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Anti-LAG-3 with or without anti-PD-1 in recurrent glioblastoma: a phase 1 trial

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Lymphocyte activation gene 3 (LAG-3) is an immune checkpoint implicated in T cell exhaustion and a potential therapeutic target in glioblastoma (GBM). We conducted a multicenter, open-label, phase 1 study with sequential allocation to evaluate the safety and preliminary activity of the anti-LAG-3 antibody relatlimab, administered alone or with the anti-programmed cell death protein 1 (PD-1) antibody nivolumab, in patients with recurrent GBM. Forty-six patients were treated (23 per cohort). The primary endpoint of safety was met, with maximum tolerated doses of 800 mg relatlimab for monotherapy and 160 mg relatlimab/240 mg nivolumab for combination therapy. Treatment-related grade 3–4 adverse events occurred in 6 of 23 patients receiving combination therapy and were not observed with monotherapy. Neoadjuvant administration was associated with increased intratumoral $CD8^+$ T cell infiltration for both monotherapy and combination therapy. Exploratory analyses suggested that tumors with elevated baseline interferon signaling and increased T cell clonality were enriched among patients with durable responses to combination therapy. Twelve-month overall survival was 34.8% with relatlimab alone and 52.2% with combination therapy; however, this study was not designed to assess efficacy. These findings demonstrate an acceptable safety profile and provide preliminary immunologic and clinical signals supporting further evaluation of LAG-3 blockade in GBM. ClinicalTrials.gov identifier: NCT02658981.

GBM is refractory to standard oncologic treatments with median survival of 14 months[1] despite maximal safe resection, aggressive radiotherapy and temozolomide chemotherapy. Immuno-oncologic therapies targeting immune checkpoint molecules PD-1 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) have not improved outcomes for GBM[2–4], although this strategy has substantially improved survival in many other cancer types[5–7]. Barriers to effectiveness of these checkpoint inhibitor therapies may include multiple factors, including a tumor microenvironment (TME) defined by profound immune cell exhaustion and marked by the elevated expression of multiple other checkpoint molecules, such as LAG-3 (CD223)[8,9]. In the present study, we assessed the safety and immunologic impact of targeting the LAG-3 checkpoint with or without concurrent targeting of the PD-1 checkpoint.

In cancer, LAG-3 is thought to promote immunosuppression by decreasing the proliferation of $CD4^+$ effector T cells, inhibiting $CD8^+$

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T cell antitumoral cytotoxic function and recruiting regulatory T (T_{reg}) cells[10,11]. LAG-3 upregulation in the TME is associated with poor treatment outcomes, and its upregulation after anti-PD-1 monoclonal antibody (mAb) therapy has implicated it as a mediator of acquired immune checkpoint inhibition resistance[12]. The recently reported RELATIVITY-047 trial in patients with metastatic melanoma evaluated combination immune checkpoint blockade and demonstrated greater than two-fold improvement in progression-free survival (PFS) with relatlimab plus nivolumab compared with nivolumab alone (10.12 months versus 4.63 months)[13]. Preclinical data in murine glioma models suggest that this combination may be effective in GBM as well[10].

Here we present the results of an open-label, multicenter phase 1 trial testing anti-LAG-3 mAb (relatlimab, BMS-986016) alone or in combination with anti-PD-1 mAb (nivolumab, BMS-936558) in patients with first-time recurrent GBM (NCT02658981). Our primary endpoint was safety to determine a maximum tolerated dose (MTD) for relatlimab given either as monotherapy or combined with nivolumab in patients with recurrent GBM. A window of opportunity arm where patients received either neoadjuvant relatlimab monotherapy or combination therapy with nivolumab prior to surgery was also included. Secondary objectives were site-determined 1-year PFS and overall survival (OS) rates as well as radiographic response per modified Response Assessment in Neuro-Oncology (mRANO) criteria. Exploratory objectives for this study involved cellular and molecular assessments of relatlimab alone and in combination with nivolumab on infiltrating immune cell populations in the context of survival outcomes.

Results

Patient baseline characteristics and treatment disposition

A total of 46 patients were enrolled in the Adult Brain Tumor Consortium (ABTC) 1501 trial. All patients had histopathologic confirmation of GBM at diagnosis and had radiographically diagnosed recurrence after prior standard treatment with radiation therapy and temozolomide. Of those patients, 9 of 46 (19.6%) had undergone previous subtotal resection, and 37 of 46 (80.4%) had received gross total resection for their surgery at time of diagnosis. The first patient was enrolled on 9 September 2016, and the last patient was enrolled on 29 April 2020. The trial is completed. Clinical characteristics of all 46 patients are described in Table 1.

Patients were sequentially allocated to either relatlimab monotherapy or anti-CD137 mAb therapy initially as part of the ABTC 1501 trial (Fig. 1). Once the MTD of relatlimab monotherapy was determined, combination therapy was started with eventually 23 patients receiving relatlimab monotherapy (17 adjuvant, six neoadjuvant) at starting dose 80 mg and subsequent dose escalation to 800 mg. Twenty-three patients were given combination relatlimab and nivolumab therapy (16 adjuvant, seven neoadjuvant), with relatlimab dosages escalating from 80 mg to 160 mg and nivolumab dosage remaining at 240 mg (Fig. 1). Neoadjuvant therapy was given within 10 ± 3 days prior to the date of surgery. All patients who received neoadjuvant therapy went on to also have adjuvant therapy with the same treatment schema as adjuvant-only patients. All patients were required to receive at least one dose of the intervention treatment to qualify for the study.

Safety

Following National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 5.0 guidelines, grade 1–4 adverse events observed with relatlimab monotherapy and with relatlimab/nivolumab combination therapy are summarized in Table 2. All reported adverse events were deemed by site investigators to be possibly, probably or definitely attributable to the investigational agents. No patients treated with relatlimab monotherapy experienced a dose-limiting toxicity (DLT) (0/23, 0%) (Table 2), whereas 6 of 23 patients (26%) receiving combination therapy developed a DLT (Table 2). These events included worsened cerebral edema in 2 patients treated with 80 mg or 160 mg relatlimab, respectively, in combination with 240 mg nivolumab; grade 3 muscle weakness in 1 patient receiving 80 mg relatlimab plus 240 mg nivolumab; grade 3 hypertension in 1 patient receiving 160 mg relatlimab plus 240 mg nivolumab; grade 3 syncope in 1 patient receiving 160 mg relatlimab plus 240 mg nivolumab; and grade 3 thyroiditis in 1 patient receiving neoadjuvant 160 mg relatlimab plus 240 mg nivolumab (Table 2). Of note, both patients who experienced worsened cerebral edema were put on a short course of dexamethasone for symptomatic control.

