Neuro-Oncology

XX(XX), 1–14, 2024 | https://doi.org/10.1093/neuonc/noae177 | Advance Access date 16 October 2024

Intracranial administration of anti-PD-1 and anti-CTLA-4 immune checkpoint-blocking monoclonal antibodies in patients with recurrent high-grade glioma

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Abstract

Background. Recurrent high-grade glioma (rHGG) lacks effective life-prolonging treatments and the efficacy of systemic PD-1 and CTLA-4 immune checkpoint inhibitors is limited. The multi-cohort Glitipni phase I trial investigates the safety and feasibility of intraoperative intracerebral (iCer) and postoperative intracavitary (iCav) nivolumab (NIVO) ± ipilimumab (IPI) treatment following maximal safe resection (MSR) in rHGG.

Materials and methods. Patients received 10 mg IV NIVO within 24 h before surgery, followed by MSR, iCer 5 mg IPI and 10 mg NIVO, and Ommaya catheter placement in the resection cavity. Biweekly postoperative iCav administrations of 1–5–10 mg NIVO (cohort 4) or 10 mg NIVO plus 1–5–10 mg IPI (cohort 7) were combined with 10 mg IV NIVO for 11 cycles.

Results. 42 rHGG patients underwent MSR with iCer NIVO + IPI. 16 pts were treated in cohort 4 (postoperative iCav NIVO at escalating doses) while 28 patients were treated in cohort 7 (intra and postoperative iCav NIVO and escalating doses of IPI). The most common TRAE was fatigue; no grade 5 AE occurred. Dose-limiting toxicity was grade 3 neutrophilic pleocytosis (4 pts) receiving iCav NIVO plus 5 or 10 mg IPI. PFS and OS did not significantly differ between cohorts (median OS: 42 [95% CI 26–57] vs. 35 [29–40] weeks; 1-year OS rate: 37% vs. 29%). Baseline B7–H3 expression significantly correlated with worse survival. OS compared favorably to a historical pooled cohort ($n = 469$) of Belgian rHGG pts treated with anti-VEGF therapies (log-rank $P = .015$).

Conclusion. Intraoperative iCer IPI + NIVO with postoperative iCav NIVO ± IPI up to biweekly doses of 1 mg IPI + 10 mg NIVO is feasible and safe, showing encouraging OS in rHGG patients. *ClinicalTrials.gov registration: NCT03233152*

Key Points

- Intraoperative intracerebral injection of ICI can be combined with intracavitary administration.
- Combined intracranial injection of nivolumab and ipilimumab is feasible and safe.
- Intracranial injection of ICI is associated with encouraging survival.

High-grade gliomas (HGG) are the most common primary malignancies of the central nervous system. Glioblastoma (GBM, WHO 2021 grade 4, IDH-wildtype) and grade 4 astrocytoma (WHO 2021, IDH-mutant, 1p/19q intact) are grade 4 gliomas that are characterized by a poor prognosis and almost universal fatality related to cancer death.[1] The incidence of GBM

Importance of the Study

Despite the current standard of care multidisciplinary frontline therapy, progression of high-grade glioma during or following primary treatment is inevitable. Current therapies offer only a modest chance for temporary disease control, highlighting the urgent need for innovative approaches. This study is the first to demonstrate the feasibility and safety of combining intraoperative intracerebral injections with postoperative intracavitary administration (through an Ommaya reservoir) of the immune checkpoint inhibitors nivolumab and ipilimumab. The encouraging 1- and 2-year survival rates justify the further investigation of intracranial administration of immune checkpoint inhibitors in patients with resectable recurrences of grade 4 glioma.

in North America and Europe ranges from 2 to 6 cases per 100 000 people.[2] The standard of care for HGG at initial diagnosis consists of "maximal-safe" surgical resection of the gadolinium-enhancing tumor mass, followed by radiotherapy with concomitant and/or adjuvant temozolomide chemotherapy.[3] The added value of tumor treating fields remains controversial and is not available in many EU member states. Despite multimodality treatment, over half of GBM patients experience disease progression within 9 months following the initiation of therapy, with a median progression-free survival (PFS) of 6.9 months and a median overall survival (OS) of 14.6 months.[4,5] Patients diagnosed with high-grade astrocytoma have a longer PFS compared to GBM, with a 5-year OS-rate of 55%.[6] Salvage therapy for recurrent HGG (rHGG) consists of re-resection and/or re-irradiation when feasible, and systemic treatments including alkylating chemotherapies (eg lomustine) or bevacizumab (a VEGF-neutralizing monoclonal antibody that did not receive approval by the European Medicine Agency in rHGG). The overall response rate for salvage chemotherapy is 5–10%, with a 6-month PFS rate of 9–21% and a median OS of 25–30 weeks.[7,8] Despite bevacizumab improving PFS compared to lomustine, OS was not improved in patients with recurrent GBM.[9] Currently, no salvage treatment has significantly increased OS for rHGG patients in a prospective randomized clinical trial, highlighting the crucial need for innovative, safe, and effective therapies.

In recent years, the administration of therapeutic monoclonal antibodies (mAbs) that block the function of immune checkpoint receptors (: immune checkpoint blockade, ICB) has been highly successful in the treatment of various cancer types.[10] While preclinical models of HGG showed promising potential for such ICB strategies, prospective clinical trials using nivolumab (NIVO) or pembrolizumab (PD-1 blocking mAbs) for newly diagnosed or rHGG failed to demonstrate sufficient clinical activity. Consequently, no ICB therapy is currently available for HGG patients.[11–13] Also, combinatorial approaches of ICB with VEGF(R)-inhibition (eg bevacizumab, axitinib) were unsuccessful.[14,15] Taking into account that: (1) therapeutic ICBs cannot readily cross the blood–brain barrier (typically a 1:100 concentration ratio between plasma and CSF),[16] that (2) the dose/effect and –/toxicity ratio are critical for the clinical activity of anti-CTLA-4 mAbs,[17] that (3) CTLA-4 blockade is effective in preclinical models of HGG,[18] that (4) the therapeutic ratio for CTLA-4 blockade is improved by intratumoral injection in preclinical models,[17–19] and that (5) durable PD-1 receptor occupancy in circulating T cells as well as clinical effectiveness have been demonstrated with low dose of intravenously administered nivolumab,[20–23] our research group started exploring the feasibility of combining intravenous (IV) anti-PD-1 mAb (nivolumab, [Opdivo™]) with intracranial administration of both NIVO and an anti-CTLA-4 mAb (ipilimumab, IPI, [Yervoy™]) in rHGG patients in an adaptive phase I clinical trial program (the Glitipni trial; ClinicalTrials.gov ID NCT03233152)[16–19].

Between December 2016 and April 2023, 122 patients with rHGG were treated in the multi-cohort Glitipni trial. In the first 2 study cohorts, it was found that intraoperative injection of NIVO plus IPI into the brain tissue lining the resection cavity, followed by IV dosing of NIVO is safe and doubled the 1- and 2-year survival rates as compared to a large historical cohort of Belgian rHGG patients treated with anti-VEGF(R) therapies.[24] Expression of the B7-H3 immune checkpoint was identified as the most important prognostic/predictive biomarker.

Here, we present the results of patients with rHGG in cohorts 4 and 7 of the Glitipni trial. These patients underwent intracerebral injection of IPI and NIVO at the end of the surgical resection for their rHGG, followed by implantation of an Ommaya reservoir. The follow-up treatment consisted of IV NIVO combined with the iCav administration of NIVO alone (cohort 4) or NIVO + IPI (cohort 7).

