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Neurotoxicities Associated with Immune Checkpoint Inhibitor Therapy

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Table of Content

1 Abstract

1.1 Abstract (English)

Cancer therapies have experienced significant advances in recent years. While conventional cytotoxic chemotherapy has long been the cornerstone for the treatment of many tumor entities, uprising immunotherapies have revolutionized the therapeutic landscape. Among them, immune checkpoint inhibitors (ICIs) with their demonstrated increased overall survival rates and response rates in cancer patients are now FDA-approved for metastatic melanoma and multiple other malignancies. Despite their clinical benefit in cancer therapies, ICIs can induce unique autoimmune-like toxicities known as immune-related adverse events (irAEs), which can involve any organ system including the nervous system. Although neurotoxicities are rare complications of ICI therapy they are often severe and can lead to long-term disability or even death if left untreated. Neurological irAEs exhibit a broad spectrum of clinical presentations affecting the entire nervous system. Diagnosing neurological irAEs is often challenging as symptoms and laboratory findings can be uncharacteristic for common neurological disorders and clinical experience with ICI-mediated toxicities is still limited. In light of expanding clinical indications for ICIs, physicians will encounter ICI-mediated neurotoxicities more frequently. Thus, thorough characterizations of the diverse set of neurological irAEs are essential for optimal patient care, the prevention of severe ICI-mediated complications, and the development of diagnostic and therapeutic algorithms. This work portrays the clinical presentation, management and outcome of neurological irAEs following ICI therapies.

Patients with neurotoxicities related to ICIs who presented at the Yale New Haven Hospital between January 2014 and June 2018 were retrospectively identified from the quality control database. A comprehensive chart review was performed and data regarding patient demographics, medical history, ICI regimen and neurotoxicity were recorded. In total, 18 patients with neurological irAEs following ICI therapy for melanoma, small cell lung cancer, non-small cell lung cancer, and Merkel-cell carcinoma were identified. Neurotoxicities included central nervous system disorders comprising central demyelinating disorder,

autoimmune encephalitis predominantly affecting the grey matter, and aseptic meningitis. Peripheral nervous system toxicities included sensorimotor polyneuropathy and myasthenia gravis. Cases of hypophysitis were also recorded. Time to onset of neurological irAEs ranged from 1 to72 weeks with a median of five weeks. In all patients ICIs were held and steroids initiated. Additional immunomodulatory therapies were required in nine patients. Sixteen of 18 patients showed neurological improvement. Fourteen patients had high-grade neurotoxicity (grade 3-4), six of whom deceased due to cancer progression, while none of the low-grade neurotoxicity patients (grade 1-2) died. High-grade neurotoxicity was identified as a negative prognostic marker for overall survival ($p = 0.046$).

This work shows that neurotoxicities present early-onset, rapidly progressive complications of ICIs with a broad spectrum of clinical phenotypes affecting the central nervous system, peripheral nervous system, and neuroendocrine system. A high index of caution for neurological irAEs is warranted throughout ICI therapy as timely diagnosis and management can reduce morbidity and mortality. Randomized clinical trials are needed to develop standardized diagnostic and therapeutic algorithms of ICI-induced neurotoxicities.

1.2 Abstract (German)

In den letzten Jahren wurden bei den therapeutischen Möglichkeiten von Tumorerkrankungen grundlegende Fortschritte erzielt. Während die konventionelle zytotoxische Chemotherapie lange Zeit Grundpfeiler in der Behandlung verschiedener Tumorentitäten war, hat der klinische Einsatz von Immuncheckpoint-Inhibitoren (ICI) die therapeutische Landschaft in den letzten Jahren revolutioniert. ICI zeigen hohe Überlebensraten und therapeutische Ansprechraten in Tumorpatienten*innen und sind bereits von der FDA zur Behandlung metastasierender Melanome sowie weiterer Malignitäten zugelassen. Trotz des klinischen Vorteils in der Tumortherapie können ICI zu autoimmun-ähnlichen Toxizitäten in allen Organsystemen, inklusive dem Nervensystem führen. Diese werden als immun-vermittelten Nebenwirkungen (irAEs) bezeichnet. Obwohl ICI-vermittelte Neurotoxizitäten selten sind, verlaufen sie häufig schwer und können zu langfristigen Schäden und sogar Todesfällen führen. Neurologische irAEs zeigen ein breites Spektrum klinischer Präsentationen und manifestieren sich im gesamten Nervensystem. Aufgrund der häufig unspezifischen Symptome und Laborveränderungen, die einem definierten neurologischen Syndrom oft nicht eindeutig zugeordnet werden können, ist die Diagnosestellung herausfordernd und die klinische Erfahrung mit ICI-induzierten Neurotoxizitäten weiterhin limitiert. In Anbetracht des zunehmenden klinischen Einsatzes von ICI werden behandelnde Ärzt*innen häufiger mit neurologischen irAEs konfrontiert werden. Eine genaue Charakterisierung ICI-vermittelter Neurotoxizität ist entscheidend für eine optimale Patientenversorgung sowie die Entwicklung diagnostischer und therapeutischer Algorithmen. Diese Arbeit analysiert den klinischen Phänotyp, das Management und das Outcome ICI-vermittelter Neurotoxizität.

Patienten mit Neurotoxizitäten infolge von ICI, die im Yale New Haven Hospital zwischen Januar 2014 und Juni 2018 vorstellig waren, wurden durch eine retrospektive Recherche der qualitätskontrollierten Datenbank identifiziert. Es folgte eine umfassende Analyse der Patientenakten, bei der Daten bezüglich Patientendemographie, medizinischer Krankengeschichte, ICI Therapieschema und Neurotoxizität erfasst wurden.

Insgesamt wurden 18 Patienten mit Neurotoxizität infolge von ICI Therapie zur Behandlung verschiedener Tumorerkrankung, inklusive des Melanoms, des kleinzelligen und nicht-kleinzelligen Lungenzellkarzinoms und des Merkel-Zell-Karzinoms, identifiziert. Neurotoxizitäten umfassten zentral demyelinisierende Erkrankungen, Autoimmunenzephalitiden mit vorwiegender Affektion der grauen Substanz, die aseptische Meningitis, sensomotorische Polyneuropathien, Myasthenia gravis und Hypophysitiden. Die mediane Zeit bis zum Auftreten neurologischer irAEs betrug fünf Wochen (Intervall 1-72). Bei allen Patienten wurde die Therapie mit ICI pausiert und eine immunsuppressive Therapie mit Kortikosteroiden begonnen. Eine zusätzliche immunmodulatorische Therapie war bei neun Patienten erforderlich. Insgesamt sprachen 16 von 18 Patienten auf die immunsuppressive Therapie an. Von allen 14 Patienten, die schwere Neurotoxizität (Grad 3-4) entwickelten, verstarben sechs bis zum Ende des Beobachtungszeitraums, während keiner der Patienten mit leichter Neurotoxizität (Grad 1-2) verstarb. Schwere Neurotoxizität wurde als negativer prognostischer Marker für die Gesamtüberlebensdauer identifiziert ($p = 0.046$).

Neurotoxizitäten sind früh manifestierende, rasch progrediente Komplikationen unter ICI Therapie mit einem breiten Spektrum an klinischen Präsentationen, welche das zentrale und periphere Nervensystem sowie das neuroendokrine System betroffen können. Ein hohes Maß an Aufmerksamkeit für neurologische irAEs ist während sowie nach ICI Therapie geboten, um zeitnah diagnostische und therapeutische Maßnahmen zur Prävention substantieller Morbidität und Mortalität einzuleiten. Randomisierte prospektive klinische Studien sind notwendig, um standardisierte diagnostische und therapeutische Algorithmen für ICI-vermittelte Neurotoxizitäten zu entwickeln.