Table 1 | Clinical demographics

	All patients (*n*=46)
Characteristic	**Value**
Median age (range), years	55 (21.9–76.8)
Race, number (%)	
White	42 (91.3)
Gender, number (%)	
Male	29 (63.0)
Female	17 (37.0)
KPS, number (%)	29 (63.0)
90–100	17 (37.0)
70–80	
IDH, number (%)	
IDH-WT	40 (87.0)
IDH-MT	6 (13.0)
MGMT, number (%)	
Unmethylated	26 (56.5)
Methylated	20 (43.5)
Measurable disease, number (%)	46 (100)
Steroids, number (%)	
Yes	6 (13.0)
Anticonvulsant, number (%)	28 (60.9)
Yes	
Histology prior study, number (%)	
Glioblastoma multiforme (grade 4)	46 (100)
Surgery, number (%)	
Subtotal resection	9 (19.6)
Gross total resection	37 (80.4)
Prior surgery: median (range)	1 (1–3)
Prior radiation therapy, number (%)	46 (100)
Prior temozolomide, number (%)	46 (100)

Baseline demographic and clinical characteristics of patients enrolled in the ABTC 1501 trial.

Tumor outcome radiographic response

Tumor response to treatment was evaluated under mRANO criteria[14] to account for pseudoprogression and delayed responses to immunotherapy by requiring confirmation of progression for a 6-month post-treatment evaluation window (Extended Data Fig. 1). Several patients who underwent relatlimab and nivolumab combination therapy demonstrated persistent or increasing contrast enhancement within the first 3 months of starting treatment, followed by near-total resolution of all enhancing disease that corresponded with prolonged OS. Although many patients had a significant period of disease control as assessed by imaging, upon central review no patients exhibited a

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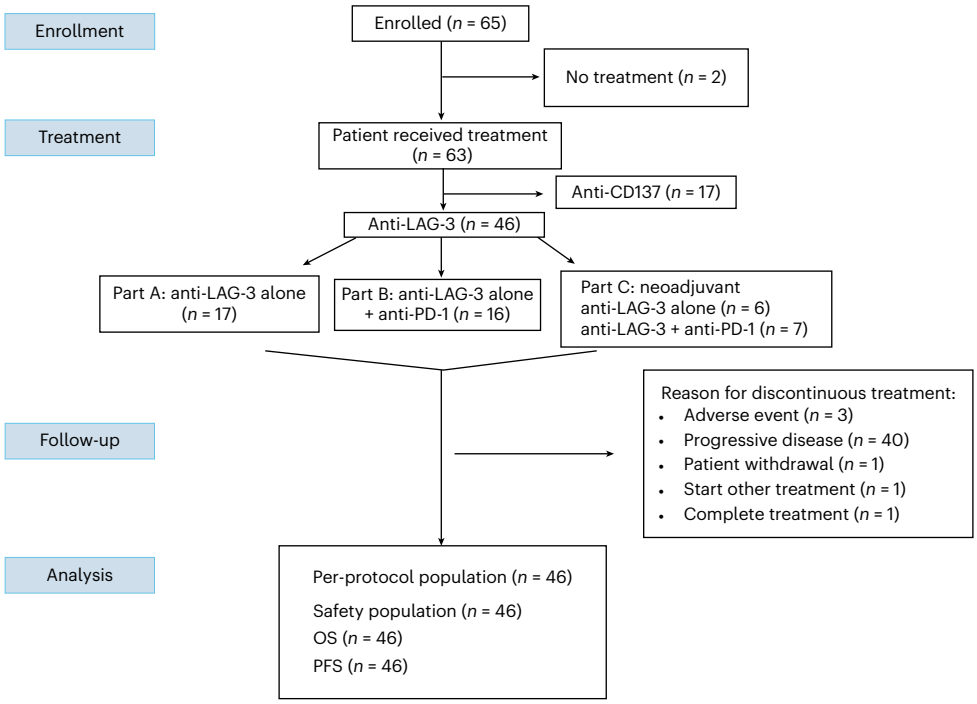

Fig. 1 | ABTC 1501 clinical trial design. Schematic overview of the multicenter phase 1 trial design illustrating sequential allocation and dose escalation of relatlimab. Patients were enrolled across three study components: adjuvant monotherapy dose escalation, adjuvant combination therapy dose escalation and neoadjuvant therapy followed by adjuvant treatment. Neoadjuvant therapy consisted of a single dose administered prior to surgical resection.

confirmed partial response or complete response. Pseudoprogression, defined as initial progressive enhancement within the first 6 months of treatment followed by disease stability or regression on the confirmation scan, was noted in eight of 17 patients in the adjuvant relatlimab group, in nine of 16 patients in the adjuvant combination relatlimab and nivolumab group, in five of six patients in the neoadjuvant relatlimab group and in three of seven patients in the neoadjuvant combination relatlimab and nivolumab group per mRANO guidelines (Extended Data Fig. 1).

Immune correlative data

To correlate immunological changes in the TME with patients who had clinical response to LAG-3 blockade, we used several immune correlative studies, including immunofluorescence, gene expression profiling, T cell clonality assays and spatial proteomics through multiplexed ion beam imaging by time-of-flight (MIBI-TOF).

Immunofluorescence analysis. Neoadjuvant therapy involving relatlimab demonstrated increased infiltration of $CD8^+$ T cells into the TME at the time of surgical resection (Fig. 2a,b). When comparing neoadjuvant treatment samples with archival samples without immunotherapy treatment, exposure to relatlimab and nivolumab combination therapy in particular was associated with an increase in the number of infiltrating $CD8^+$ T cells as well as upregulation of LAG-3 and PD-1 expression (Fig. 2b).

However, correlating the immunophenotype of the TME to clinical response demonstrates that infiltration of $PD\text{-}1^+$, $LAG\text{-}3^+$ or $PD\text{-}1^+LAG\text{-}3^+$ T cells in the resected tumor samples do not correlate with survival among patients receiving relatlimab monotherapy or combination relatlimab/nivolumab therapy (Fig. 2c–f). Of note, although PD-1 and LAG-3 are typically co-expressed on T cells in the setting of exhaustion[15], neoadjuvant therapy resulted in $CD8^+$ T cell recruitment with both LAG-3 and PD-1 co-expression as well as some cells with only LAG-3 or PD-1 expression (Fig. 2g,h).

Gene expression profiling. To investigate baseline differential gene expression between long-term survivors (OS > 24 months) and short-term survivors (OS < 9 months) treated with adjuvant (relatlimab/nivolumab combination therapy), we performed gene expression profiling using NanoString nCounter transcriptomic analysis on formalin-fixed paraffin-embedded (FFPE) biopsy specimens collected from three short-term survivors and four long-term survivors prior to the initiation of immunotherapy. Analysis of gene expression profile revealed 56 differentially expressed genes (DEGs) between groups ($P < 0.05$), with 34 genes showing higher expression and 22 showing lower expression in long-term survivors (Fig. 3a,b). Gene set enrichment analysis (GSEA) of type I (IFNα) and type II (IFNγ) interferon signaling pathways demonstrated enrichment in responder patients, reaching significance with Wald test statistics ($P < 0.05$) (Fig. 3c).