Materials and Methods

Clinical Trial Design and Patient Eligibility

This study is a nonrandomized, open-label, multi-cohort, and single-center phase I clinical trial conducted at the Universitair Ziekenhuis Brussel (UZ Brussel). This analysis focuses on 2 cohorts (cohort 4 and cohort 7) within the multi-cohort Glitipni trial. The primary objective is to assess the feasibility and safety of intra and postoperative administration of IPI and NIVO in patients diagnosed with resectable rHGG. A "classical 3 + 3" phase I trial design was used to guide patient recruitment, followed by a cohort expansion when considered appropriate. Three escalating dose levels were predetermined for postoperative intracavitary (iCav) NIVO (: cohort 4) and for postoperative iCav IPI (: cohort 7). This trial was registered on ClinicalTrials.gov as NCT03233152.

Written informed consent for study participation was obtained from all enrolled patients prior to any study-related procedures. No compensation was offered to

participants. Ethical approval for this study was granted by the medical ethics committee of UZ Brussel. A copy of the Clinical Protocol with the full study eligibility criteria is available in the Supplementary Information.

Adult patients (18 years or older) diagnosed with a recurrence of a previously histologically confirmed HGG (CNS WHO 2021 grade 3 or 4) and who were amenable to a gross-total resection of the tumor (with an anticipated acceptable risk for postoperative neurological deficits), were eligible for study participation. Patients required an Eastern Cooperative Oncology Group Performance Status of ≤2 and adequate hepatic, renal, and bone marrow function. Patients with histopathologically proven lower-grade gliomas that demonstrated transformation to an HGG on brain imaging were also found eligible.

The main exclusion criteria included the requirement for systemic corticosteroids (at doses of >8 mg daily methylprednisolone or equivalent, ie ~1–2 mg dexamethasone) or other immunosuppressive medications within 14 days prior to enrolment and prior immunotherapy with anti-PD-1, -PD-L1, -CTLA-4 mAb, or any other immune checkpoint inhibitors. Additional exclusion criteria were the presence of active auto-immune disease, prior immunodeficiency syndromes, persisting toxicities from prior treatments, diagnosis of any other malignancy within the last 5 years, bleeding or thrombotic disorders, and problematic wound healing.

Treatment Plan

On day 1 of the treatment schedule, patients received a fixed dose of 10 mg NIVO via a 15-min IV infusion. Within 24 h, a maximal safe surgical resection of the tumor was performed, guided by 5-Amino-Levulinic-Acid (5-ALA) fluorescence. After obtaining hemostasis, 5 mg of ipilimumab (: 1 ml, 50 mg/10 mL solution), followed by 10 mg of NIVO (: 1 mL, 40 mg/4 mL solution), were injected into the tissue lining the resection cavity, using a total of 20–30 injections. The injections were administered using a tuberculin needle inserted to a depth of 3–5 mm, evenly distributed to cover the entire resection cavity wall. Functional areas were avoided by assessing the white matter anatomy, and integrated as tracts into the navigation system.

At the end of the neurosurgical intervention, an Ommaya reservoir was implanted subcutaneously with the catheter in connection with the resection cavity for the intra and postoperative iCav injections. Intraoperative treatment via the Ommaya reservoir was administered exclusively to cohort 7. Patients in dose levels 1, 2, and 3 received 1-, 5-, and 10-mg of IPI, respectively, in a total volume of 2 mL. Subsequently, 10 mg of NIVO was administered through the Ommaya reservoir, and the catheter was flushed with 1.5 mL of NaCl 0.9%.

Postoperative treatment was administered biweekly for up to 22 weeks following surgery, up to a total of 11 postoperative administrations. Patients received the iCav administrations via the Ommaya reservoir alongside a fixed dose of 10 mg NIVO via a 15-min IV infusion. In cohort 4, the dose of NIVO administered through the Ommaya reservoir was escalated from 1- to 5- and a maximum of 10 mg. In cohort 7, 10 mg of NIVO was combined with 1-, 5-, or 10-mg of IPI administered through the Ommaya reservoir.

A schematic representation of the treatment schedule is shown in Supplementary Figure 1.

Treatment was terminated early in case of confirmed disease recurrence or progression, unacceptable toxicity, or patient refusal to continue study treatment. Continuation of study treatment after the first documented disease progression was permitted if the investigator deemed it to be of clinical benefit for the patient.

Assessment of Tumor Response and Toxicity

Baseline T1 gadolinium-enhanced MRI and [^{18}F]-FET-PET/CT were conducted within 28 days prior to initiating the study treatment. Tumor response assessments were performed by T1 gadolinium-enhanced MRI within 48 h postoperatively, 2 weeks postoperatively (prior to the postoperative treatment administration), 6 weeks postoperatively, and every 6 weeks thereafter until the end of the treatment. Further follow-up was scheduled every 12 weeks. Additional on-treatment follow-up imaging with [^{18}F]-FET-PET/CT was performed as clinically indicated. Tumor responses and progression of disease were defined according to the response assessment for neuro-oncology (iRANO) criteria.[25]

Safety was assessed continuously throughout the treatment phase up until 1 month after the last study treatment administration. Clinical, hematological, and biochemical parameters were assessed before each administration of the study treatment. Adverse events (AE) were classified by type, frequency, and severity according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 5.0.

Objectives and Statistical Analysis

The primary objectives of this study were to establish the safety and feasibility of the experimental treatment regimen. Treatment-emergent adverse events were classified and graded for severity according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 5.0. Descriptive statistics were used to portray demographics, treatment disposition, and safety data.

The secondary endpoints PFS and OS were assessed using Kaplan–Meier probability analysis. Statistical analyses were performed using SPSS Statistics Version 29.0.2.0 (IBM) and GraphPad Prism v10.0.2 (for translational data). Figure legends indicate tests used to assess statistical significance.

A post hoc exploratory analysis of "PFS2" was conducted. PFS2 is defined as the duration between the initial progression on study treatment and the subsequent progression event after transitioning to a low-dose bevacizumab regimen (400 mg initial loading dose followed by 100 mg every 4 weeks). PFS2 was only considered if patients had clinical and/or radiological benefit (defined as at least stable disease according to RANO criteria) from bevacizumab.

The database was locked on the 17th of July 2024.

Tissue Analysis

Tumor tissue was collected from all patients at the time of their surgical intervention. Hematoxylin & eosin (HES) staining, and immunohistochemistry (IHC) analyses were performed on formalin-fixed, paraffin-embedded (FFPE) tumor tissues. Next-generation sequencing (NGS) was performed on all resected tumor tissue. Details on IHC for PD-L1 and B7-H3 as well as on NGS can be found in Supplementary Information.

Cerebrospinal Fluid Analysis

Cerebrospinal Fluid (CSF) Collection and Storage. —When considered feasible, CSF samples were collected during surgery or bi-weekly thereafter using the Ommaya reservoir, before incave administration of NIVO and/or IPI. CSF samples were collected up to 11 times during postoperative study treatments or until disease progression.

Pharmacokinetic Analysis of NIVO and IPI in CSF and Serum. —NIVO and IPI concentration were determined in CSF and plasma samples using a NIVO and IPI quantitative enzyme-linked immunosorbent assay (ELISA, both from IBL International GmbH). Only samples on time points where study treatment was administered 2 weeks prior to sampling and had concentrations above the respective lower detection limit were included. CSF samples with a clear yellow-red color, indicative of blood contamination, were excluded from further analysis. The median and range concentration (µg/mL) of NIVO and/or IPI were calculated in CSF and serum per dose level for cohorts 4 and 7.