2 List of Abbreviations

A	Atezolizumab
AChR	Acetylcholine receptor
ACTH	Adrenocorticotropic hormone
AGNA-1	Anti-glial nuclear antibody type 1
ADEM	Acute demyelinating encephalomyelitis
AEGM	Autoimmune encephalitis predominantly affecting the grey matter
AID	Autoimmune disorder
CK	Creatine kinase
CNS	Central nervous system
CSF	Cerebral spinal fluid
CTCAE	Cancer Institute's Common Terminology Criteria for Adverse Events
CTE	Number of cycles to neurotoxicity event
CTLA-4	Cytotoxic T-lymphocyte-associated antigen 4
DM1	Diabetes mellitus type 1
ECG	Electrocardiogram
EEG	Electroencephalography
EMG	Electromyography
FDA	U.S. Food and Drug Administration
GAD65	Glutamic acid decarboxylase 65 antibody
GKRS	Gamma knife radiosurgery
I	Ipilimumab
ICI	Immune checkpoint inhibitor
ICU	Intensive care unit
IrAEs	Immune-related adverse events
IV	Intravenous
IVIG	Intravenous immune globulin
LDH	Lactate dehydrogenase
MG	Myasthenia gravis
Mo	Months
MP	Methylprednisolone
MRI	Magnetic resonance imaging

MS	Multiple sclerosis
N	Nivolumab
NA	Not available
NCS	Nerve conduction study
NSCLC	Non-small cell lung cancer
NTX	Neurotoxicity
OCB	Oligoclonal bands
OS	Overall survival
P	Pembrolizumab
PD-1	Programmed cell death 1
PD-L1	Programmed cell death ligand 1
PND	Paraneoplastic neurological disorders
PNP	Paraneoplastic
PNS	Peripheral nervous system
RNS	Repetitive nerve stimulation
SCLC	Small cell lung cancer
SPEP	Serum protein electrophoresis
TSH	Thyroid-stimulating hormone
WBC	White blood count
WNL	Within normal limits
Y	Years

3 Comprehensive Summary of the Publication

3.1 Introduction

Immune checkpoint inhibitors (ICIs) are powerful immunotherapeutic agents that have transformed cancer therapy.[1] They have shown high response rates in patients with metastatic melanoma and various other advanced malignancies. ICIs are now FDA-approved for melanoma, non-small cell lung cancer (NSCLC), urothelial cancer and head and neck cancers, among others.[2-5] Due to the expanding trial landscape for ICIs, their clinical indication is expected to grow.[6]

ICIs are monoclonal antibodies that promote an immune response against tumor-specific antigens by blocking coinhibitory regulators of T-cell activation.[7] Identified binding targes of ICIs include the coinhibitory immune checkpoint receptors cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), programmed cell death 1 (PD-1), and its ligand, programmed cell death ligand 1 (PD-L1). While CTLA-4 is found on activated T cells, PD-1 is expressed on activated T cells, B cells, monocytes, and natural killer cells.[8,9] PD-L1 is expressed on antigen presenting cells and has further been identified in several cancer cells.[10] The CTLA-4 and PD-1/PD-L1 pathways are essential for maintaining peripheral immune tolerance. Activating these pathways inhibits T-cell function *via* a negative feedback mechanism, thereby preventing autoimmunity.[11,12] Various tumor cells can hijack and potentiate CTLA-4 and PD-1/PD-L1 signaling which leads to T-cell exhaustion. Escaping T-cell mediated immune destruction enables unchecked cancer proliferation and survival.[7,10]
ICIs interfere with these interactions and antagonize the inhibitory signal, which potentiates a destructive antitumor T cell immune response.[13] Yet, the upregulation of immune pathways by ICIs is unspecific. The administration of ICIs therefore pose the risk of provoking immune-related adverse events (irAEs).[14] Possible pathophysiological mechanisms of irAEs include: (i) the activation of T-cells with cross-reactivity between tumor antigens and endogenous antigens, (ii) the direct effect of ICIs binding to tissue antigens, (iii) the increased B-cell-mediated autoantibody production, and (iv) the increased production of pro-inflammatory cytokines.[15]

The spectrum of ICI-mediated irAEs is broad. Any organ system can potentially be affected. The most commonly observed irAEs involve the skin, the gastrointestinal tract, the liver, and the endocrine system. For example, melanoma-associated hypopigmentation, colitis, hepatitis and thyroid dysfunction have been linked to ICI therapy.[16] While neurological irAEs are relatively rare complications of ICIs, they can be particularly severe compared to irAEs in other organ systems and can even be life-threatening if left undiagnosed or untreated.[17,18] The cumulative incidence of ICI-mediated neurotoxicities has been reported to be approx. 3.8 % with anti-CTLA-4, 6.1 % with anti-PD-1 and 12.0 % with combination therapy, with severe neurotoxicities occurring in less than 1.0 % of all cases.[17] Neurotoxicities can involve the entire nervous system, including the central nervous system (CNS), the peripheral nervous system (PNS), and the neuroendocrine system.[17]

At present, there is a paucity of literature regarding the clinical characteristics and long-term sequelae of ICI-induced neurotoxicities. Current practical guidelines for the management of neurological irAEs are mostly derived from clinical observations and based on expert consensus.[19] Diagnosing ICI-mediated neurotoxicities is often challenging due to uncharacteristic symptoms and laboratory changes. Neurological irAEs should be categorized following existing diagnostic groups, although they might constitute their own disease entity requiring specific diagnostic and therapeutic algorithms.

The intensified application of ICIs for cancer therapies is expected to increase the prevalence of neurotoxicities. Clinicians need to be aware of treatment-related symptoms and their appropriate management to systematically reduce morbidity and mortality. The present work characterizes neurological irAEs, their clinical presentation, diagnostic findings, management, overall outcome, and prognostic markers. Further defining the clinical spectrum of ICI-mediated neurotoxicities will pave the way to raise awareness among treating physicians and foster early disease management to keep leveraging the advantages of ICIs at a much reduced patient risk.

3.2 Results

In the present work 18 patients with neurotoxicity following anti-PD-1 (pembrolizumab, nivolumab), anti-PD-L1 (atezolizumab) or anti-CTLA-4 (ipilimumab) therapy were retrospectively identified from the database of the Section of Neuro-Oncology and the Neuromuscular Clinic of the Department of Neurology at the Yale School of Medicine. Most neurotoxicities were reported following combination therapy with ipilimumab and nivolumab (*n* = 7), followed by anti-PD-1/PD-L1 and anti-CTLA-4 monotherapy. Melanoma was the most frequently observed cancer (*n* = 10). Patients further received ICIs for SCLC, NSCLC and Merkel-cell carcinoma. Neurotoxicities occurred after a median of 5 weeks (range 1-72) of ICI therapy. Neurotoxicities were graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.[20] The scale spans five grades of increasing severity, corresponding to mild (1), moderate (2), severe (3), life-threatening (4) and death related to adverse events (5). Low-grade neurotoxicity was defined as grade 1 and 2 and high-grade neurotoxicity comprised grade 3 and 4. High-grade neurotoxicity developed in 14 patients, three of whom had a preexistent neurological autoimmune disorder (AID), including myasthenia gravis (MG) and multiple sclerosis (MS). Low-grade neurotoxicities developed in four patients. Fifteen patients experienced other concurrent non-neurological irAEs.

CNS disorders predominated the patient population (*n* = 9) and comprised central demyelinating disorders (*n* = 5), autoimmune encephalitis predominantly affecting the grey matter (AEGM, *n* = 3), and aseptic meningitis (*n* = 1). Central demyelinating disorders included three cases of high-grade neurotoxicity, presenting as an exacerbation of MS in a patient with known relapsing-remitting MS, optic neuritis, and acute demyelinating encephalomyelitis (ADEM). The remaining two cases were low-grade neurotoxicity, including a demyelinating overlap syndrome and focal demyelinating encephalitis. While magnetic resonance imaging (MRI) findings consistent with demyelination aided in the diagnosis, brain biopsy was required in two cases to confirm demyelination and exclude brain metastasis or radiation necrosis.

AEGM were all high-grade neurotoxicities. In one patient glutamic acid decarboxylase 65 antibody (GAD65) was detected in the serum, another had paraneoplastic anti-glial nuclear antibody type 1 (AGNA-1) in the cerebral spinal fluid (CSF), and the third patient remained antibody negative. All patients presented with subacute encephalopathy.
Finally, aseptic meningitis was identified as high-grade CNS toxicity. The patient presented with fever and headache. CSF revealed mild lymphocytic pleocytosis with elevated protein levels without the detection of a viral or bacterial infection.

PNS toxicities comprised sensorimotor peripheral neuropathy (*n* = 2), MG (*n* = 3) and MG with concomitant myositis and myocarditis (*n* = 1).
Peripheral neuropathies were all high-grade neurotoxicity and diagnosis was based on clinical exam findings, demonstrating gait ataxia, areflexia, and diminished sensation in the lower extremities and electrodiagnostic findings supportive of sensorimotor axonal-demyelinating polyneuropathies. Although one patient received previous platinum-based chemotherapy, the onset of polyneuropathy immediately after ICI initiation supported an immune-mediated pathogenesis.
All four patients with ICI-mediated MG had high-grade neurotoxicity, three of whom were positive for anti-acetylcholine receptor antibodies (anti-AChR). The main presenting symptoms were generalized muscle weakness and fatigability. Two seropositive patients progressed to severe myasthenic crisis requiring ICU admission and ventilatory support for respiratory weakness. One seropositive MG patient had overlapping myositis and myocarditis. In the case of seronegative generalized MG, diagnosis was supported by electrophysiologic findings demonstrating decrement in repetitive nerve stimulation.