To further explore transcriptional changes induced by neoadjuvant treatment, we performed gene expression profiling on FFPE biopsy specimens obtained from three patients before and after neoadjuvant relatlimab and nivolumab combination therapy. This analysis identified 67 DEGs ($P < 0.05$), with 63 genes increased and four decreased after treatment (Fig. 3d,e). Multiple genes associated with interferon signaling and antigen presentation, including CXCL9, CCL5, GZMA, STAT1 and HLA family members, showed consistent trends toward upregulation in patients after treatment, with Wald test statistics showing significance ($P < 0.05$) (Fig. 3f).

T cell clonality. We analyzed T cell receptor (TCR) sequences from FFPE specimens of four long-term responders (patients surviving >24 months) and five non-responders (patients surviving <9 months). A statistically significant increase in clonality ($P < 0.05$) was observed in these long-term responders compared with non-responders, suggesting enhanced monoclonal expansion of T cell clones in responders (Extended Data Fig. 2a). This likely reflects a targeted immune response against specific tumor antigens. To assess T cell expansion

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Table 2 | Safety profile and DLTs for patients treated with relatlimab monotherapy and combination therapy

(A)				
Anti-LAG-3 adverse events	**Grade 1 (*n*)**	**Grade 2 (*n*)**	**Grade 3 (*n*)**	**Total (*n*=23)**
Amylase (elevated)	1			1 (4%)
Alanine aminotransferase (elevated)		1		1 (4%)
Alkaline phosphatase (elevated)	1			1 (4%)
Anemia	2			2 (9%)
Aspartate aminotransferase (elevated)	1			1 (4%)
Blurred vision	2			2 (9%)
Cognitive disturbance	1			1 (4%)
Confusion		1		1 (4%)
Dizziness	1			1 (4%)
Dry mouth	1			1 (4%)
Fatigue	3	2		5 (22%)
Gait disturbance	1			1 (4%)
Generalized muscle weakness	1			1 (4%)
Headache	2	3		5 (22%)
Hyperglycemia	1			1 (4%)
Hypernatremia	1			1 (4%)
Hypertension	1			1 (4%)
Hyperthyroidism	1			1 (4%)
Hyponatremia	1			1 (4%)
Leukocytosis			1	1 (4%)
Lipase (elevated)	4			4 (17%)
Lymphopenia		3		3 (13%)
Memory impairment		1		1 (4%)
Muscle weakness (right-sided)		1		1 (4%)
Nausea	1	2		3 (13%)
Platelets (decreased)	1			1 (4%)
Pruritus			1	1 (4%)
Rash (maculopapular)	1			1 (4%)
Seizure	1			1 (4%)
Serum amylase (elevated)	2			2 (9%)
T4 (thyroxine) (decreased)	1			1 (4%)
Urinary urgency	1			1 (4%)
Visual field deficit		1		1 (4%)
Vomiting	1			1 (4%)
White blood cell (decreased)	1			1 (4%)

(B)					
Anti-LAG-3 + anti-PD-1 adverse events	**Grade 1 (*n*)**	**Grade 2 (*n*)**	**Grade 3 (*n*)**	**Grade 4 (*n*)**	**Total (*n*=23)**
Alanine aminotransferase (elevated)	3		1		4 (17%)
Anemia	3				3 (13%)
Anorexia	1				1 (4%)
Anxiety	1				1 (4%)
Arthritis	1				1 (4%)
Aspartate aminotransferase (elevated)	2		1		3 (13%)
Ataxia		1			1 (4%)
Autoimmune disorder		1			1 (4%)
Burning sensation bilaterally	1				1 (4%)
Bronchial obstruction	1				1 (4%)

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Table 2 (continued) | Safety profile and DLTs for patients treated with relatlimab monotherapy and combination therapy

(B)					
Anti-LAG-3 + anti-PD-1 adverse events	**Grade 1 (*n*)**	**Grade 2 (*n*)**	**Grade 3 (*n*)**	**Grade 4 (*n*)**	**Total (*n*=23)**
Cardiac troponin I (elevated)	1				1 (4%)
Cognitive disturbance	1				1 (4%)
Confusion			1		1 (4%)
Constipation		1			1 (4%)
Cough	1				1 (4%)
Dermatitis	2				2 (9%)
Dehydration		1			1 (4%)
Diarrhea	4				4 (17%)
Dizziness	1				1 (4%)
Dysarthria		1			1 (4%)
Dysgeusia	1				1 (4%)
Dysphasia		1			1 (4%)
Edema (cerebral)			1[a]	1[a]	2 (9%)
Facial nerve disorder	1				1 (4%)
Fall	1				1 (4%)
Fatigue	7	3			10 (43%)
Fever	1	1			2 (9%)
Folliculitis	1				1 (4%)
Gait disturbance		2			2 (9%)
Generalized muscle weakness		1			1 (4%)
Headache		3			3 (13%)
Hyperglycemia	1				1 (4%)
Hypertension			1[a]		1 (4%)
Hypoalbuminemia	1				1 (4%)
Hypocalcemia	1				1 (4%)
Hyponatremia			1		1 (4%)
Hypophosphatemia	3				3 (13%)
Hypotension	1	1			2 (9%)
Hypothyroidism	3	2			5 (22%)
Increased falls/ataxia		1			1 (4%)
Increase in number of bowel movements	1				1 (4%)
Lipase (elevated)	2	1		1	4 (17%)
Lymphadenitis	1				1 (4%)
Lymphopenia	4	4			8 (35%)
Memory impairment	1		1		2 (9%)
Mucosal infection		1			1 (4%)
Muscle weakness (left-sided or right-sided)	1		1[a]		2 (9%)
Nausea	3	3			6 (26%)
Parainfluenza		1			1 (4%)
Pericardial effusion			1		1 (4%)
Thrombocytopenia	2	1			3 (13%)
Pleural effusion			1		1 (4%)
Pruritus	1				1 (4%)
Purpura	1				1 (4%)
Rash (maculo-papular)	1	1			2 (9%)
Seizure	1				1 (4%)
Serum amylase (elevated)	1		1		2 (9%)

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Table 2 (continued) | Safety profile and DLTs for patients treated with relatlimab monotherapy and combination therapy

(B)					
Anti-LAG-3 + anti-PD-1 adverse events	**Grade 1 (*n*)**	**Grade 2 (*n*)**	**Grade 3 (*n*)**	**Grade 4 (*n*)**	**Total (*n*=23)**
Skin infection		1			1 (4%)
Syncope			1[a]		1 (4%)
Thyroiditis			1[a]		1 (4%)
TSH (low)	1				1 (4%)
TSH (high)	1				1 (4%)
Vomiting		1			1 (4%)
Watery eyes	1				1 (4%)
White blood cell (decreased)	3				3 (13%)