Determination of Nucleated Cells in CSF. —The total count of nucleated cells in the CSF was determined by an Latinity analyzer (Abbott) or manual counting using a Bruker chamber. The percentage of immune cell subsets (lymphocytes, neutrophils, and eosinophils) in the CSF was assessed using Cytosines.

Cytokine/Chemokine Measurement in CSF. —CSF samples were analyzed for cytokine/chemokine content using a custom 10-plex (IL-4, IL-6, IL-8, IL-12p70, IL-18, IP-10/CXCL10, MCP-1/CCL2, MIP-1β/CCL4, TNFα, and sB7-H3) and 5-plex (IN, IL-10, MIP-1α/CCL3, MIG/CXCL9, and RANTES/CCL5) U-plex assay (MesoScale Diagnostics).

Results

Patient Baseline Characteristics

Between August 2019 and April 2021, 44 patients were enrolled sequentially in cohort 4 (C4; $n = 16$) and cohort 7 (C7; $n = 28$) of the Glitipni clinical trial. Baseline patient characteristics and their prior therapies are summarized in Table 1.

Treatment Disposition

A total of 42 patients (32 male) initiated the study treatment, receiving the predefined pre and intraoperative doses of IV and iCer NIVO and iCer IPI. Two patients in cohort 7 did not initiate intra and postoperative study treatment due to rapid progressive disease with corticosteroid dependency (1 pt) and a suspected meningeal bacterial infection observed during surgery (1 pt). The postoperative iCav administrations were initiated in 37 patients and repeated biweekly for up to 11 cycles, concurrently with 10 mg of IV NIVO.

In cohort 4 ($n = 16$), the postoperative IV NIVO administrations were combined with iCav administrations of 1 mg, 5 mg, or 10 mg NIVO in 3-, 3-, and 9 patients, respectively. The median number of postoperative IV/iCav NIVO administrations was 7 (3–7), 7 (2–11), and 2 (1–11), respectively. In total, 4 patients completed the planned therapy. Eleven patients discontinued study treatment prematurely, in 8 patients (50%) because of tumor progression, and in 3 patients (19%) because of adverse events.

In cohort 7 ($n = 28$), the postoperative iCav administrations consisted of 10 mg NIVO and 1 mg, 5 mg, and 10 mg of IPI in 8, 5, and 9 patients, respectively. The median number of iCav IPI and NIVO administrations was 5 (2–12), 5 (2–11), and 5 (3–12), respectively. In total, 4 patients (14%) completed the planned therapy. Early treatment discontinuation occurred in 18 patients (62%), in 16 patients (55%) due to tumor progression, and in 2 patients (7%) due to adverse events.

Patient flow and treatment disposition are provided in Figure 1. In total, 8 patients received the complete study treatment, with 4 patients in each cohort.

Safety

The most frequent TRAEs per cohort are shown in Table 2. The most commonly reported treatment-related AEs (TRAEs) were fatigue and headache, affecting 24 and 19 patients, respectively. There were no unexpected adverse events (AE) related to the intraoperative treatment. In cohort 7, dose-limiting toxicity manifested as transient symptomatic grade 3 aseptic neutrophilic pleocytosis in the CSF in 1 patient receiving 5 mg and in 3 patients receiving 10 mg of postoperative iCav IPI (Supplementary Figure 5). Patients presented with clinical deterioration, fever (3 patients), and CSF samples expressing elevated neutrophils associated with negative CSF bacterial cultures. All patients recovered following the initiation of corticosteroids and/or antibiotics. Antibiotics were administered when infection was suspected, based on the clinical presentation and pending CSF bacterial culture results. In 6 of the 42 patients (C4: $n = 3$; C7: $n = 3$), a grade 3 or 4 catheter-related infection was identified, leading to the surgical removal of the Ommaya reservoir in all patients. Across both cohorts, the study treatment was discontinued in 10 patients because of adverse events. In cohort 4, 1 patient (C4-2) could not receive the postoperative study treatment because of increased intracerebral edema and clinical deterioration. The postoperative treatment (after receiving the first iCav postoperative dose) was discontinued early in 3 patients

Table 1. Baseline Patient Characteristics

Baseline patient characteristics			
		Cohort-4 ***n*** = 16 (%)	Cohort-7 ***n*** = 28 (%)
Age	Median (range)	56 (42–77)	60 (32–74)
Gender	Male/female	12 (75)/4 (25)	20 (71)/8 (29)
ECOG PS	0/1/2	6 (37)/7 (44)/3 (19)	21 (75)/7 (25)/0
Corticosteroids (≤8mg methylprednisolone) at treatment start		3 (19)	4 (14)
Prior therapy at primary diagnosis			
Surgery	Resection/biopsy	16 (100)/0	28 (100)/0
Systemic therapy	Concomitant RT/TMZ + adjuvant TMZ	14 (87)	26 (93)
	Other	2 (13)	2 (7)
Prior therapy at recurrent disease			
Surgery + RT/TMZ + adjuvant TMZ		0	1 (4)
Surgery + TMZ + immunotherapy		1 (6)	0
Surgery + chemotherapy (TMZ/lomustine/carboplatine)		4 (25)	7 (25)
Surgery + immunotherapy		1 (6)	0
Surgery		2 (12)	1 (4)
Chemotherapy (TMZ/lomustine)		0	2 (7)
None		8 (50)	17 (61)
Molecular data of patients that received the intraoperative treatment		***n*** **= 16 (%)**	***n*** **= 26 (%)**
MGMT-methylation status	Methylated	7 (44)	5 (19)
	Unmethylated	4 (25)	19 (73)
	Unknown	5 (31)	2 (8)
IDH-mutation status	IDH wild-type	13 (81)	17 (65)
	IDH1 R132H mutant	1 (6)	1 (4)
	Unknown	2 (13)	8 (31)
Pathogenic mutations detected	Yes	14 (88)	18 (69)
	No	2 (12)	8 (31)
Pathogenic mutations	TERT c.-124C >T	8	12
	PTEN	4	10
	TP53	3	9
	TERT c.-146C >T	4	5
	EGFR	2	5
	NF1	1	4
	ATM	0	3
	ATRX	1	2

Abbreviations: ECOG PS: Eastern Cooperative Oncology Group Performance Score; IDH: isocitrate dehydrogenase; MGMT: O(6)-methylguanine-DNA-methyltransferase; RT: radiotherapy; TMZ: temozolomide.

due to 2 cases of catheter-related infections and 1 case of increased cerebral edema. In cohort 7, 4 patients did not initiate the postoperative study treatment due to various causes: 2 cases of neutrophilic pleocytosis (C7-2, C7-3), 1 case of a catheter-related infection (C7-1), and 1 case of increased intracerebral edema (C7-1). The postoperative treatment was discontinued early due to increased cerebral edema in 1 case and neutrophilic pleocytosis in another (C7-3). There was 1 patient (C7-3) who experienced simultaneous neutrophilic pleocytosis with disease progression (confirmed bone metastasis). Importantly, no grade 5 AEs were reported throughout the study.