Low-grade ICI-induced hypophysitis (*n* = 3) was observed following anti-CTLA-4 monotherapy or combination therapy for metastatic melanoma. Commonly reported symptoms were fatigue, headache, insomnia, confusion, and proximal muscle weakness. Decreased serum levels of pituitary hormones supported the diagnosis of hypophysitis-induced anterior pituitary insufficiency. MRI of the

brain was performed to rule out metastasis and in one case MRI findings were consistent with pituitary inflammation.

In all patients, ICIs were discontinued followed by corticosteroid therapy. Additional immunosuppression was administered with infliximab (n = 1) and intravenous immunoglobulins (IVIG, n = 9). Neurotoxicities completely resolved in five patients, partially resolved in 11 patients, and persisted in the two remaining patients with ICI-induced polyneuropathy. Long-term therapy comprising monthly IVIG and daily oral pyridostigmine for MG or hormone substitution for anterior pituitary insufficiency was required in six patients.

Twelve patients were alive at database closure. The remaining six patients, all with high-grade irAEs, had died due to cancer progression. Overall survival estimates from ICI infusion to death of any cause or last follow-up were calculated along with predictors of outcome using Kaplan-Meier survival analysis and log-rank test. The significance level was set to $p \leq 0.05$ for all tests. Median follow-up was 19 months (range 2-80). Presentation with high-grade neurotoxicity (grade 3-4, n = 14) negatively impacted OS (p = 0.046) when compared to patients with low-grade neurotoxicity (grade 1-2, n = 4).

3.3 Discussion

This work characterizes the clinical spectrum of neurological irAEs based on a patient cohort with a diverse set of neurotoxicities, affecting the CNS, the PNS and the neuroendocrine system. The results suggest that ICI-induced neurotoxicities may increase morbidity and mortality.

3.3.1 Prognostic marker for survival in patients with neurotoxicity

High-grade neurotoxicity is identified as a negative prognostic indicator for OS. Although the ultimate cause of death in patients with high-grade neurotoxicities was progression of the underlying cancer, this finding suggests that high-grade neurotoxicity may impact OS due to uncontrolled tumor progression in response to ICI discontinuation. The role of neurotoxicities emphasize the importance of

optimized therapeutic algorithms to overcome neurotoxicities without impairing cancer therapies.

3.3.2 Central nervous system toxicity

CNS disorders are rare complications of ICIs with an estimated incidence ranging from 0.4 % to 1.0 %.[21] Clinical studies investigating ICI-induced CNS disorders are sparse and most reported evidence is based on smaller case series and case reports.[22-25] Previous studies predominantly reported neurotoxicities involving the PNS.[17,26] This work extends the spectrum of known neurotoxicities by contributing several cases of CNS toxicities.

ICI-induced central demyelinating disorders can develop *de novo* or as a flare of preexisting neurological AIDs. Relevant differential diagnoses include metastatic disease or radiation necrosis in recipients of gamma knife radiosurgery (GKRS). Radiographic distinction of these entities can be challenging[27] and might require histopathologic investigation *via* brain biopsy as seen in this cohort. Increased rates of radiation necrosis after GKRS in patients with ICI therapy compared to cytotoxic chemotherapy have been reported.[28] The mechanism by which ICIs contribute to the development of radiation necrosis is not fully understood, yet most likely involves radiation-induced disruption of the blood brain barrier, resulting in the facilitated interaction of peripheral immune cells with neuronal antigens thus promoting an inflammatory response.[29,30]

ICI-induced autoimmune encephalitis presented with an alteration in consciousness, confusion, memory dysfunction, accompanied by headaches, and hallucinations. Differential diagnoses are broad and include classic paraneoplastic neurological disorders (PND). In this work, the autoimmune antibody GAD-65 and paraneoplastic antibody AGNA-1 were identified in patients with encephalitis following ICI therapy. Whether ICIs mount *de novo* autoimmunity or unmask preexisting autoimmune and paraneoplastic syndromes is still unknown. However, the detection of autoimmune encephalitis and paraneoplastic antibodies following ICI exposure suggests that these

etiologies are not mutually exclusive. The frequency of anti-neuronal antibodies associated with ICI-mediated encephalitis is currently underexplored as antibody diagnostics, including both serum and CSF paraneoplastic panel, are rarely reported. Determining a baseline serologic profile of patients with cancers commonly associated with paraneoplastic neurological disorders may aid in predicting the risk for developing these adverse events. The high mortality of encephalitis[18] requires early recognition and appropriate intervention with corticosteroids as most of the cases are steroid-responsive.

3.3.3 Peripheral nervous system toxicity

The most commonly reported ICI-induced PNS toxicities are peripheral neuropathies, followed by myasthenic syndromes.[31-33]

ICI-induced peripheral neuropathies can cause axonal or demyelinating damage patterns and present acutely or subacutely.[31] Peripheral neuropathies are often associated with long-term sequelae due to permanent motor or sensory deficits[31] that are not responsive to steroid therapy as seen in this cohort.

MG is a severe complication of ICIs with an estimated mortality rate of 30.4 %.[32] The risk for myasthenic crisis, overlapping myositis, and myocarditis is higher for neurological irAEs than for idiopathic MG.[26,33,34] Especially immune-mediated myocarditis is associated with a severe clinical course and high mortality.[35] Patients with ICI-mediated MG often show residual neurological symptoms requiring long-term immunomodulatory therapy and in some cases additional symptomatic therapy with pyridostigmine.[33]

3.3.4 Neuroendocrine toxicity

Hypophysitis is a more common ICI-induced neuroendocrine toxicity with an estimated incidence of 3.3 %, especially following anti-CTLA-4 therapy used singly or in combination.[36] Clinical presentation of consequent hypopituitarism includes fatigue, dizziness, and headache. Often, anterior pituitary insufficiency is permanent, requiring long-term hormonal substitution.[37]

3.3.5 Diagnostic investigations of ICI-induced neurotoxicity

The timely diagnosis of ICI-induced neurotoxicities is complicated by the clinical presentation and diagnostic findings that are unspecific and do not necessarily fit the typical characteristics of defined neurological syndromes. Important differential diagnoses include cancer progression, paraneoplastic syndromes, metabolic disorders, infections, or complications related to other non-ICI therapies. Diagnostic evaluations of neurological irAEs should include MRI studies as imaging abnormalities demonstrating leptomeningeal enhancement, enlargement, or enhancement of the anterior pituitary gland or demyelination were described in aseptic meningitis, hypophysitis, and central demyelinating disorders, respectively. Further, imaging studies were used to exclude alternative diagnoses for the observed neurological symptoms, especially metastatic diseases. CSF analysis may further aid in the diagnosis of ICI-induced CNS disorders, revealing lymphocytic pleocytosis or elevated protein levels in aseptic meningitis or encephalitis.

3.3.6 Management of ICI-induced neurotoxicity

Patients need to be educated about symptoms related to ICI-mediated neurotoxicities and understand that the timing of presentation is highly variable, ranging from a few weeks to several months from ICI initiation and can occur even after discontinuation of ICI therapy.[38] However, this work demonstrates that an early presentation within a few weeks after ICI infusion is more common. These observations highlight the importance of constant vigilance for signs and symptoms of neurotoxicities throughout and after cessation of ICI therapy.

The most important measures to reduce morbidity and mortality in patients treated with ICIs are close clinical monitoring and adequate adverse event management. Multinational and multidisciplinary organizations such as the *American Society of Clinical Oncology* (ASCO) have developed comprehensive therapeutic outlines for ICI-induced toxicities to facilitate diagnosis and management.[19] Specific therapies depend on the respective phenotype and severity of the neurotoxicity. Steroids are the mainstay of therapy, as most

neurological irAEs are responsive to steroids.[26] The timely resolution of neurological symptoms to steroids can further aid in diagnosing an ICI-mediated etiology. As most of the patients in this cohort had improvements of their neurological symptoms upon holding ICIs and commencing steroids, a low threshold to begin immunosuppressive measures in patients with grade > 1 neurotoxicities should be established. In certain steroid-refractory cases, permanent drug cessation or additional IVIG, plasmapheresis or more aggressive immunosuppressants may be indicated to prevent permanent damage to the nervous system.[19,26] Treatment algorithms optimizing neurological outcome without compromising the effect of cancer therapy remain to be established. With respect to the latter, until now there has been no clear evidence that systemic steroid therapy affects the anticancer effect of ICIs or negatively impacts overall survival in oncologic patients with irAEs.[39,40]

3.4 Conclusion

In conclusion, ICIs can induce severe, potentially long-lasting and life-threatening neurological irAEs. In the era of emerging immunotherapies for cancers, neurological irAEs will be more frequently encountered and increasingly recognized. Defining the broad spectrum of neurotoxicities is warranted to facilitate diagnosis and management of irAEs for an optimized patient outcome. Randomized prospective studies are needed to develop diagnostic and treatment algorithms to provide optimized patient care. Predictive biomarkers would help to identify patients at risk for severe immune-mediated neurotoxicity.