(C)							
Time	**Phase**	**Dosage**	**Adverse event**	**Grade**	**attLAG-3**	**attPD-1**	**DLT**
Cycle 2, day 15	1	Anti-LAG-3 80 mg; anti-PD-1 240 mg	Cerebral edema	4	Possible	Probable	Yes
End of cycle 2	1	Anti-LAG-3 80 mg; anti-PD-1 240 mg	Muscle weakness (left)	3	Possible	Possible	Yes
End of cycle 2	1	Anti-LAG-3 160 mg; anti-PD-1 240 mg	Cerebral edema	3	Possible	Possible	Yes
End of cycle 1	1	Anti-LAG-3 160 mg; anti-PD-1 240 mg	Hypertension	3	Possible	Possible	Yes
End of cycle 2	1	Anti-LAG-3 160 mg; anti-PD-1 240 mg	Syncope	3	Possible	Possible	Yes
30-day follow-up	Neoadjuvant	Anti-LAG-3 160 mg; anti-PD-1 240 mg	Thyroiditis	3	Possible	Possible	Yes

(A,B) Summary of grade 1–4 adverse events, categorized according to NCI CTCAE version 5.0, in patients receiving relatlimab monotherapy (A) or relatlimab/nivolumab combination therapy (B). Data include patients treated in both adjuvant and neoadjuvant settings. (C) Summary of DLTs and corresponding dose levels observed during combination therapy. [a]Dose-limiting toxicity.

in circulation after immunotherapy, we analyzed TCRβ sequences from peripheral circulating T cells in one responder patient and in one non-responder patient. Compared with the non-responder patient, the long-term survivor demonstrated expansion of intratumoral T cell clones (Extended Data Fig. 2b). In the responder patient, peripheral T cell expansion was noted with as few as two immune checkpoint inhibitor infusions, indicating that these T cells had robust proliferative potential (Extended Data Fig. 2c).

Spatial analysis involving immune populations for GBM. Given our findings of increased T cell clonality, we also found that there was upregulation of genes associated with antigen presentation on myeloid cells that was positively correlated with long-term survivors (Fig. 4a,b). Myeloid cells capable of antigen presentation can directly activate T cells with clonal expansion[16]. We performed MIBI-TOF analysis on four patient samples and found that there was robust intratumoral infiltration of $CD68^+$ macrophages throughout the entire tumor with concentrated infiltration of lymphoid cells, largely consisting of B cells, in perivascular spaces (Fig. 4c,d). This observation is consistent with previous studies reporting increased B cell infiltration in the TME after immune checkpoint blockade, including PD-1-directed therapies[17]. Further phenotypic resolution of B cell subsets was limited by tissue availability and marker constraints. Highlighting the importance of the myeloid compartment for immunotherapy response, patients who received neoadjuvant relatlimab with or without nivolumab exposure, as well as those who went on to demonstrate long-term response, exhibited a substantial presence of myeloid cells, including $CD68^+$ myeloid cells and activated microglia. Conversely, short-term survivors had a dearth of intratumoral microglia at the time of surgery while maintaining a population of immunosuppressive $CD163^+$ myeloid cells (Fig. 4e). When comparing this sample of patients to a larger cohort of 15 standard-of-care patients with GBM, long-term survivors and the neoadjuvant patient had a pattern of increased microglia, non-immunosuppressed myeloid and lymphoid cell populations when compared with all other patient populations (Fig. 4f).

Antitumor response

Survival outcomes in this phase 1 study were reported to assess unexpected deleterious effects of therapy and to support hypothesis-generating analyses examining associations between immune responses and tumor control. The 12-month OS rate for all patients who received relatlimab monotherapy was 34.8% compared with 52.2% for patients who received combination therapy (Fig. 5a). Patients who received neoadjuvant relatlimab monotherapy had a median OS of 8.6 months (similar to adjuvant therapy (95% confidence interval: 2.6–23.6)), and the median OS for patients treated with neoadjuvant combination relatlimab and nivolumab therapy was 16.7 months (95% confidence interval: 6.8–21.0) (Extended Data Fig. 3a). The adjuvant combination arm had four long-term survivors with OS > 24 months and corresponding radiographic improvement in tumor burden (Extended Data Fig. 1).

Given that the primary goal of the study was safety, the study size was not designed for a stable estimation of OS across treatment arms but was used to inform selection of a regimen for future study. However, it should be noted that the combination therapy group exhibited longer treatment durations compared with those receiving relatlimab alone, with fewer patients in the combination group requiring switch to a new therapy (Fig. 5b). The presence of the small cohort of neoadjuvant patients did not appear to uniformly alter treatment duration relative to adjuvant therapy.

Discussion

In GBM, LAG-3 expression is highly represented across infiltrating immune populations within the TME. Preclinical studies have

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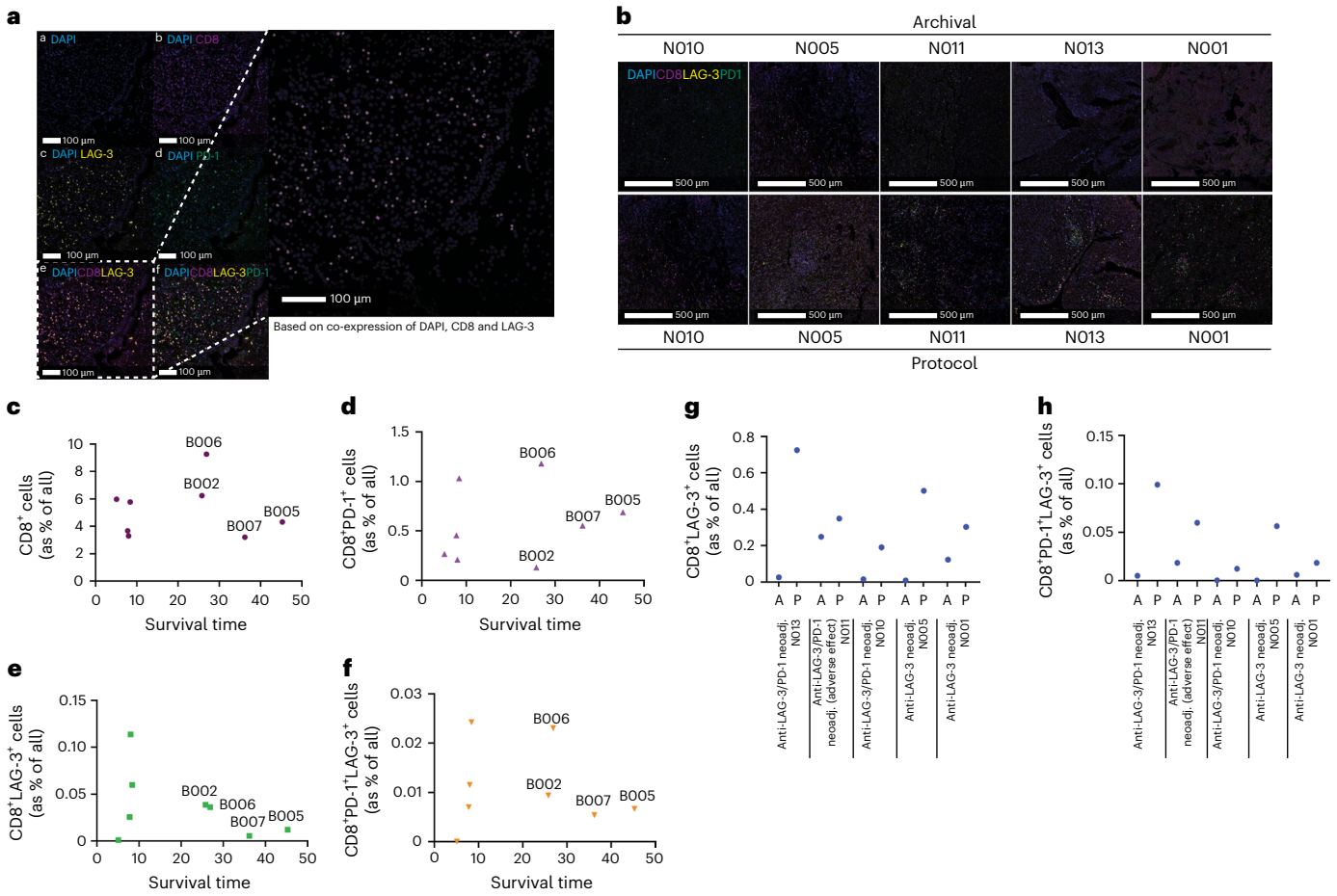