Clinical Outcome

At database lock, 3 patients from cohort 7 were alive of whom 1 patient remains progression-free, more than 2 years after initiating study treatment. All 3 had an IDH wild-type glioblastoma. Noteworthy is that 1 of the surviving

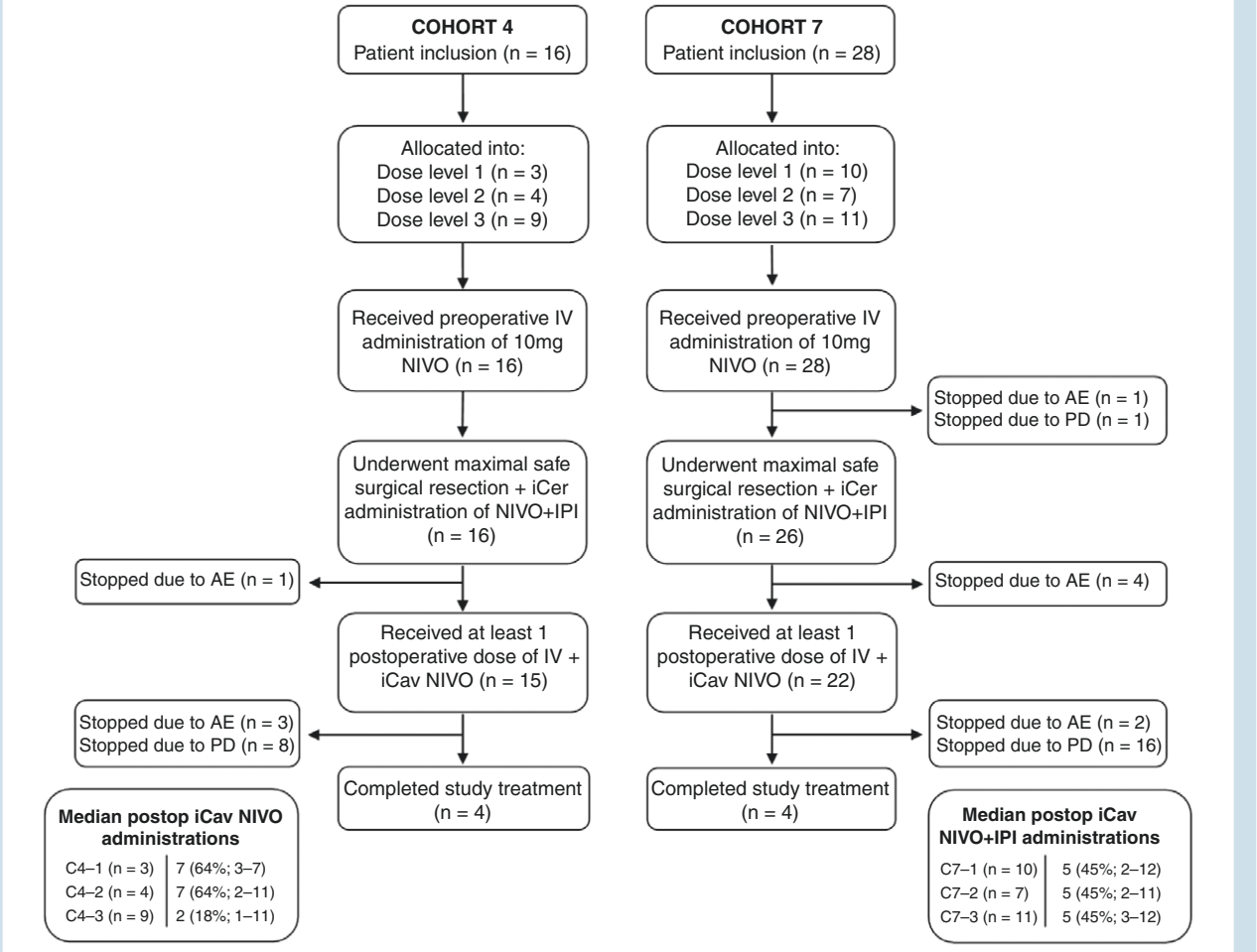

Figure 1. Patient flow and treatment disposition. Two patients in cohort 7 did not initiate intra and postoperative study treatment due to rapid progressive disease with corticosteroid dependency (1 pt) and a suspected meningeal bacterial infection observed during surgery (1 pt). In cohort 4, 1 patient was unable to receive the postoperative study treatment due to increased intracerebral edema and clinical deterioration. In cohort 7, 4 patients did not receive the postoperative study treatment due to various causes: 1 case of an Ommaya-related infection (C7-1), 1 case of increased intracerebral edema (C7-1), and 2 cases of neutrophilic pleocytosis (C7-2 and C7-3). In total, 8 patients received the complete study treatment, with 4 patients in each cohort. Postop: postoperative; iCav: intracavitary injection through an Ommaya reservoir; IV: intravenous; Mdn: median; admin: administration(s); PD: progression disease; AE: adverse event.

patients was diagnosed with disease progression 12 weeks after the initiation of the study treatment. A low-dose bevacizumab regimen was initiated and, at the latest follow-up, more than 3 years later, remains free from progression. The third surviving patient was diagnosed with symptomatic aseptic neutrophilic pleocytosis 2 weeks after the intraoperative treatment and received no postoperative study treatment. One year later, this patient was diagnosed with progressive disease and received IV NIVO to which no response was observed. All patients from cohort 4 have died. All except 1 death in both cohorts were related to disease progression. One patient died from a SARS-CoV-2 infection (COVID-19), without evidence of tumor progression on an MRI of the brain. PFS and OS are shown in Figure 2. There were no significant differences in survival between the patients treated in cohorts 4 and 7, regardless of the dose level of NIVO or IPI. There was no correlation between survival and baseline blood LDH levels or cellular composition of the CSF.

On an exploratory basis, OS was compared to (1) the previously reported survival of patients treated in the Glitipni clinical trial who did not receive postoperative iCav administrations of NIVO or NIVO plus IPI,[24] and (2) a large pooled historical cohort of 469 Belgian patients with recurrent glioblastoma who were treated in 3 prospective phase II clinical trials (investigating bevacizumab, axitinib, and avelumab).[15,26–28] OS did not differ significantly from the study patients who did not receive postoperative iCav NIVO or NIVO + IPI. All cohorts from the Glitipni study (regardless of whether postoperative iCav treatment was administered) had a superior OS when compared to the historical pooled cohort. When pooled (Figure 2D), the OS of the patients treated in Glitipni cohorts 1,2,4, and 7 ($n = 69$) was significantly better

Table 2. Treatment-Related Adverse Events Reported Over 5% in Cohort 4 and Cohort 7

Treatment-related adverse events									
Adverse event (CTCAE 5.0)	Total	Cohort 4 (*n* = 16)				Cohort 7 (*n* = 28)			
	(*n* = 146)	All grades (*n*)	Grade 1	Grade 2	Grade 3	All grades (*n*)	Grade 1	Grade 2	Grade 3/4
Fatigue	24	9	7	2	0	15	10	3	2
Headache	19	7	5	1	1	12	8	4	0
Fever	17	5	5	0	0	12	11	1	0
Nausea	10	3	1	2	0	7	5	2	0
Cerebral edema	10	5	2	1	2	5	0	1	4
Dysphasia	6	3	2	1	0	3	0	3	0
Seizure	6	5	3	1	1	1	1	0	0
Confusion	6	1	0	1	0	5	4	1	0
Catheter-related infection	6	1	0	0	1	5	0	0	5
Anorexia	5	1	0	1	0	4	4	0	0
Vomiting	5	1	1	0	0	4	3	1	0
Periorbital edema	4	1	1	0	0	3	3	0	0
Neutrophilic pleocytosis	4	0	0	0	0	4	0	0	4
Cerebrospinal fluid leakage	3	3	1	2	0	0	0	0	0
Rash maculo-papular	3	0	0	0	0	3	2	1	0
Arterial hypertension	3	0	0	0	0	3	0	2	1
Hemineglect	3	1	1	0	0	2	0	1	1
Cognitive disturbance	3	2	1	1	0	1	0	1	0
Arthralgia	3	1	1	0	0	2	2	0	0
Hypertension	3	0	0	0	0	3	0	2	1

compared to the historical pooled cohort (log rank descriptive *P*-value: .0002).