4 Publication List

Duong SL, Barbiero FJ, Nowak RJ, Baehring JM. Neurotoxicities associated with immune checkpoint inhibitor therapy. J Neurooncol. 2021 Apr;152(2):265-277. doi: 10.1007/s11060-021-03695-w.

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CLINICAL STUDY

Neurotoxicities associated with immune checkpoint inhibitor therapy

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Abstract

Introduction Immune checkpoint inhibitors (ICIs) have emerged as a promising class of cancer immunotherapies. Neurotoxicities are uncommon, but often severe, and potentially fatal complications of ICIs, and clinical experience is limited. The aim of this study is to further define the clinical spectrum and outcome of ICI-mediated neurotoxicities.

Methods Patients with ICI-associated neurotoxicities were identified from retrospective review of the quality control database at a single institution. Data regarding demographics, medical history, clinical presentation, diagnosis, management and outcome were recorded.

Results We identified 18 patients with neurotoxicity following ICI therapy with pembrolizumab, nivolumab, atezolizumab, or ipilimumab for a diverse set of malignancies. Neurotoxicities comprised central demyelinating disorder (28%), autoimmune encephalitis predominantly affecting the grey matter (17%), aseptic meningitis (6%), myasthenia gravis (MG) (17%) with concurrent myositis (6%), sensorimotor polyneuropathy (11%) and hypophysitis (17%). Median time to onset of neurotoxicities was 5 weeks (range 1–72). All patients discontinued ICIs and received steroids with additional immunomodulation required in 9 patients, resulting in improvement for 16 of 18 patients. Grade 3–4 neurotoxicity developed in 14 patients, of whom 6 had died at database closure. Grade 3–4 severity negatively impacted overall survival (OS) ($p = 0.046$).

Conclusions ICI-mediated neurotoxicities present early, are rapidly progressive and include a diverse phenotype affecting the CNS, PNS and neuroendocrine system. A high level of vigilance is warranted, as early diagnosis and targeted treatment can substantially prevent morbidity and mortality. Prospective clinical trials are warranted to assess optimized management of ICI-induced neurotoxicities.

Keywords Neurologic immune-related adverse events · Immunotherapy · Cancer · PD1 · CTLA4

Introduction

Immune checkpoint inhibitors (ICIs) have emerged as a potent class of immunotherapeutic agents and are now FDA-approved for an increasing number of advanced malignancies [1–3]. Further ongoing trials are anticipated to expand their indications [4]. ICIs constitute monoclonal antibodies that block negative regulators of T cell activation by targeting the cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), programmed cell death 1 (PD-1) and its ligand programmed cell death ligand 1 (PD-L1). This process potentiates a destructive antitumor T cell immune response [5]. Given their mechanism of action it has come as no surprise that unique autoimmune toxicities, termed immune-related adverse events (irAEs) have been described in ICI recipients [6]. ICI-related neurotoxicities are uncommon and the reported cumulative incidence of severe neurotoxicities is estimated at less than 1% [7]. Neurotoxicities can involve the CNS, the PNS and the neuroendocrine system and may be associated with substantial morbidity and mortality if left untreated [7–10]. At present, the pathogenesis of ICI-related neurotoxicities has not been fully understood and data regarding the clinical characteristics and long-term outcome are limited with most evidence derived from isolated

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case reports and small case series [11–14]. This study characterizes patients who presented to the Yale New Haven Hospital with neurotoxicities related to ICIs. We describe the clinical presentation, management and overall outcome of ICI-induced neurotoxicities to further define the diverse toxicity profile of ICIs.

Material and methods

Patient selection

We identified patients with neurotoxicities following treatment with ICIs targeting PD-1 (pembrolizumab, nivolumab), PD-L1 (atezolizumab) or CTLA-4 (ipilimumab) from the quality control database of the Section of Neuro-Oncology, Yale Cancer Center, New Haven, CT and the Neuromuscular Clinic, Department of Neurology, Yale School of Medicine using the keywords: "PD-L1", "PD-1", "pembro", "atezo", "ipilimumab", "CTLA-4", "CTLA", "immune checkpoint", "ICI" and "checkpoint inhibitors". Patients encountered between 01/2014 and 06/2018 were included. We performed a comprehensive chart review and recorded patient demographics, medical history, ICI characteristics and neurotoxicity data.

Neurotoxicity assessment

We defined immune-related neurotoxicity as an autoimmune disorder related to ICI exposure. Neurologic symptoms attributed to tumor progression or systemic treatment other than ICIs were excluded. Neurotoxicities were graded retrospectively on the basis of chart review according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 [15]. The scale ranges from 1 to 5. Grades 1 and 2 are considered low-grade and grades 3 and 4 as high-grade neurotoxicity. Grade 5 describes death related to the adverse event.

Statistical analysis

Descriptive statistics were calculated for all patient data. Categorical variables are reported in absolute numbers and relative frequencies. Continuous variables are described by medians and ranges are given. Baseline patient characteristics were compared using Fisher's exact test for dichotomous categorical variables and the Wilcoxon rank sum test for continuous variables. For survival analysis, patients were followed until death or database closure. Patients alive at last follow-up were censored. Overall survival (OS) was defined as the time from ICI initiation to the date of death of any cause or last follow-up. Survival estimates and prognostic markers of outcome were calculated using the Kaplan–Meier method and log-rank test. p-values <0.05 were considered significant for all tests. All analyses were performed with the IBM SPSS Statistics software, version 25 [16].

Results

Study cohort

We identified 18 patients with ICI-mediated neurotoxicities at our institution (Table 1). Among those, one case (patient 3 in Table 2) had already been reported previously [17]. Four of 18 patients (22%) developed low-grade neurotoxicity (grade 1–2), whereas high-grade neurotoxicity (grade 3–4) developed in 14 (78%) patients. Most neurotoxicities were associated with ipilimumab (anti-CTLA-4) and nivolumab (anti-PD-1) therapy in combination or sequentially ($n=7$; 39%), followed by anti-PD-1/PD-L1 (pembrolizumab, nivolumab, atezolizumab) and anti-CTLA-4 (ipilimumab) monotherapy. The most common underlying malignancy was melanoma ($n=10$; 56%). Patients were further treated for small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC) and Merkel-cell carcinoma. All patients had advanced stage disease with brain metastases present in 5 out of 18 (28%) patients. Eight patients (44%) of the study cohort had received prior therapy such as radiation, surgery or systemic therapies for the underlying malignancy. Patients with local tumor resection outside the nervous system were not listed. Neurological autoimmune disorders (AID) were preexistent in three patients (17%). All of them developed high-grade neurotoxicity, including generalized myasthenia gravis (MG), ocular MG and multiple sclerosis (MS). We found no significant differences while comparing the toxicity groups (grade 1–2 versus grade 3–4) with patient demographics, cancer type, CNS metastasis, previous therapies, preexistent neurological AID or ICI regimen, albeit the frequency of these factors was low in our cohort.

Neurotoxicity phenotypes

Neurotoxicities affected the CNS, PNS and neuroendocrine system (Fig. 1). The median time from ICI administration to onset of neurotoxicities was 5 weeks (range 1–72) and the median number of ICI cycles received was 2 (range 1–36). Patients 5, 7 and 8 who developed neurotoxicities at least 6 months after ICI initiation (see Table 2) had intermittent study drug discontinuation due to non-neurologic irAEs or changed from monotherapy to combination therapy. CNS toxicities predominated and included central demyelinating disorder ($n=5$; 28%), autoimmune encephalitis predominantly affecting the grey matter (AEGM, $n=3$; 17%) and aseptic meningitis ($n=1$; 6%) (Fig. 1a). Among them,

Table 1 Baseline characteristics associated with neurotoxicity grade

Neurotoxicity grade	Grade 1–2 ($n=4$)	Grade 3–4 ($n=14$)	Total ($n=18$)	p value
Median age (range), years	62.5 (58–66)	63 (46–79)	63 (46–79)	0.789
Sex, male, *n* (%)	2 (50)	8 (57)	10 (56)	1.0
PD-1 and CTLA-4, *n* (%)				1.0
Nivolumab + Ipilimumab	2 (50)	5 (36)	7 (39)	
PD-1/PD-L1, *n* (%)				0.588
Pembrolizumab	0	4 (29)	4 (22)	
Nivolumab	1 (25)	2 (14)	3 (17)	
Atezolizumab	0	1 (7)	1 (6)	
CTLA-4, *n* (%)				1.0
Ipilimumab	1 (25)	2 (14)	3 (17)	
Cancer type, *n* (%)				
Melanoma	3 (75)	7 (50)	10 (56)	0.588
SCLC	1 (25)	3 (21)	4 (22)	1.0
NSCLC	0	3 (21)	3 (17)	1.0
Merkel-cell carcinoma	0	1 (7)	1 (6)	1.0
CNS metastasis[a], *n* (%)	1 (25)	4 (29)	5 (28)	1.0
Prior therapy, *n* (%)				
CNS radiosurgery/surgical resection	0	2 (14)	2 (11)	1.0
Systemic therapy[b]	1 (25)	4 (29)	5 (28)	1.0
Two or more	0	1 (7)	1 (6)	1.0
None	3 (75)	7 (50)	10 (56)	0.588
Neurological AID[c], *n* (%)	0	3 (21)	3 (17)	1.0