Fig. 2 | Neoadjuvant therapy enhances intratumoral T cell infiltration. **a**, Representative mIF images from a patient treated with neoadjuvant relatlimab/nivolumab, showing nuclear staining (DAPI) and expression of CD8, PD-1 and LAG-3. **b**, Comparative mIF images from paired tumor samples obtained before treatment and after neoadjuvant combination therapy. **c**–**f**, Correlation of intratumoral $CD8^+$ T cell subsets ($CD8^+$ (**c**), $CD8^+PD\text{-}1^+$ (**d**), $CD8^+LAG\text{-}3^+$ (**e**) and $CD8^+PD\text{-}1^+LAG\text{-}3^+$ (**f**)) expressing PD-1 and/or LAG-3 with OS; patients receiving adjuvant anti-LAG-3 and anti-PD-1 therapy who survived longer than the median are highlighted. **g**,**h**, Quantification of PD-1 and LAG-3 expression patterns on $CD8^+$ T cells ($CD8^+LAG\text{-}3^+$ (**g**) and $CD8^+PD\text{-}1^+LAG\text{-}3^+$ (**h**)) in patients before and after neoadjuvant (neoadj.) anti-PD-1 and anti-LAG-3 therapy. A, archival; P, protocol.

demonstrated that tumor-infiltrating lymphocytes co-expressing LAG-3 and PD-1 exhibit a functionally exhausted phenotype and that dual checkpoint targeting can restore antitumor immunity and improve survival in experimental models[10]. Accordingly, the rationale for therapeutically targeting LAG-3 in GBM is grounded in convergent immunophenotypic and functional studies demonstrating widespread LAG-3 expression and its association with immune dysfunction in these tumors[10,18,19].

In the present study, the primary endpoint of safety was met; relatlimab monotherapy and combination relatlimab with nivolumab were well tolerated with the dosages described. The only grade 3 or 4 adverse events occurred in combination arms involving both nivolumab and relatlimab, which included cerebral edema (2), hypertension (1), syncope (1), unilateral muscle weakness (1) and thyroiditis (1). Although our combination relatlimab dose was kept low due to studies at that time showing myocardial toxicity, pharmacokinetic studies have since shown that the receptor occupancy was only around 75%. Bristol Myers Squibb has since worked with 480 mg dosages of combination relatlimab therapy safely in other trials, which are being used for our current Alliance trial (A07221).

Although the study was not designed or powered to formally assess survival, the combination relatlimab and nivolumab arm demonstrated a 12-month OS of 52% (95% confidence interval: 31–73), with five of 46 patients (11%) exhibiting extended survival beyond 24 months. These patients went on to have additional therapies, including radiation, temozolomide, bevacizumab and other chemotherapeutic and immunotherapeutic agents (Extended Data Table 2). As a descriptive comparison to previous phase 2 survival data within the ABTC consortium, patients treated with nivolumab monotherapy had a 12-month OS rate of 22% (95% confidence interval: 15–30) (Extended Data Fig. 3b). This comparison should only be taken as contextual reference and not as a formal historical control. It is important to note that these observations cannot be disentangled from subsequent therapies and selection bias and do not imply clinical benefit. As such, this observation of increased antitumor efficacy with combination therapy is being followed up with a phase 2 study through the Alliance (A077201) to formally assess survival.

In assessing our immunologic correlative data, the neoadjuvant relatlimab and nivolumab samples suggested that targeting these checkpoint molecules results in increased intratumoral infiltration of $PD\text{-}1^+$ and $LAG\text{-}3^+CD8^+$ T cells. This has been similarly seen in trials, particularly in the neoadjuvant setting for non-small cell lung cancer[20] and melanoma[21]. The mechanisms behind this higher prevalence of intratumoral T cells are thought to involve either (1) in situ proliferation/expansion of existing T cells from increased activation or (2) improved T cell trafficking due to boosted chemokine signaling from inhibitory checkpoint blockade[22]. Notably, early increased infiltration of T cells alone did not correlate with improved survival unless