Baseline Molecular Tumor Characteristics and Correlation with Survival

The NGS analysis of DNA extracted from the resected tumor tissue in patients who received the intraoperative study treatment detected pathogenic mutations in 32 out of 42 patients (76.2%) (Table 1). The most common mutation was the TERT c.-124C >T mutation, found in 20 patients (57.1%; 8 in C4 and 12 in C7). This was followed by the PTEN mutation, detected in 14 patients (40%; 4 in C4 and 10 in C7). The TP53 mutation was identified in 12 patients (34.3%; 3 in C4 and 9 in C7) and the IDH1 R132H mutation was present in 2 patients (5.7%; 1 in C4 and 1 in C7). No unexpected mutations were detected.

The immunophenotype was assessed by PD-L1 (cohort 4: $n = 16$; cohort 7: $n = 26$) and B7-H3 (cohort 4: $n = 15$; cohort 7: $n = 26$) score. PD-L1 was expressed in both immune cells and tumor cells. This was evaluated with a Tumor Area Positivity (TAP) score, a visual estimation method that combines the scoring of tumor cells and immune cells. Most PD-L1-positive immune cells were located perivascular and were intermixed with PD-L1-negative immune cells. In cases with a TAP score of around 5–10%, the staining was predominantly seen in immune cells. Conversely, cases with a TAP score greater than 10% displayed staining in both immune cells and tumor cells.

B7-H3 expression was observed in endothelial cells (EC) in all cases. Additionally, 44% (7/16) of cases showed strong B7–H3 expression in at least 25% of the tumor cells in cohort 4 and in 11% (3/26) of cases in cohort 7 (Figure 3A). The percentage of B7–H3-positive tumor cells was assessed using the TPS (Figure 3B and C). An inverse relationship was noted between B7–H3-positive tumor areas and PD-L1-positive areas (Figure 3D).

When comparing scores between both cohorts, B7–H3 was significantly higher expressed in tumors from cohort 4 compared to cohort 7, while no difference was observed regarding PD-L1 expression (Figure 3A).

Kaplan–Meier survival analysis showed that the absence of B7–H3 staining was associated with prolonged OS in the pooled cohort 4 + 7 (Figure 3E, log-rank $P < .001$). This was also observed for cohort 4 separately (Figure 3F, log-rank $P < .001$), but not for cohort 7 (Figure 3G, log-rank $P = .094$). For PD-L1 staining, no correlation between the PD-L1 expression level and OS was seen for the pooled cohort 4 + 7 (Figure 3H, log-rank $P = .089$) or for cohort 4 (Figure 3I, log-rank $P = .671$), while in cohort 7 low expression of PD-L1 correlated with improved OS (Figure 3J, log-rank $P = .033$).

CSF Analysis

Pharmacokinetic Analysis of NIVO and IPI in CSF and Plasma.—NIVO and IPI were measured in CSF and plasma following iCav administration of escalating doses. In cohort 4 (iCav NIVO at escalating doses), CSF NIVO was low

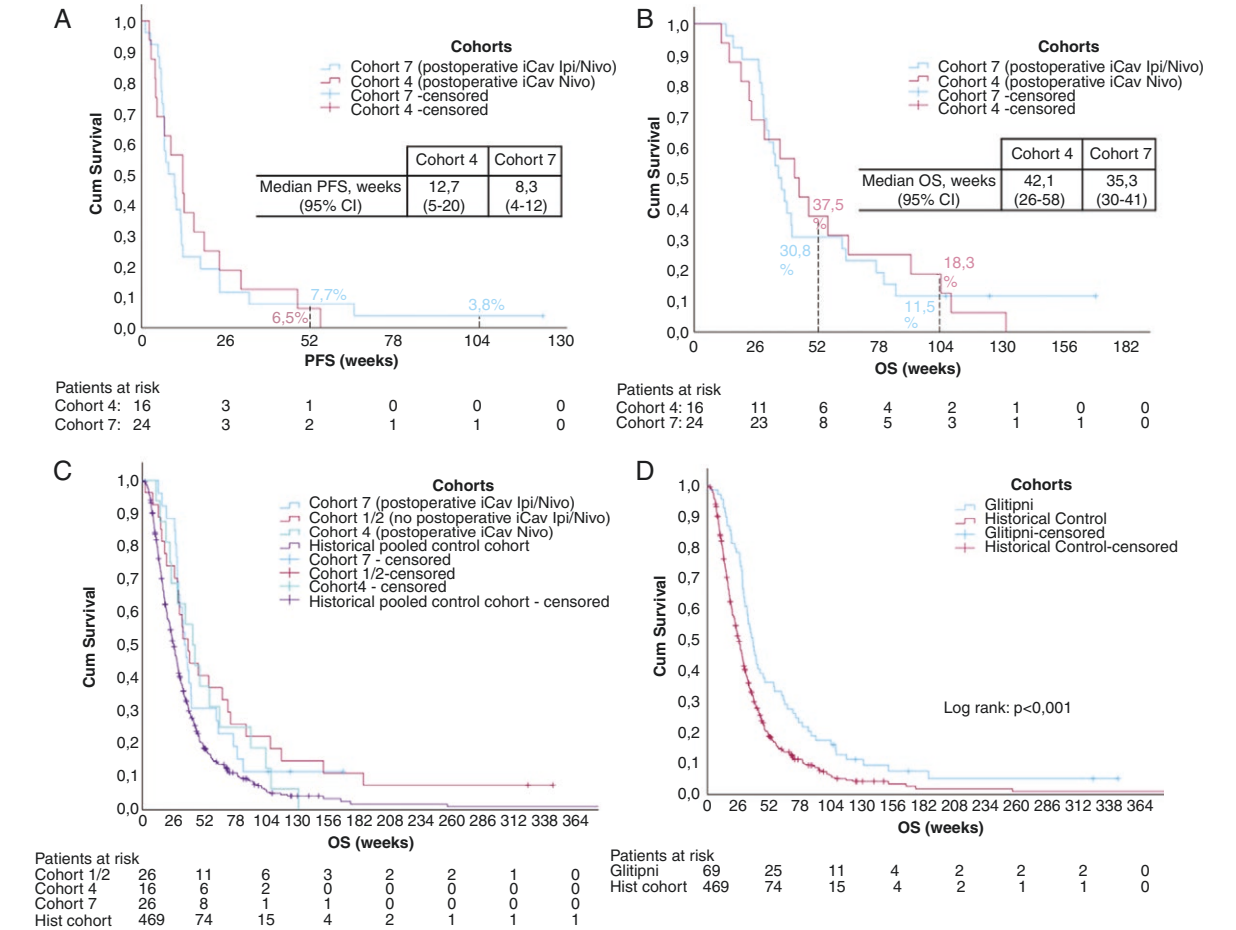

Figure 2. Progression-free survival and overall survival. PFS (A) and OS (B) probability according to Kaplan–Meier for cohort 4 with intraoperative NIVO + IPI followed by postoperative iCav NIVO (n = 16), or cohort 7 with postoperative NIVO + IPI(n = 26). (C) OS of patients treated in the Glitipni trial with intra plus postoperative iCav NIVO (cohort 4) or NIVO + IPI (cohort 7), intraoperative IPI or NIVO + IPI only (cohorts 1 and 2, no postoperative NIVO or IPI, n = 27), and a pooled historical cohort of Belgian patients with recurrent HGG (n = 469). (D) OS comparison between pooled Glitipni patients (n = 69) and the pooled historical cohort of Belgian patients with recurrent HGG.