Baseline characteristics of our cohort stratified by neurotoxicity. p values are given for Fisher's exact and Wilcoxon rank sum tests

AID autoimmune disorder, *CNS* central nervous system, *ICI* immune checkpoint inhibitor, *NSCLC* non-small cell lung cancer, *SCLC* small cell lung cancer

[a]Presence of CNS metastasis before ICI therapy

[b]Systemic therapy included chemotherapy or molecular targeted therapy

[c]History of neurological AID before ICI therapy included generalized and ocular MG and MS

seven were high-grade toxicities (Fig. 1b). PNS toxicities ($n=6$; 33%) included MG ($n=3$; 17%), MG complicated by myositis and myocarditis ($n=1$; 6%) and sensorimotor polyneuropathy ($n=2$; 11%), of which all were high-grade toxicities. Neuroendocrine toxicity comprised hypophysitis ($n=3$, 17%), with one case of high-grade severity. Concurrent non-neurologic irAEs were observed in 15 patients (83%), presenting as fatigue ($n=8$), fever ($n=2$), skin rash ($n=4$), vitiligo ($n=2$), sicca-syndrome ($n=1$), transaminitis ($n=4$), diabetes mellitus type 1 (DM1; $n=1$), adrenalitis ($n=1$), hypothyroidism ($n=2$), colitis ($n=1$), pancreatitis ($n=1$) and arthralgia ($n=3$). Table 2 summarizes characteristics, diagnostic evaluations, management and outcome of ICI-related neurotoxicity in our cohort.

Central demyelinating disorder ($n=5$)

Central demyelinating disorder was diagnosed in patients 1–5. Onset occurred at a median of 15 weeks (range 3–36). Patient 1, with preexistent relapsing-remitting MS, experienced a flare following atezolizumab. Patients 2–5 developed new onset demyelinating disorders. Patient 2 had optic neuritis, manifesting as bilateral vision loss. Patient 3 with acute demyelinating encephalomyelitis (ADEM), presented with subacute encephalopathy. Patient 4, diagnosed with a demyelinating overlap syndrome, developed dysesthesias in the extremities and proximal muscle weakness. Patient 5 had a focal demyelinating encephalitis, presenting with leg heaviness. MRI abnormalities consistent with demyelinating lesions were present in four of five patients. Patients 3 and 5 required histopathologic confirmation due to the close proximity of new demyelinating lesions to previous gamma knife radiosurgery (GKRS) sites.

Autoimmune encephalitis predominantly affecting the grey matter ($n=3$)

AEGM developed in patients 6–8 with metastatic lung cancer and diagnosis was supported by a combination of serology, MRI, cerebral spinal fluid (CSF) and EEG findings.

Table 2 Clinical characteristics, diagnostic investigations, management and outcome of ICI-mediated neurotoxicities

Case/sex/age[a], y	NTX	NTX grade	ICI	CTE	Cancer	Diagnostic assessment		Management	Outcome	Survival/follow-up, mo
						MRI	Other tests			
1[b]/M/46	Multiple sclerosis relapse	3	A	1	NSCLC	MRI brain + spine: new enhancing cerebral lesions and optic nerve enhancement	CSF: OCB positive; serology: WNL	IV MP 1 g/day for 3 days, then prednisone taper over 1 mo	Resolved	Alive/11
2/F/61	Bilateral optic neuritis	3	I + N	6	Melanoma	MRI brain: optic nerve enhancement	CSF: lymphocytic pleocytosis 130 WBC/μl, protein 165 mg/dl, OCB negative, anti-NMO and PNP antibody negative; serology: WNL	IV MP 1 g/day for 5 days, IVIG 2 g/kg over 2 days, then prednisone taper over 1 mo, IV infliximab 10 mg/kg one dose for worsening symptoms	Improved with sequelae	Dead/19
3/F/77	Acute demyelinating encephalo-myelitis	3	I	4	Melanoma	MRI brain: edema in GKRS site, FLAIR signal in parietal, frontal lobe and optic nerve	CSF: mild lymphocytic pleocytosis (exact count unknown), OCB negative; EEG: WNL; brain biopsy: demyelination with inflammatory cells	IV dexamethasone 10 mg one dose, then IV MP 1 g/day for 5 days	Improved with sequelae	Dead/8
4/F/58	Overlapping demyelinating syndrome	2	I + N	2	Melanoma	MRI brain + spine: no metastasis	Serology: PNP and autoimmune neuropathy antibody negative; EMG and NCS: WNL	Prednisolone taper over 1 mo, IV MP 2 mg/kg one dose for worsening symptoms	Resolved	Alive/19
5/F/66	Subacute focal demyelinating encephalitis	2	N	15	SCLC	MRI brain: rim-enhancing lesion close to GKRS site	Brain biopsy: demyelination with inflammatory cells	IV MP 1 g/day for 5 days, then prednisone taper over 1 mo	Resolved	Alive/32

Table 2 (continued)

Case/sex/age[a], y	NTX	NTX grade	ICI	CTE	Cancer	Diagnostic assessment		Management	Outcome	Survival/ follow-up, mo
						MRI	Other tests			
6/M/57	Autoimmune limbic encephalitis (GAD65 positive)	3	N	1	SCLC	MRI brain: FLAIR signal in mesial temporal lobes	CSF: lymphocytic pleocytosis 9 WBC/μl; PNP antibody negative, anti-GAD65 not tested in CSF; serology: anti-GAD65 positive, PNP antibody negative; EEG: temporal lobe hyperexcitability	IV MP 1 g/day, IVIG 2 g/kg over 5 days, then prednisone taper over 6 mo and monthly IVIG 2 g/kg, levetiracetam for seizure prophylaxis	Improved with sequelae	Dead/20
7/M/64	Autoimmune paraneoplastic limbic encephalitis (AGNA1 positive)	4	N	36	SCLC	MRI brain + spine: no metastasis	CSF: lymphocytic pleocytosis 13 WBC/μl, OCB positive, PNP: anti-AGNA1 positive; SPEP: WNL; EEG: polymorphic delta activity pronounced over fronto-temporal lobe	IV MP 1 g/day and IVIG 2 g/kg over 5 days, then prednisone taper over 1 mo, levetiracetam for seizures prophylaxis	Improved with sequelae	Dead/28
8/F/71	Autoimmune limbic encephalitis (antibody negative)	3	I + N	2	NSCLC	MRI brain: FLAIR signal in temporal lobes and thalami	CSF: lymphocytic pleocytosis 100 WBC/μl, protein 180 mg/dl, PNP antibody negative; EEG: NA	IV MP 1 g/day for 5 days, empiric antimicrobial coverage (no infection confirmed)	Resolved	Alive/7
9/F/56	Aseptic meningitis	3	P	1	NSCLC	MRI brain: no new metastasis	CSF: lymphocytic pleocytosis 340 WBC/μl, protein 84 mg/dl, glucose 55 mg/dl	Empiric antimicrobial coverage (no infection confirmed), IV dexamethasone 8 mg/day	Resolved	Alive/2

Table 2 (continued)