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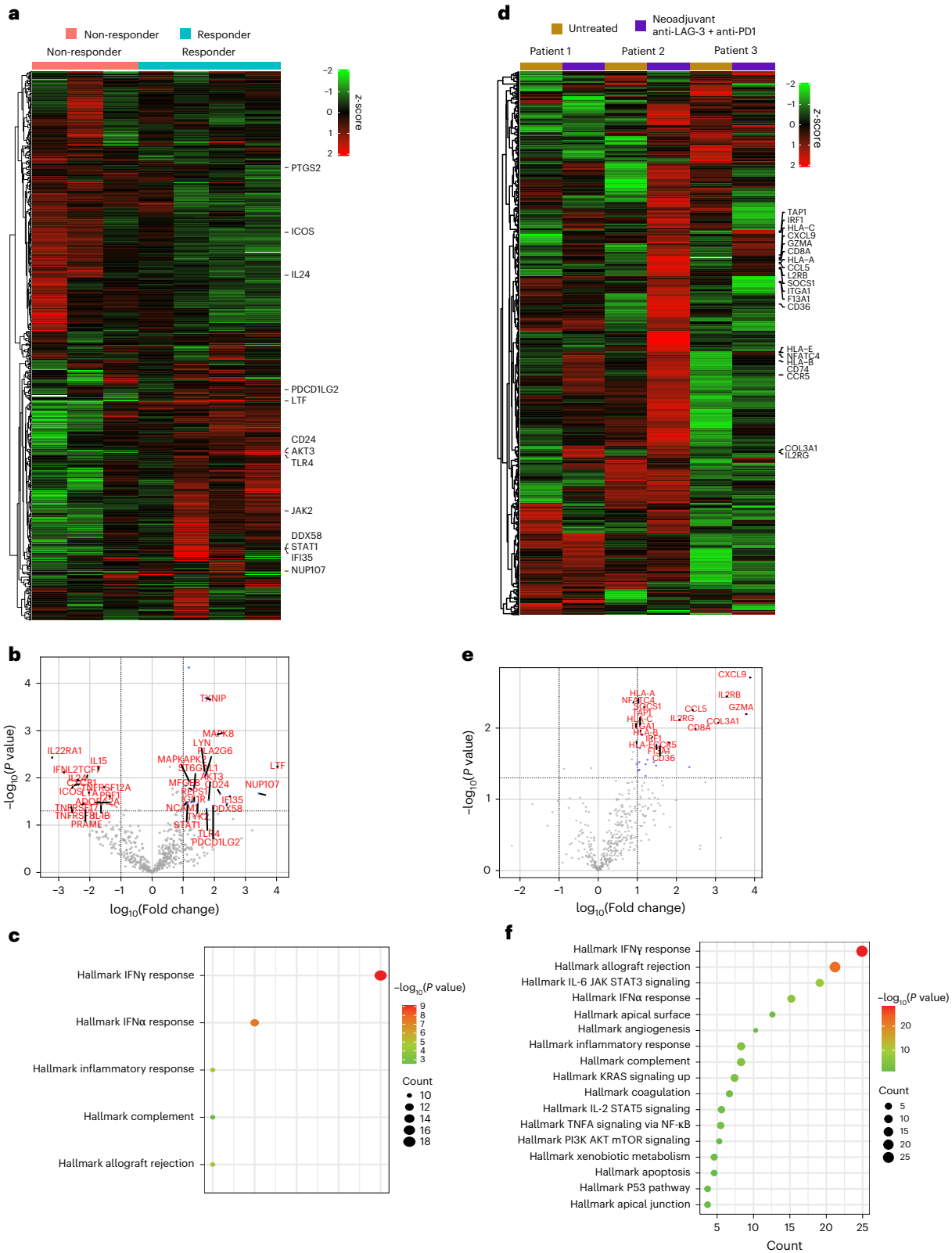

Fig. 3 | Interferon signature of tumor at time of first surgery is associated with clinical response. a, Heatmap showing normalized gene expression of genes with differential expression ($P < 0.05$) between long-term survivors (OS > 24 months) and short-term survivors (OS < 9 months) at baseline. **b**, Volcano plot displaying $\log_2$(fold change) versus $-\log_{10}$(P value) for baseline differential gene expression. Differential expression analysis was performed using NanoString nSolver Advanced Analysis. Statistical significance was assessed using two-sided t-tests, and $P < 0.05$ is considered signficant. **c**, GSEA showing enrichment of type I (IFNα) and type II (IFNγ) interferon signaling pathways in long-term survivors at baseline. **d**, Heatmap showing normalized gene expression of genes with differential expression ($P < 0.05$) before and after neoadjuvant relatlimab and nivolumab combination therapy. **e**, Volcano plot displaying $\log_2$(fold change) versus $-\log_{10}$(P value) for differential gene expression before and after neoadjuvant treatment. Statistical significance was assessed using two-sided t-tests, and $P < 0.05$ is considered significant. **f**, GSEA pathway analysis demonstrating enrichment of interferon signaling pathways after neoadjuvant treatment.

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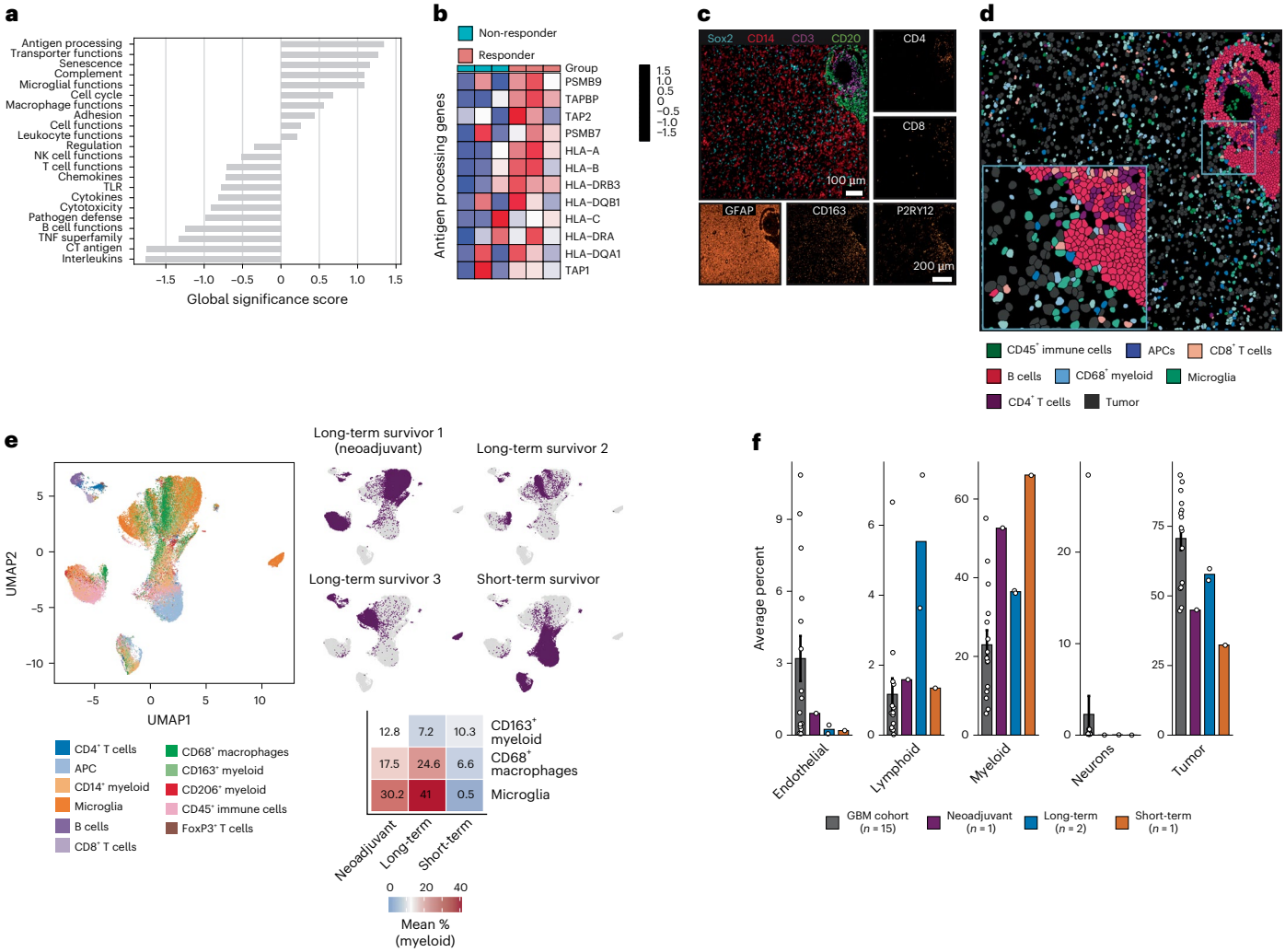

