate double inhibition and small dots single inhibition. The encephalomyelitis patient is indicated by a big red dot. Panel B shows no difference in $CD4^{+}FoxP3^{+}$ cells or in $CD4^{+}CD25^{high}CD127^{low}$ regulatory T cells after immunosuppression (Wilcoxon matched-pairs signed rank test).NS – non significant.

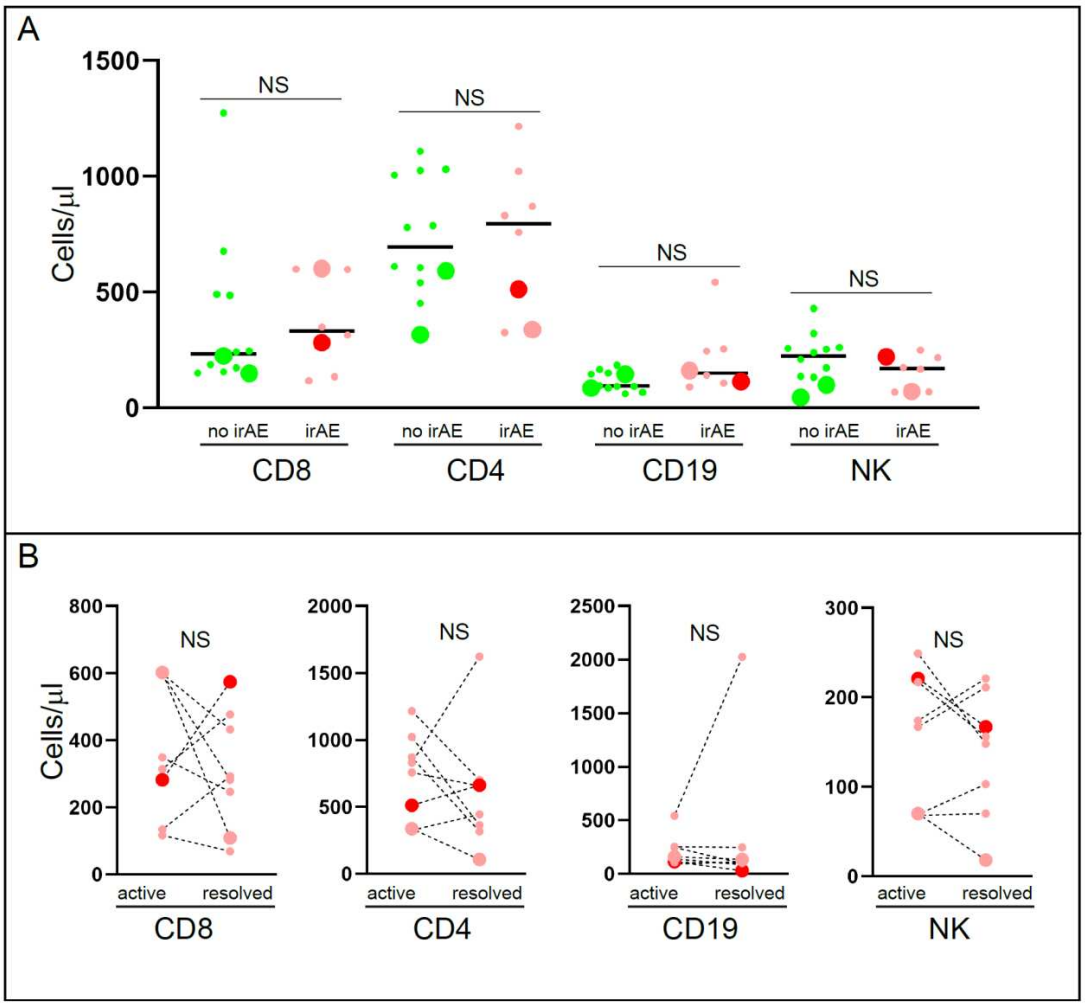
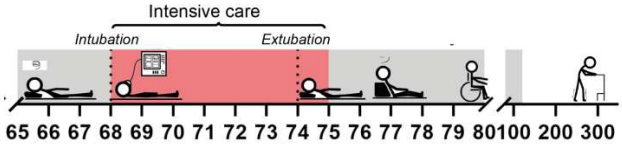