Case/sex/age[a], y	NTX	NTX grade	ICI	CTE	Cancer	Diagnostic assessment		Management	Outcome	Survival/follow-up, mo
						MRI	Other tests			
10[b]/M/75	Myasthenic crisis	4	P	2	Melanoma	MRI chest: no thymoma	Serology: anti-AChR antibody positive	ICU, BPAP, IV MP 1 g/day for 5 days, IVIG 2 g/kg over 4 days, then prednisone taper over 10 mo and monthly IVIG 2 g/kg	Improved with sequelae	Alive/3
11/M/62	Myasthenic crisis	4	P	1	Melanoma	NA	Serology: anti-AChR antibody positive	ICU, BPAP, IV MP 1 g/day for 3 days, IVIG 2 g/kg over 5 days, then prednisone taper (duration unknown)	Improved with sequelae	Alive/2
12[b]/M/79	Generalized myasthenia gravis	3	P	1	Merkel-cell carcinoma	MRI brain: no metastasis	Serology: anti-AChR antibody negative; RNS: decrement; EMG: consistent with MG	IV MP 1 g/day for 3 days, IVIG 2 g/kg over 5 days, prednisone tapered down to 20 mg/day, IVIG 1 g/kg every 3 weeks, pyridostigmine 180 mg/day	Improved with sequelae	Dead/12
13/F/57	Generalized myasthenia gravis with myositis and subclinical myocarditis	3	I + N	1	Melanoma	NA	Serology: anti-AChR antibody positive, myositis antibody negative; CK 9000 U/l, troponin T elevated, LDH 938 IU/l, AST 202 IU/l, ALT 289 IU/l	IV MP 1 g/day for 3 days, IVIG 2 g/kg over 4 days, pyridostigmine 90 mg/day, then prednisone taper over 1 mo	Improved with sequelae	Alive/5
14/F/59	Sensorimotor polyneuropathy	3	I + N	1	SCLC	MRI brain + spine: no metastasis	Serology: PNP antibody negative; NCS: severe sensorimotor axonal-demyelinating neuropathy	IV MP 1 g/day for 3 days, then prednisone tapered down to 10 mg/day (duration unknown), IVIG 2 g/kg over 5 days for worsening symptoms	Ongoing	Alive/7

Table 2 (continued)

Case/sex/age[a], y	NTX	NTX grade	ICI	CTE	Cancer	Diagnostic assessment		Management	Outcome	Survival/ follow-up, mo
						MRI	Other tests			
15/M/72	Sensorimotor polyneuropathy	3	I+N	6	Melanoma	MRI brain: no metastasis	CSF: lymphocytic pleocytosis 29 WBC/μl, protein 88 mg/dl; NCS: severe sensorimotor axonal-demyelinating neuropathy	IVIG 2 g/kg over 3 days every 2 weeks and prednisone taper over 2.5 mo	Ongoing	Alive/30
16/M/65	Hypophysitis	2	I	3	Melanoma	MRI brain: no metastasis	CSF: WNL; TSH low, free T4 WNL, cortisol low	Prednisone taper over 3 mo, L-thyroxine 100 μg/day	Improved with sequelae	Alive/80
17/M/60	Hypophysitis	2	I+N	3	Melanoma	MRI brain: pituitary inflammation	TSH WNL, total and free T4 low, AM cortisol and ACTH low	IV MP 1 mg/kg single dose, prednisone tapered down to 10 mg/day, L-thyroxine 100 μg/day	Improved with sequelae	Alive/75
18/M/75	Hypophysitis	3	I	1	Melanoma	MRI brain: no metastasis	TSH low, total and free T4 high, AM cortisol and ACTH low, abnormal ACTH stimulation test	Prednisone tapered down to 5 mg/day, L-thyroxine daily (dose unknown)	Improved with sequelae	Dead/24

A atezolizumab, *AB* antibody, *ACTH* adrenocorticotropic hormone, *AGNA1* anti-glial nuclear antibody type 1, *CTE* number of cycles to NTX event, *CSF* cerebrospinal fluid, *EMG* electromyography, *GKRS* gamma knife radiosurgery, *I* ipilimumab, *IV* intravenous, *IVIG* intravenous immunoglobulin, *MG* myasthenia gravis, *MP* methylprednisolone, *mo* months, *MRI* magnetic resonance imaging, *MS* multiple sclerosis, *N* nivolumab, *NA* not available, *NCS* nerve conduction study, *NSCLC* non-small cell lung cancer, *NTX* neurotoxicity, *OCB* oligoclonal bands, *P* pembrolizumab, *PNP* paraneoplastic, *RNS* repetitive nerve stimulation, *SCLC* small cell lung cancer, *SPEP* serum protein electrophoresis, *WBC* white blood count, *WNL* within normal limits, *y* years

[a]Age at time of cancer diagnosis

[b]Patients with preexisting autoimmune neurologic disorder experiencing relapses under ICI therapy

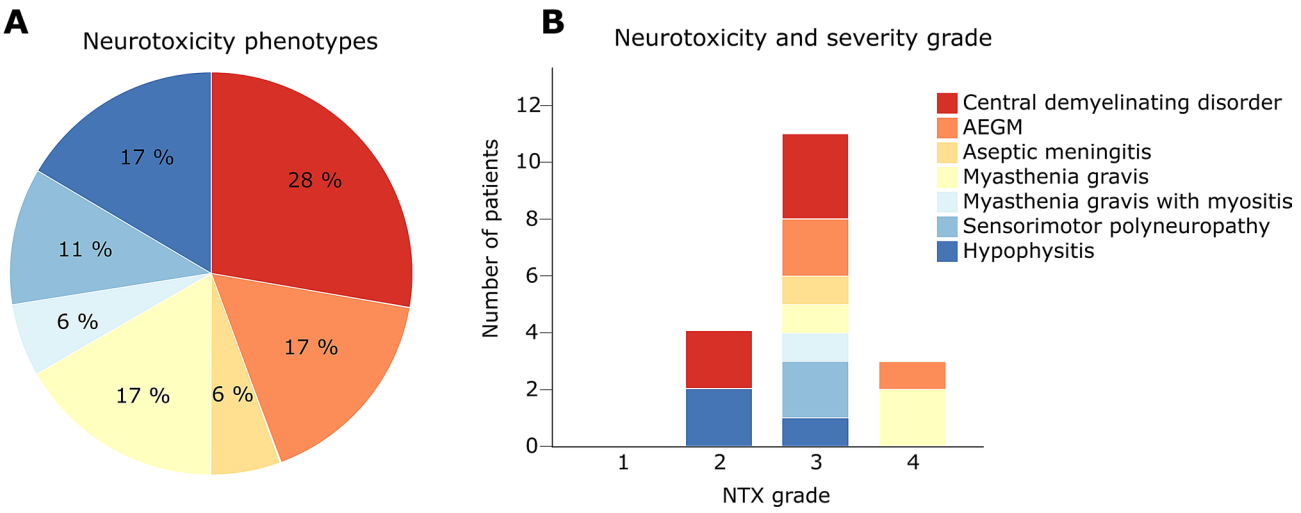

Fig. 1 Characteristics of neurotoxicity (NTX). (**a**) Pie chart depicting the different phenotypes of NTX associated with ICIs ($n=18$). Central demyelinating disorders were the most frequent neurotoxicities ($n=5$; 28%). (**b**) Stacked bar chart showing number of patients with NTX phenotypes and their respective severity grade. High-grade neurotoxicities ($n=14$; 78%) were more common than low-grade NTX ($n=4$; 22%). Note that the legend applies to both panels. *AEGM* autoimmune encephalitis predominantly affecting the grey matter

Time to symptom onset was at 5, 24 and 72 weeks after ICI initiation. All patients presented with subacute encephalopathy. Limbic system involvement was suggested by psychiatric symptoms. Patient 6 with glutamic acid decarboxylase 65 antibody (GAD65) positive autoimmune limbic encephalitis, also developed new-onset insulin dependent DM1 following 1 cycle of ICI. Patient 7 was diagnosed with anti-glial nuclear antibody type 1 (AGNA1) positive limbic encephalitis. Patient 8 had antibody negative autoimmune limbic encephalitis.

Aseptic meningitis ($n=1$)

Patient 9 developed aseptic meningitis 3 weeks following pembrolizumab therapy and presented with fever and headache.

Myasthenia gravis ($n=3$) and myasthenia gravis with concurrent myositis ($n=1$)

High-grade MG was diagnosed in patients 10–13 and presented with a median time to onset of 13 days (range 4–29). Patients 10 and 11 had life-threatening myasthenic crisis with respiratory failure. Notably, patient 10 had preexistent anti-AChR-antibody positive generalized MG, stably controlled on immunosuppression. Patient 12, with a previous episode of ocular myasthenia, developed generalized MG. Patient 13, with a family history of MG, developed generalized MG with concurrent myositis. Clinically, the patient presented with diplopia, ptosis, muscle weakness with fatigability and myalgia. Laboratory work-up demonstrated elevated creatine kinase, lactate dehydrogenase, liver transaminases and cardiac troponin T. Concomitant immune-mediated subclinical myocarditis was suspected as ECG and echocardiogram were negative for any acute pathology or structural damage. Serologic tests revealed anti-AChR antibodies in three out of four patients (patients 10, 11 and 13).

Sensorimotor axonal-demyelinating polyneuropathy ($n=2$)

Patients 14 and 15 were diagnosed with sensorimotor axonal-demyelinating polyneuropathy, based on clinical and electrodiagnostic evaluation, following 3 and 12 weeks of combined nivolumab and ipilimumab therapy, respectively. Although previous treatment with carboplatin and cisplatin was reported in patient 14, the onset of neurotoxicity immediately after ICI initiation supported an immune-related pathogenesis.