Fig. 4 | Myeloid antigen presentation and spatial immune organization. **a**,**b**, Differential gene expression (**a**) and heatmap of antigen processing and presentation genes between responder and non-responder patients (**b**). **c**,**d**, MIBI-TOF images showing high perivascular myeloid and lymphoid cell infiltration (**c**) and mosaic depiction showing distinct spatial immune cell clustering (**d**). **e**, The multicolored uniform manifold approximation and projection (UMAP) represents different immune populations (legend is on the bottom right of the UMAP), and the four purple-encoded maps show the same UMAP colored by unique patients. Heatmap shows selected myloid polulations (as percent of the total myeloid population) in neoadjuvant (survival was 18 months), long-term and short-term responders. **f**, Cell proportion analysis shows endothelial, lymphoid, myeloid, neuronal and tumor cell populations across neoadjuvant, long-term and short-term survivor samples alongside a cohort of 15 archival treatment-naive patients with GBM for reference. Each dot represents an individual patient. Error bars show s.e.m. Center line shows mean. No statistical analysis was performed due to limited numbers. APC, antigen-presenting cell.

also seen in the context of increased baseline interferon signatures and increased T cell clonality in the TME at the time of surgery. It is also possible that the impact of changes occurring after radiation is obscured by an association, at least in part, with the later effects during the period of adjuvant therapy. We did not have tissue available to assess this relationship as intracranial biopsy during this period is not standard of care.

When we compared the immune profiles of samples (from the time of first diagnosis) in an exploratory group of long-term and short-term survivors with gene expression profiling, we found that the tumors of responders at baseline trended toward a higher inflammatory TME phenotype (defined by upregulation of type I and II interferon genes, antigen presentation genes and increased T cell clonality) in the long-term survival patients (>24 months). In light of recent studies[23–26], this increase in interferon pathways as a positive prognostic indicator emerges as thematic and may have utility as a biomarker for future immunotherapy trials. Regarding other potential biomarkers, we observed a statistically significant increase in T cell clonality from the baseline FFPE samples of long-term responders. In one long-term survivor, we also found that there was robust expansion of T cells in the periphery after only two doses of relatlimab and nivolumab combination therapy (Extended Data Fig. 2c).

Immunologically, increased T cell clonality and expansion typically accompany the presence of activated antigen-presenting myeloid cells. In the long-term survivors, we observed a trend of elevated expression of antigen presentation pathways, suggesting that relatlimab may affect the myeloid compartment and that antigen presentation is also important in GBM for responding to relatlimab therapy. Finally, from our MIBI-TOF analysis, it appears that there is a subset of microglia and lymphoid cells that are present in responders that are not present in short-term survivors. Further investigation into the role of LAG-3 in myeloid cells is required, as recent studies have demonstrated the importance of this cell population in propagating immunosuppression in GBM[27,28].

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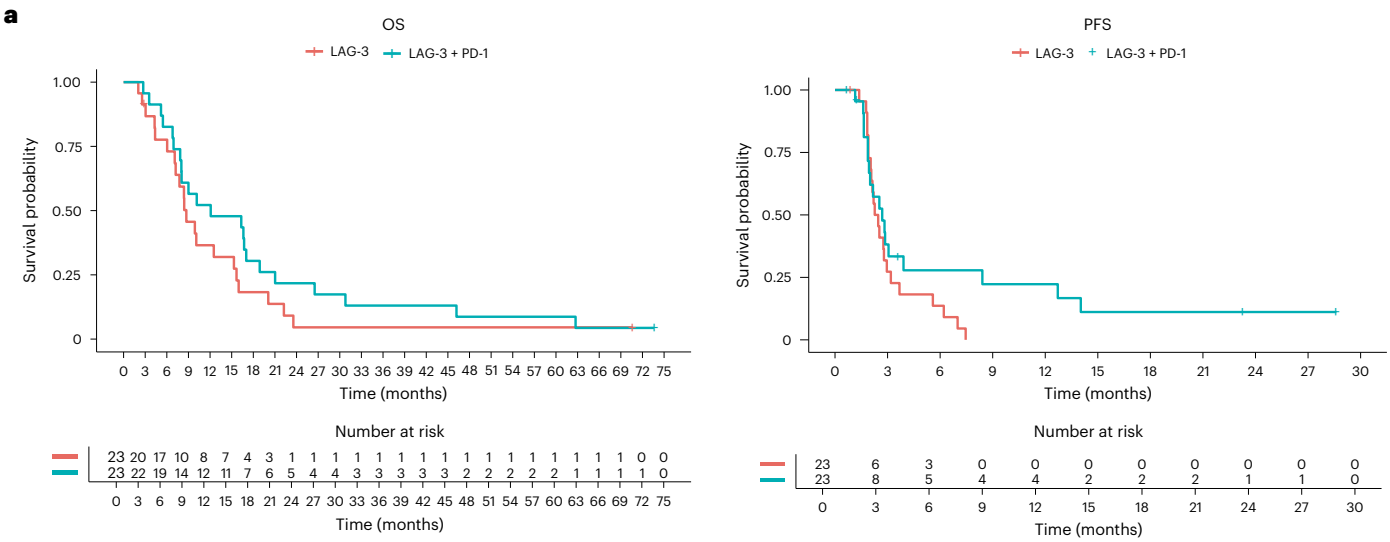

Treatment group	Number of patients	Number of deaths	Number of censored	mPFS	mPFS 95% CI	mOS	mOS 95% CI
LAG-3	17	15	2	2.2	1.9–3.2	8.7	6.0–15.3
LAG-3 + PD-1	16	15	1	2.1	1.6–3.9	8.5	5.5–17.0
Neoadjuvant LAG-3	6	6	0	2.6	1.8–6.2	8.6	2.6–23.6
Neoadjuvant LAG-3 + PD-1	7	7	0	3.1	2.5–12.7	16.7	6.8–21.0