Figure S8. Total number of immune cells during active encephalomyelitis and other irAEs

Panel A shows no difference in total numbers of CD8 cytotoxic T cells, CD4 T helper cells, B cells, or natural killer cells (NK) in patients with (pink dots) or without immune related adverse events (green dots)(Mann Whitney test).c Big dots indicate double inhibition and small dots single inhibition. The encephalomyelitis patient is indicated by a big red dot. Panel B shows no change in any of these cell populations after immunosuppression (Wilcoxon matched-pairs signed rank test). NS – non significant.

SUPPLEMENTARY TABLES

Table 1. Laboratory analyses of cerebrospinal fluid during intensive care and recovery

				
Laboratory test (reference values)	**Day 68**	**Day 72**	**Day 75**	**Day 268**
GFAP (<1250 ng/L)		92800	30400	490
NFL (<1850 ng/L)		85400	174000	4580
Tau protein (<400 ng/L)		484	1730	240
Albumin (<420 mg/L)	2880	814.8	505.8	170.6
Qalb (<10.2 x 10^{-3})	106.8 x 10^{-3}	29.1 x 10^{-3}	18.7 x 10^{-3}	5.9 x 10^{-3}
S-100B(<1,7 µg/L)		2.79	1.07	1.13
IgG (<60 mg/L)		359.6	255.2	34.6
IEF (bands in CSF only)		4 - 5	4 - 5	8 - 10
VZV	Negative			
HSV type 1	Negative			
HSV type 2	Negative			
Enterovirus	Negative			
CNS-infection, 14 agents*	Negative			
Bacterial culture	Negative			
16S rRNA gene	Negative			
Glucose (<2/3 of blood glc)L	Normal			
Lactate (0.5 - 1.8 mM/L)	5.1			
Lymphocytes (<4 x 10^6/L)	16		11	15
Monocytes(<3 x 10^6/L)	<3		<3	<3
Neutrophils (<3 x 10^6/L)	<3		<3	<3
Erythrocytes (<5 x 10^6/L)	24		42	<5
IL-1β (<5 pg/mL)		<5	5.4	8.2
IL-2 (<16 pg/mL)		<16	<16	<16
IL-4 (<16 pg/mL)		<0.25	<0.25	<0.25
IL-5 (<8 pg/mL)		3.9	<3.9	<3.9
IL-6 (<50 pg/mL)		12	3.4	4.2
IL-8 (<90 pg/mL)		360	190	150
IL-10 (<20 pg/mL)		12	9	5.9
IL-12 (<7.8 pg/mL)		<7.8	<7.8	<7.8
IFN-γ (<7.8 pg/mL)		22	<7.8	<7.8
GM-CSF (<7.8 pg/mL)		<7.8	<7.8	<7.8
TNF-α (<25 pg/mL)		17	10	8.2
IL-18 (140-500 pg/mL)			65	

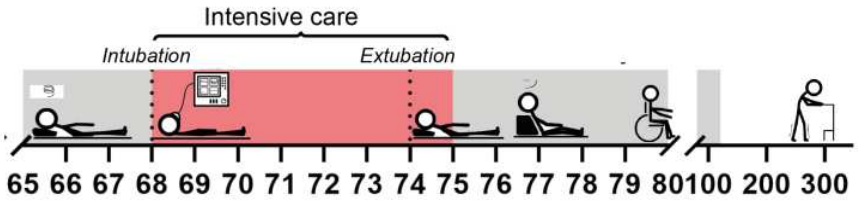
GFAP, Glial fibrillary acidic protein; NFL, Neurofilament light polypeptide; Qalb, albumin quotient (CSF/serum); IEF, Isoelectric Focusing; VZV, Varicella zoster virus; HSV, Herpes simplex virus ; rRNA, Ribosomal ribonucleic acid; IL, Interleukin; IFN-γ, Interferon gamma; GM-CSF, Granulocyte-macrophage colony-stimulating factor; TNF-α, Tumor necrosis factor alpha.