(median range 0.02–0.08 µg/mL) and remained consistent across dose levels (Figure 4A, Supplementary Figure 2). In cohort 7 (iCav NIVO 10 mg + IPI at escalating doses), CSF NIVO remained low (median 0.04–0.14 µg/mL). Plasma NIVO in cohort 7 was higher than in CSF (6.25 µg/mL in the 5 mg IPI group and 4.95 µg/mL in the 10 mg IPI group), with a possible trend toward higher levels with increasing IPI dose. CSF IPI was mostly undetectable (<0.1 µg/mL), with low levels (median 0.17–0.49 µg/mL) in some samples. Plasma IPI was higher than expected, with the highest median level (2.58 and 2.74 µg/mL) in the 5–10 mg IPI group. Both NIVO and IPI plasma levels tended to increase over time with multiple iCav doses. A detailed description of pharmacokinetic analysis can be found in Supplementary results.

Effect of Treatment on Cellular Composition CSF.—Generally, no major differences in the number of nucleated cells in the CSF between the different cohorts or dose levels of iCav treatment were observed. In cohort 4, a trend to a higher number of nucleated cells could be observed in patients treated with 1 mg of iCav NIVO, however, this might be attributed to the low sample size (n = 2 in 1 mg, n = 2 in 5 mg, and n = 6 in 10 mg dose level) and high interpatient variability (Supplementary Figure 3, panel A).

The number of nucleated cells in the CSF was higher during the first study treatments in both cohorts regardless of the dose level and diminished upon study treatments (Supplementary Figure 3; panel B).

Within the population of nucleated cells in CSF, lymphocytes were the most abundant across both cohorts throughout the study treatment (Supplementary Figure 3, panel C). While the number of nucleated cells between the different dose levels did not vary, the proportion of lymphocytes in CSF was significantly higher at the highest dose levels (5 and 10 mg) of iCav administration in both cohorts. Patients who developed neutrophilic pleocytosis were not included in the above analysis and are discussed separately in more detail.

Cytokine Analysis of CSF.—Biochemical analysis of the CSF showed a total protein content above the upper limit of normal in a large proportion of patients (Supplementary

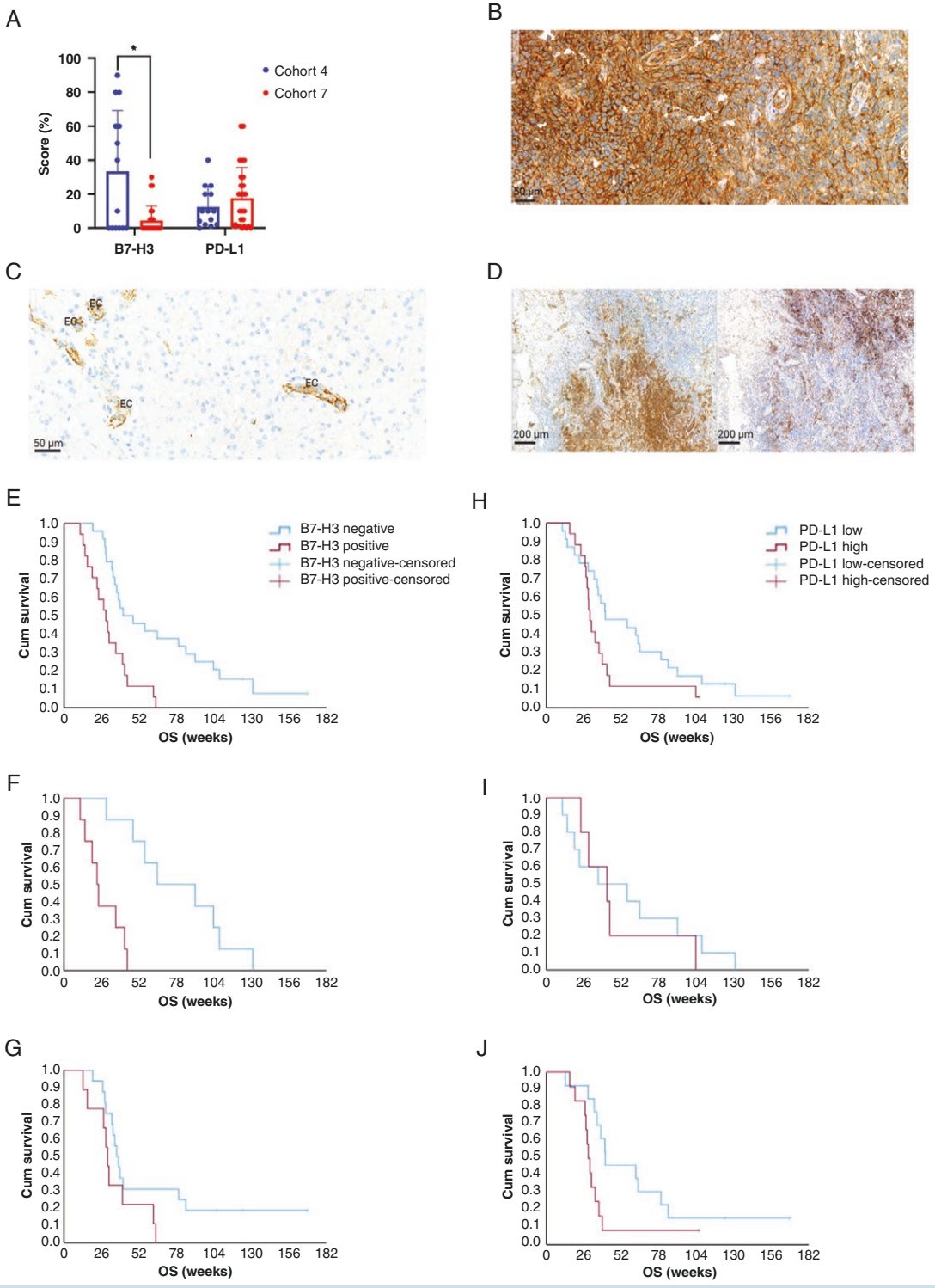

Figure 3. Expression of B7-H3 and PD-L1 in resected tumor samples and correlation with survival. (A) Comparison of the B7-H3 and PD-L1 expression scores in tumor samples from cohort 4 and cohort 7. A Mann–Whittney *U*-test was performed to evaluate differences between cohorts. *P*-values < .05 were considered significant. **P* < .05. (B) Representative image of a B7-H3 positive case. The tumor cells show a strong membrane staining and the TPS for the whole sample was 80%. 20× magnification, scale bar 50 µm. (C) Representative image of a B7-H3 negative case. Only the EC are stained, while the tumor cells do not show membrane staining or show a very weak cytoplasmic blush (negative). 20× magnification, scale bar 50 µm. (D) The region with an inverse relationship between B7-H3 (left) and PD-L1 (right). The sections are serial sections. The B7-H3 positive region in the lower half of the left figure is PD-L1 negative, while the B7-H3 negative region is PD-L1 positive. 5× magnification, scale bar 200 µm. (E–G) Probability for OS according to B7-H3 expression score in the pooled cohort 4 + 7 (E), and cohort 4 (F) and cohort 7 (G) separately. (H–J) Probability for OS according to PD-L1 expression score in the pooled cohort 4 + 7 (H), and cohort 4 (I) and cohort 7 (J) separately.