Hypophysitis ($n=3$)

Hypophysitis developed in patients 16–18 with metastatic melanoma within 4–7 weeks of ICI therapy. Symptoms included fatigue, headaches, insomnia, confusion and proximal muscle weakness. Diagnosis of hypophysitis induced hypopituitarism was established by low serum levels of pituitary hormones, while patient 17 also had MRI findings consistent with pituitary inflammation.

Clinical outcome and prognostic factors

In all cases, ICIs were held and corticosteroids started. Corticosteroids were commonly administered as pulse-dose IV

methylprednisolone (Table 2), followed by a slow prednisone taper over at least 4 weeks. Additional immunosuppression with intravenous immunoglobulins (IVIG) was administered in 9 out of 18 patients (50%) and patient 2 also received infliximab. Six out of the cohort (33%) continued maintenance therapy: patients 10, 12 and 13 with MG received IVIG or pyridostigmine and patients 16–18 remained on long-term hormone substitution for hypopituitarism. Sixteen out of 18 patients (89%) were responsive to immunosuppressive therapy with complete resolution of neurotoxicity in five patients (28%) (Fig. 2a).

Anti-PD-1 monotherapy was resumed in two patients (4 and 17), both with low-grade neurotoxicity and prompt improvement following immunosuppression. No neurologic and non-neurologic irAEs developed with resumption of anti-PD-1 monotherapy. However, patient 4 with subsequent anti-PD-1/CTLA-4 combination therapy experienced grade 4 transaminitis, leading to permanent ICI discontinuation. At the time of database closure, 12 patients were alive (67%) and 6 (33%) had died, all of whom were among the high-grade neurotoxicity group. The fatalities were due to progression of the underlying cancer. Median follow-up was 19 months (range 2–80). The median OS for the cohort was not reached, as more than half of the patients were still alive at the end of follow-up (Fig. 2b). High-grade neurotoxicity ($n=14$) was a significant negative prognostic factor for OS ($p=0.046$) when compared to patients with low-grade neurotoxicity ($n=4$) (Fig. 2c). OS did not significantly differ between CNS and non-CNS toxicity ($p=0.202$) (Fig. 2d).

Discussion

While ICIs have revolutionized the field of cancer immunotherapy [18], they can be complicated by serious neurotoxicities [1, 9, 19, 20], and clinical experience with the toxic potential of ICIs is still limited. Thus, a comprehensive definition of the diverse set of neurotoxicities

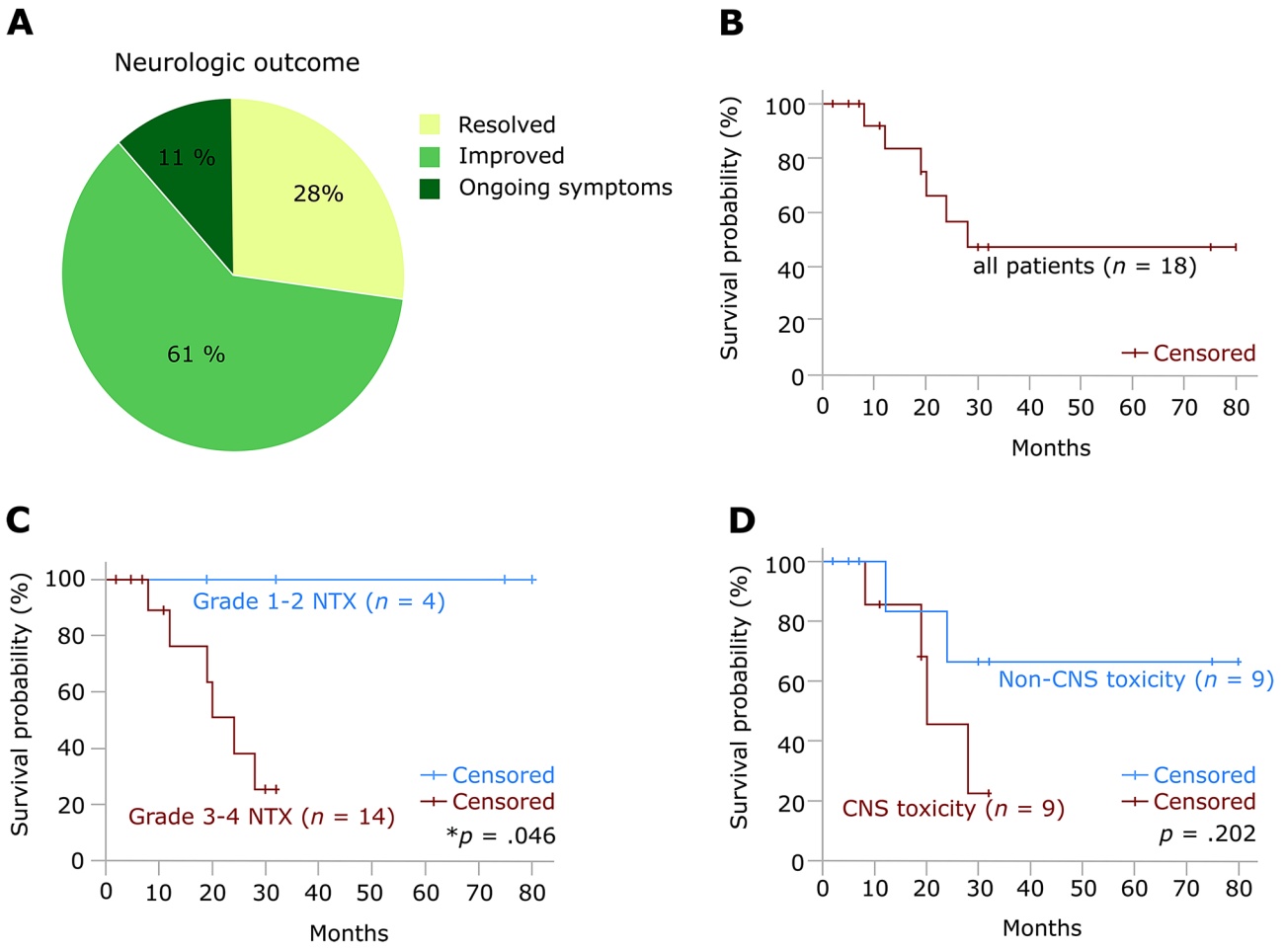

Fig. 2 Neurologic outcome and prognostic marker for OS in patients with neurotoxicity (NTX). (**a**) Pie chart depicting the neurologic outcome following immunosuppressive management of NTX ($n=18$). (**b**) Kaplan–Meier estimates of OS in this study cohort ($n=18$). (**c**) OS curves stratified by grade of neurotoxicity. (**d**) OS curves comparing CNS and non-CNS toxicity. Tick marks indicate censored patients

is required to increase awareness among clinicians and facilitate management for a favorable patient outcome. To the best of our knowledge, we present one of the largest single-center case series to date with long-term follow-up of ICI-related neurotoxicities. Contrary to previously published studies, our cohort comprised patients with various types of cancers undergoing different ICI regimens, thus reducing selection bias in this study.

We observed highly variable neurotoxic phenotypes. Clinical presentation can be non-specific including headaches or fatigue, leading to a lack of recognition of these events as ICI-related neurotoxicities [21]. While neurotoxicities have mostly been reported to involve the PNS [7, 22], inflammatory CNS disorders were predominant in our series. Inflammatory CNS disorders are rare complications with a reported incidence from 0.4% to 1.0% [23], yet more studies report ICI-mediated demyelinating diseases, autoimmune encephalitis predominantly affecting the grey matter and aseptic meningitis [24–26].

Central demyelinating diseases associated with ICIs can occur de novo or present as an exacerbation of a preexisting condition and have to be distinguished from brain metastasis or radiation necrosis. In prior recipients of GKRS, distinction of ICI-induced demyelination, radiation necrosis, or relapsed tumor may be difficult [27] and require histopathologic confirmation as seen in our cohort. Such cases with demyelination in close proximity to previously irradiated areas raise the question whether radiosurgery increases susceptibility to ICI-associated CNS toxicity. Several studies have demonstrated that radiation-induced disruption of the blood brain barrier facilitates exposure of neural antigens to peripheral immune cells, promoting humoral autoimmunity [11, 28, 29]. Further investigation is required to explore the interplay of radiosurgery and ICIs in the development of CNS inflammation.

ICI-mediated autoimmune encephalitis has to be distinguished from classic paraneoplastic neurologic disorders (PND). Certain characteristics more indicative of an ICI-mediated etiology have been observed in our cohort and include: (1) the development of autoimmune encephalitis late in the course of cancer whereas classic PNDs usually precede cancer diagnosis, (2) the onset of neurotoxicity following ICI exposure, demonstrating timely association with the agent, and (3) the responsiveness to immunosuppression whereas tumor-induced PNDs are commonly refractory to these measures, but may improve with cancer therapy [30]. On the other side, the observation of a positive classic paraneoplastic AGNA-1 antibody shortly after ICI initiation suggests that ICIs may be able to unmask paraneoplastic encephalitis, indicating that these etiologies are not mutually exclusive.