*Filmarray meningitis panel: Herpes simplex type 1 and 2, Varicella zooster virus, Enterovirus, Human herpes virus 6, Human parechovirus, Cytomegalovirus, Streptococcus pneumonia, Hemophilus

Table S2. Antibodies used for flow cytometry

antigen	fluorochrome	isotype	Manufacturer	clone
CD4	APC-H7	IgG1	BD	SK3
CD25	BV421	IgG1	Biolegend	BC96
CD25	APC	IgG1	BD	2A3
CD127	PerCP-Cy5.5	IgG1	BD	HIL-7R-M21
CD45RO	FITC	IgG2a	Biolegend	UCHL1
PD-1	PE	IgG1	Biolegend	EH12.2H7
Foxp3	PE	IgG2a	eBioscience	PCH101
CTLA-4	biotin	IgG2a	BD	BNI3
CCR4	PE-Cy7	IgG1	Biolegend	L291H4
CCR6	APC	IgG2b	Biolegend	G034E3
CXCR3	BV421	IgG1	Biolegend	G025H7
CD8	PE-Cy7	IgG1	BD	RPA-T8
CD3	APC-H7	IgG1	BD	SK7
CD4	BV421	IgG1	BD	SK3
CD3	BV421	IgG1	BD	UCHT1
CD28	APC	IgG1	Biolegend	CD28.2
TIM-3	BV421	IgG1	Biolegend	F38-2E2
ICOS	PerCP-eFluor710	IgG1	eBioscience	ISA-3
T-bet	PE	IgG1	Biolegend	4B10
Granzyme B	PerCP-Cy5.5	IgG1	Biolegend	QA16A02
EOMES	eFluor660	IgG1	eBioscience	WD1928
PD-L1	APC	IgG2b	Biolegend	29E.2A3
isotype	APC	IgG2b	Biolegend	MPC-11
isotype	PerCP-Cy5.5	IgG1	Biolegend	MOPC-21
isotype	PE	IgG1	Biolegend	MOPC-21
isotype	eFluor660	IgG1	eBioscience	

Table S3. Brain damage markers in blood during intensive care and recovery



Laboratory test (reference value)	Day 65	Day 70	Day 71	Day 78	Day 110	Day 119	Day 251
GFAP (<210 ng/L)			12208	1091.8	405.9	291	80
NFL (< 30 ng/L)			153.7	301.7	165.7	105.7	25.7
Tau (< 5 ng/L)			3.1	2.6	3.7	3.2	3.3
S-100B(<0.1 μg/L)	0.31	0.06	0.03	0.02	0.04	0.04	

GFAP, Glial fibrillary acidic protein; NFL, Neurofilament light polypeptide.

Table S4. No autoantibodies in blood or cerebrospinal fluid during active encephalomyelitis

Antinuclear antibody screen (blood)	**Result**
ANA	Negative
Anti-SS-A60	Negative
Anti-SS-A52	Negative
Anti-SS-B	Negative
Anti-Sm	Negative
Anti-RNP	Negative
Anti-Scl-70	Negative
Anti-Jo-1	Negative
Anti-dsDNA	Negative
Anti Cent B	Negative
Anti-Ribo P	Negative
	Negative
Antibodies associated with autoimmune encephalitis (cerebrospinal fluid)	
Anti-NMDA-receptor	Negative
Anti-LGI1	Negative
Anti-CASPR2	Negative
Anti-GABA-B	Negative
Anti-VGCC PQ-typ	Negative
Anti-AMPA 1/2-receptor	Negative
Anti-DPPX	Negative
Antibodies against neuronal antigens (cerebrospinal fluid)	
Anti-CV2	Negative
Anti-Amphiphysin	Negative
Anti-Ma2/Ta	Negative
Anti-Tr	Negative
Anti-Recoverin	Negative
Anti SOX1	Negative
Anti-Zic4	Negative
Anti-Aquaporin-4 (NMO)	Negative
Paramalignant antibodies (cerebrospinal fluid)	
Anti-Hu Ri Yo	Negative
Anti-PCA2	Negative