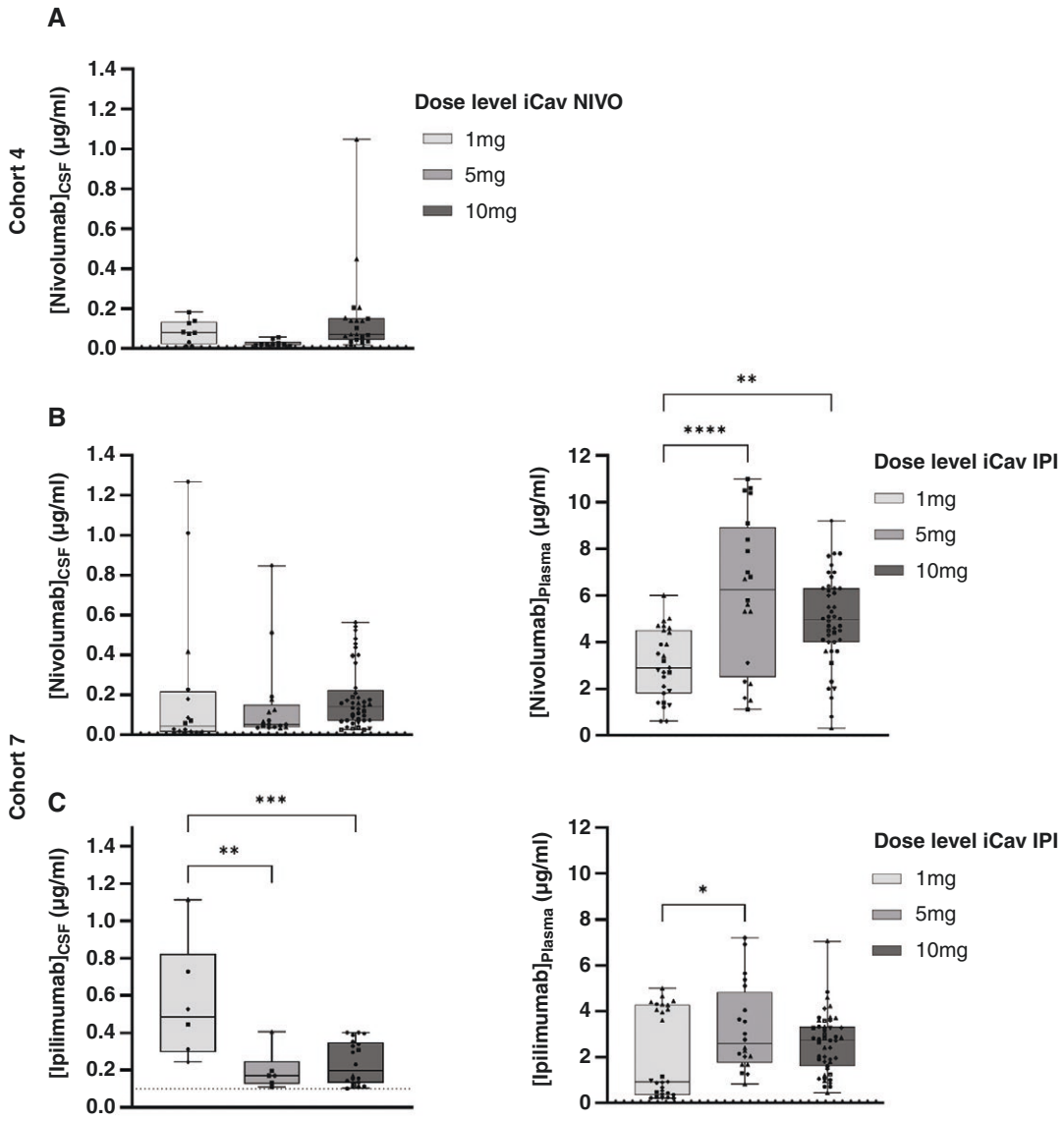

Figure 4. Pharmacokinetic determination of nivolumab and ipilimumab concentrations in CSF and plasma. (A) NIVO concentrations (µg/mL) in CSF samples of patients ($n = 8$) enrolled in cohort 4 per dose level 1 mg ($n = 2$), 5 mg ($n = 2$), and 10 mg ($n = 4$) of iCav NIVO treatment. Data is depicted as median and range with individual sample concentrations as dots. (B) NIVO concentration (µg/mL) measured in CSF (left panel) and plasma samples (right panel) of pts enrolled in cohort 7 per dose level of iCav ipilimumab administration 1 mg, 5 mg, and 10 mg. (C) IPI concentration (µg/mL) in CSF and plasma per dose level of iCAV IPI. Graphs represent the median with range (min–max), and individual data points depict individual sample concentrations with different symbols for each patient within 1 dose level. Different dose levels of iCav administration of NIVO of IPI are depicted in the figure legend of cohorts 4 and 7 in gray scales. The dotted lines represent the lower detection limit of the assay for nivolumab (0.01 µg/mL) and ipilimumab (0.1 µg/mL). Statistical analysis by one-way ANOVA with *P*-values corrected for multiple testing between doses using Tukey-method (*$P \leq .05$; **$P \leq .01$, ***$P \leq .001$, ****$P \leq .0001$).

Table 2). Multiplex cytokine/chemokine analysis showed that IL-4, IL-12p70, and IFNγ were detected at low levels, close to the detection limit of the assay. TNFα was detected at low/moderate levels; RANTES/CCL5, MIP-1α/CCL3, MIG/CXCL9, IL-10, and IL-18 showed moderate levels. Soluble B7-H3, IL-6, and MIP-1β/CCL4 were detected at high levels, while IL-8, IP-10/CXCL10, and MCP-1/CCL2 were detected at very high levels.

Treatment significantly decreased MCP-1/CCL2 and sB7-H3 in cohort 4, and sB7-H3 in cohort 7. (Supplementary Figure 4A). A stronger decrease in TNFα along treatment correlated with improved OS, while stronger decreases of MIP-1β/CCL4 and IL-10 correlated with improved PFS. (Supplementary Figure 4B) Low baseline levels of IL-6, IL-18, and sB7-H3 correlated with improved overall survival (Supplementary Figure 4C). IL-8, IP-10/CXCL10, IL-10, MIP-1a/CCL3, and MIG/CXCL9 were significantly increased both at the early time point and at progression. MIP-1β/CCL4 was only increased at the time of progression, while TNFα was only increased at the early time point (Supplementary

Figure 4D). Neutrophilic pleocytosis coincided with high cytokine levels, particularly TNFα, MCP-1/CCL2, IL-8, IL-6, IL-18, and sB7-H3. (Supplementary Figure 5).

Discussion

To the best of our knowledge, the Glitipni trial is the first clinical trial to have shown that the injection of the immune checkpoint inhibitors, ipilimumab and nivolumab, directly into the brain parenchyma (iCer) at the end of the rHGG resection is feasible, safe and characterized by favorable overall survival of the treated patients.[24] Previously, the iCer administration approach was demonstrated to be safe and biologically active as an immunotherapeutic strategy but had thus far only been used for local administration of oncolytic viruses (sitimagene ceradenovec, TOCA-511, and DNX-2401) and CAR-NK cells.[29–32]

We here report on cohorts 4 and 7 of the Glitipni trial, where the intraoperative treatment (iCer) was combined with postoperative escalating doses of iCav PD-1 and CTLA-4 immune checkpoint inhibition (plus IV-administration of NIVO).