ICI-mediated aseptic meningitis has been previously described in the literature and in accordance with our observations, the neurotoxicity is highly responsive to steroids [9].

Myasthenia gravis has been increasingly recognized as a severe neurotoxicity with a mortality rate estimated at 30.4% [8]. For most ICI-related cases, prognosis is poor with rapid clinical deterioration, increased risk for life-threatening myasthenic crisis and concurrent myositis compared to classic MG [31, 32].

Acute sensorimotor neuropathy and hypophysitis are both more frequently encountered ICI-mediated neurotoxicities [22, 33]. These phenotypes are likely underrepresented in our cohort as we may not have been consulted on cases with mild and transient peripheral neuropathy [13, 25] or hypophysitis.

Time to onset of neurotoxicities varied greatly with a tendency for early presentation within a few weeks after ICI administration. Whether the timing of neurotoxicities depends on a specific phenotype or is associated with treatment-specific or patient-specific factors remains unclear.

Diagnosis of ICI-mediated neurotoxicities poses a great challenge as the differential diagnosis is broad, ranging from tumor progression, PND, metabolic derangements, infections and complications related to concurrent treatment modalities. Our study highlights the pivotal role of MRI in the evaluation of CNS toxicities. Meningeal enhancement, pituitary inflammation or demyelination may aid in the early diagnosis of ICI-related aseptic meningitis, hypophysitis and central demyelinating disorders, respectively.

The majority of our patients showed a favorable neurologic outcome following ICI discontinuation and steroid therapy, further providing evidence supporting the current guidelines for management of irAEs [34]. Depending on the severity of the neurotoxicity, further immunomodulation with IVIG, plasmapheresis or immunosuppressive drugs might be required [34]. Based on our observations, in patients with neurotoxicities higher than grade 1 the threshold to withhold ICIs and commence steroids or more aggressive immunosuppression should be low in order to prevent permanent damage to the nervous system [32]. It has been encouraging that a correlation between systemic steroid use and a negative impact on the anticancer effect of ICI therapy has not been clearly established [35, 36].

Data regarding the use of ICIs in patients with preexisting autoimmune neurologic conditions are limited as most clinical trials have excluded patients with known AID. Our data suggest that preexistent AID may increase the risk for high-grade toxicities. However, since most of the neurotoxicities can be successfully managed [7, 37], autoimmunity should not be an absolute contraindication for the life-prolonging effect of ICIs.

We identified high-grade neurotoxicity as a negative prognostic factor for OS. Although mortalities occurred late in the course of ICI therapy (median of 19.5 months,

range 8.0–28.0), and cause of death was the progression of the underlying malignancy, neurotoxicities may have negatively impacted OS based on the following explanations: First, high-grade neurotoxicities impose greater damage to the nervous system with the potential of progressing to a life-threatening condition. Second, the aggressive immunosuppression to control for these neurotoxicities raises the risk for opportunistic infections and metabolic derangements that again increases morbidity. Third, high-grade neurotoxicities may be too severe for ICI resumption allowing for uncontrolled tumor progression. As ICIs have demonstrated improved OS rates [1, 19, 38], the impact of ICI discontinuation due to neurotoxicities on OS is deleterious. Optimized patient support is warranted to overcome these neurotoxicities in order to resume ICIs for adequate cancer management.

Limitations

Despite the comparatively abundant patient data presented in this study, some limitations arise. Retrospective study designs are prone to selection bias. Our colleagues may have consulted us preferentially for neurotoxicities of higher grade and complexity, while low-grade or previously described neurotoxicities (hypophysitis, demyelinating polyneuropathy) might be underrepresented. While previous reports have listed non-specific presentations (headaches, dysgeusia or insomnia) as neurologic irAEs, we did not include these symptoms as a separate neurotoxicity as they are non-specific and can occur in a variety of non-neurologic disorders. Despite having a standardized grading system for irAEs, toxicity grading is subject to interobserver variability. The short follow-up period limits meaningful survival analyses, as neurotoxicities may develop over time, even after discontinuation of ICIs. The number of cases in our cohort restricts its statistical power and does not allow for firm conclusions for all studied dependencies.

Conclusion

ICIs are emerging as powerful cancer immunotherapies but are associated with a broad spectrum of early onset, rapidly progressing and potentially fatal neurotoxicities. Considering the expanding indications for ICIs, neurotoxicities are anticipated to become more prevalent. Therefore, physicians need to be familiar with these irAEs as timely management is crucial to reduce morbidity and mortality. Neurotoxicities need to be distinguished from complications of the underlying malignancy, metabolic derangements, or complications of other therapies. Overall, most ICI-mediated neurotoxicities are responsive to steroid therapy. Randomized prospective studies for diagnostic and treatment algorithms are needed to improve patient outcomes and develop predictive biomarkers for identifying patients at risk for neurotoxicities.

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Data availability Data available on request from the authors.

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethical approval Study design and methods were approved by the institutional review board of Yale School of Medicine.

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6 Detailed Declaration of Contributions to the Publication

Publication:

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The author of this thesis, Sophie L. Duong, was the main researcher in the study. Sophie L. Duong developed the working hypothesis and study design. Sophie L. Duong defined inclusion and exclusion criteria under the guidance of Prof. Joachim M. Baehring. With the assistance of Frank J. Barbiero, Dr. Richard J. Nowak, and Prof. Joachim M. Baehring, Sophie L. Duong identified patients to be included in the study. Sophie L. Duong performed a comprehensive chart review and systematically collected relevant primary patient data with support from Frank J. Barbiero. Sophie L. Duong analyzed the data and performed statistical analyses (Fisher's exact test, Wilcoxon rank sum, Kaplan-Meier method). Results were discussed with Frank J. Barbiero and Prof. Joachim M. Baehring. Sophie L. Duong designed figures and tables to visualize the study results. The manuscript was drafted by Sophie L. Duong after a comprehensive literature research and critically revised by all authors. The submission of the manuscript, as well as addressing the revisions from the peer-review process until publication in a peer-reviewed journal was executed by Sophie L. Duong under the guidance of Prof. Joachim M. Baehring. In addition, Sophie L. Duong presented the results of this study at scientific conferences (American Academy of Neurology Annual Meeting, Philadelphia, PA, USA, 2019 and American Neurological Association Annual Meeting, Atlanta, GA, USA, 2018).

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8 Curriculum Vitae

Sophie Lan-Linh Duong

Akademischer Werdegang

[redacted]

Klinische Erfahrung

[redacted]

PRAKTISCHES JAHR TERTIALE

[redacted]

[redacted]

Forschungserfahrung

[redacted]

Auszeichnungen

[redacted]

Stipendien

[redacted]

Publikationen

[1] **Duong SL**, Barbiero FJ, Nowak RJ, Baehring JM. Neurotoxicities associated with immune checkpoint inhibitor therapy. J Neurooncol. 2021 Apr;152(2):265-277. doi: 10.1007/s11060-021-03695-w.

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[3] Kuo IY, **Duong SL**, Nguyen L, Ehrlich BE. Decreased Polycystin 2 Levels Result in Non-Renal Cardiac Dysfunction with Aging. PLoS One. 2016 Apr 15;11(4):e0153632. doi: 10.1371/journal.pone.0153632.

Berlin, 04.12.2021

Ort, Datum

Sophie Duong

Unterschrift

9 Acknowledgments

[redacted]

10 Schriftliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die dem Fachbereich Medizin der Johann Wolfgang Goethe-Universität Frankfurt am Main zur Promotionsprüfung eingereichte Dissertation mit dem Titel

Neurotoxicities Associated with Immune Checkpoint Inhibitor Therapy

in dem Institut für Physiologie II (Sinnes- und Neurophysiologie) unter Betreuung und Anleitung von Prof. Dr. Jochen Roeper mit Unterstützung durch Prof. Dr. Joachim M. Baehring ohne sonstige Hilfe selbst durchgeführt und bei der Abfassung der Arbeit keine anderen als die in der Dissertation angeführten Hilfsmittel benutzt habe. Darüber hinaus versichere ich, nicht die Hilfe einer kommerziellen Promotionsvermittlung in Anspruch genommen zu haben.

Ich habe bisher an keiner in- oder ausländischen Universität ein Gesuch um Zulassung zur Promotion eingereicht. Die vorliegende Arbeit wurde bisher nicht als Dissertation eingereicht.

Vorliegende Ergebnisse der Arbeit wurden in folgendem Publikationsorgan veröffentlicht:

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Sophie Duong — (Unterschrift)