The implantation of an Ommaya reservoir and its use for repetitive administration had already been demonstrated to be feasible and safe.[33] We confirm this, although we did encounter ≥ grade 3 catheter-related infections in 14% (6/42 patients). Additionally, dose-limiting toxicity of iCav IPI was encountered as neutrophilic pleocytosis (in the absence of infection), which occurred with doses from 5 mg upward, establishing the maximum tolerated dose at 1 mg of iCav IPI Q2w. Longitudinal profiling of CSF of patients with neutrophilic pleocytosis showed a correlation between elevated IL-6, MCP-1/CCL2, TNFα, IL-8, sB7-H3, and IL-18 levels and the presence of neutrophils. These cytokines are known to play roles in neutrophil recruitment and activation, suggesting a possible mechanism for NIVO + IPI-induced neutrophilia.[34] A more detailed investigation of the different cell types in the CSF and their activation status by single-cell RNA sequencing is needed to unravel the exact mechanism of action leading to NIVO + IPI-induced neutrophilia. However, due to the low recovery of neutrophils upon cryopreservation, this would require the analysis of freshly collected CSF samples upon detection of neutrophilic pleocytosis, which we didn't perform in this trial.

Compared to our previous report on cohorts 1 and 2, where procedures were similar but no Ommaya was placed and thus postoperative iCav injection was not performed, results are similar. This seemingly indicates that the additional iCav injections do not add an extra benefit in terms of progression-free or overall survival in this patient cohort, although it should be noted that our study was not designed to investigate the impact of adding the postoperative iCav administrations on survival.[24] Although the comparison of OS with historical data should be carefully interpreted and the study was not designed to demonstrate survival benefit, a consistently favorable outcome (especially 1- and 2-year landmark survival rates) in the Glitipni trial was observed when the OS of patients treated in cohort 1, 2, 4, and 7 was compared to OS of a historical pooled control cohort. We acknowledge that patients in this historical cohort are not fully matched to patients from the Glitipni study, which constitutes a surgical series of patients with limited or no baseline corticosteroid use. The role of resection of recurrent high-grade glioma remains debatable, however. A recent report from the RANOresect group showed that only in cases of maximal resection a survival benefit is obtained.[35] Other retrospective reports have also shown an increasing survival as more of the recurrence is resected, suggesting a survival benefit for resection of recurrent glioblastoma.[36] On the other hand, an analysis from Clarke et al comparing patients in a clinical trial undergoing surgery at progression with patients only receiving medical treatment showed no difference in PFS or OS between these 2 groups.[37] It also bears noting that most reports on surgery for recurrent glioblastoma (such as the RANOresect paper) concern first recurrence. In our series, only 25/44 (57%) were operated in the trial for first recurrence. All other patients (19/44–43%) had at least a second recurrence, having already undergone surgery for recurrence before inclusion in the trial. A detailed description of the role of resection and the extent thereof, including matching with another surgical series will be reported at a later stage.

In our previous report of cohorts 1 and 2, we showed that baseline B7-H3 expression significantly correlated with worse survival, which was confirmed here for the study population of cohorts 4 and 7. B7-H3 is considered to be a gatekeeper, preventing the effectiveness of immune checkpoint blockade and is considered an emerging therapeutic target. Amongst distinct approaches targeting B7-H3, including the use of antibody-drug conjugates and bispecific T-cell engagers (BiTEs), the field of CAR-T cells particularly deserves further investigation within our unique context of locoregional administration of immune checkpoint blocking mAbs. Recently CAR-T cells against B7-H3 have proven successful in both preclinical and clinical studies, more specifically in glioblastoma.[38,39] Additional clinical trials assessing the safety and efficacy of anti-B7-H3 CAR-T cell therapy for rGBM are currently ongoing.

We observed low but detectable levels of NIVO and IPI in CSF after iCav administration, remaining stable throughout treatment with no clear dose-dependent effect. In terms of cell composition of the CSF, we observed a general increase in nucleated cells with a peak during the first study treatment cycles that diminish over time across the different dose levels in both cohorts. This increase may be due to postoperative inflammation, which can be partially explained by the predominance of lymphocytes and elevated protein counts.[40] Cytokine and chemokine levels measured in the CSF were similar to previously reported data for glioblastoma patients.[41,42] Baseline low expression of IL-6, IL-18, and sB7-H3 was associated with improved survival, which is consistent with previously reported findings indicating these cytokines are prognostic factors in glioblastoma.[43–46] We further observed that cytokines/chemokines (IL-8, IP-10/CXCL10, IL-10, MIP-1α/CCL3, MIP-1β/CCL4, and MIG/CXCL9) were significantly increased at time of progression compared to baseline, and/or at first iCav treatment administration (TNFα, IL-8, IP-10/CXCL10, IL-10, MIP-1α/CCL3, and MIG/CXCL9), indicating that disease progression might be accompanied with increased

production of these cytokines. Given the encouraging clinical results of these and previously reported cohorts, we believe that intracerebral injection following surgical resection deserves further exploration to be used in a combinatorial strategy of immunotherapy. A new clinical trial (called the NEO-GLITIPNI trial) has been initiated that will explore the feasibility of adding a 4-week IV neoadjuvant NIVO + IPI treatment phase to the regimen established in cohort 7. Also awaited are the results from patients treated in cohorts 5 and 6 of the Glitipni trial that received iCer injection of autologous myeloid CD1c(BDCA-1)+/CD141(BDCA-3) + myeloid dendritic cells.

Supplementary material

Supplementary material is available online at *Neuro-Oncology* (https://academic.oup.com/neuro-oncology).

Keywords

glioblastoma | high-grade glioma | immune checkpoint inhibition | immunotherapy | local administration

Funding

The Glitipni trial was funded by Kom op tegen Kanker (Stand up to Cancer), "the Flemish cancer society" and Stichting tegen kanker. J.D. received a personal postdoctoral mandate from Stichting tegen Kanker for his work in the Glitipni trial. Translational analyses were additionally funded by the VUB-UZ Paul De Knop fund, the FWO and F.R.S.-FNRS under the Excellence of Science (EOS) programme (FWO-EOS18; reference 40007555), Hersentumorfonds, and Wetenschappelijk Fonds Willy Gepts.

Acknowledgments

The authors would like to acknowledge Evelien Vandeurzen, Katrien Van den Bossche, and Maud Allard for their help in data management and practical organization and follow-up. We also acknowledge Hugo Vandenplas, Maya Verdeye, and Jordy Schaukens for their help in handling all tissue and CSF samples.

Conflict of interest statement

J.D. has received honoraria for advisory board participation from Miltenyi and Servier; M.K. serves as chief medical and scientific officer for Cellcarta; B.N. has received honoraria for public speaking or advisory board participation from Roche, Bristol-Myers Squibb, MSD, Novartis, AstraZeneca, and Miltenyi; All other authors report no potential conflicts of interest.

Authorship staement

Study concept and design: J.D., B.N.; Data collection: J.D., L.L., I.D., L.S., X.G., F.V., W.G., S.B., A.V., H.E., B.C., M.B., L.L., M.K., S.T., B.N.; Data analysis and interpretation: J.D., L.L., I.D., J.D'.h., L.S., X.G., S.T., B.N.; Manuscript writing: J.D., L.L., I.D., J.D'.h., L.S., X.G., S.T., B.N.; Manuscript review and approval: all authors.

Data availability

The data sets of the study are not publicly available but stored at UZ Brussel. Upon motivated request and approval from the research group and ethics committee for the specific research question, sharing of the data is possible.

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