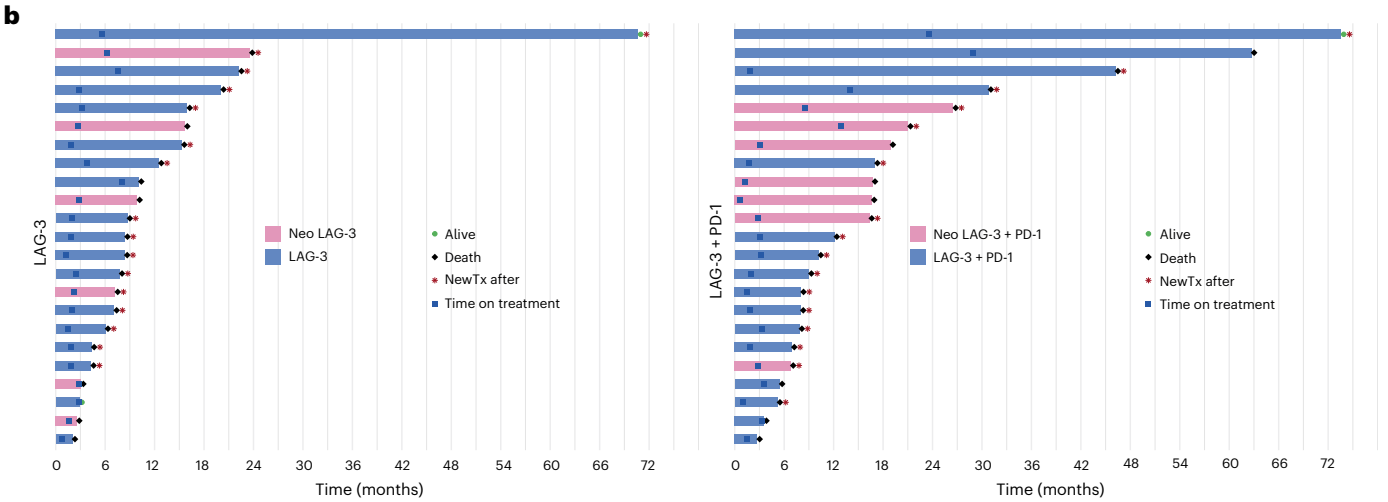

Fig. 5 | Descriptive survival outcomes and treatment exposure. a, Probability of OS and PFS according to Kaplan–Meier estimation for patients who received monotherapy or combination therapy with corresponding survival statistics. **b**, Duration and mapping of significant clinical events (alive, death, new treatment (newTx) after relatlimab or nivolumab/relatlimab initiation, time on treatment) for patients enrolled in the trial over time for adjuvant (pink) and neoadjuvant (blue) relatlimab monotherapy as well as relatlimab and nivolumab combination therapy. mOS, median overall survival; mPFS, median progression-free survival.

This study has several limitations. Survival of individual patients may have been affected by unknown selection criteria from subsequent therapies, although the impact of third-line therapy for GBM is generally modest. Additionally, only six of 46 (13%) patients required steroids prior to trial enrollment, indicating a relatively more fit population that may have biased prognosis. However, our inclusion criteria requiring a minimum Karnofsky Performance Status (KPS) of at least 60% were similar to other clinical trials studying recurrent GBM (Extended Data Table 1). Furthermore, when this trial was initiated in 2014, the World Health Organization classification of GBM had not yet incorporated *IDH*(isocitrate dehydrogenase) mutation status into its diagnostic criteria. As such, a subset of *IDH*-MT patients (6/46) was included in the trial. All patients, including those with *IDH*-MT status, have their corresponding survival data shown (Extended Data Table 2). Of note, although the study was not designed to evaluate survival outcomes or stratify by *IDH*-associated prognosis, both *IDH*-MT and *IDH*-WT patients were represented among those with long-term survival as well as those with survival less than 6 months. Specifically, survival among *IDH*-MT patients ranged from 2.7 months to 70.7 months, whereas survival among *IDH*-WT patients ranged from 2.6 months to 73.6 months.

Finally, the absence of postadjuvant tumor tissue, reflecting the clinical difficulty and procedural risk of obtaining biopsies at recurrence for immune sequencing, precluded comparative analyses of the long-term molecular effects of relatlimab monotherapy versus relatlimab/nivolumab combination therapy. Although such analyses could have yielded important insights into the biological features underlying durable clinical benefit in long-term survivors, repeat tumor sampling was not incorporated into the trial protocol.

The recent positive survival results of combination relatlimab and nivolumab therapy in melanoma[13] support investigating the targeting of LAG-3 as a therapeutic strategy for other difficult-to-treat cancers. Interest in targeting LAG-3 for GBM is also noted in the recent case report examining neoadjuvant triplet immune checkpoint blockade against CTLA-4, PD-1 and LAG-3 (ref. 29). Our correlative data suggest that relatlimab or relatlimab with nivolumab combination therapy can facilitate increased immune cell infiltration into GBM. However, increased immune cell infiltration alone is unlikely to be sufficient to generate an effective antitumor immune response. Rather, therapeutic benefit likely depends on a subset of tumors with a preexisting inflammatory phenotype—characterized by interferon-responsive gene expression, enhanced antigen presentation and increased T cell clonality—together with the presence of specific activated myeloid cell populations that support and sustain responsiveness to LAG-3 blockade.

These findings also suggest that T cell exhaustion may exist along a spectrum, ranging from earlier, more plastic dysfunctional states to late or terminally exhausted states that are less amenable to rescue with checkpoint blockade. As such, the absence of durable response in all patients may, in part, reflect differences in the timing of therapy relative to this exhaustion trajectory, although this is unlikely to be the sole explanation for heterogeneous outcomes in GBM. Continued interrogation of the TME using neoadjuvant strategies prior to surgical resection, therefore, remains particularly important, as it may help define when and in whom checkpoint blockade such as relatlimab is most likely to be effective. Our ongoing A07221 Alliance phase 2 trial (NCT06325683) will further investigate these immune correlative findings and their impact on clinical efficacy.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-026-04475-7.

References

1. Stupp, R. et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N. Engl. J. Med.* **352**, 987–996 (2005).
2. Reardon, D. A. et al. Effect of nivolumab vs bevacizumab in patients with recurrent glioblastoma: the CheckMate 143 phase 3 randomized clinical trial. *JAMA Oncol.* **6**, 1003–1010 (2020).
3. Omuro, A. et al. Radiotherapy combined with nivolumab or temozolomide for newly diagnosed glioblastoma with unmethylated MGMT promoter: an international randomized phase III trial. *Neuro Oncol.* **25**, 123–134 (2023).
4. Lim, M. et al. Phase III trial of chemoradiotherapy with temozolomide plus nivolumab or placebo for newly diagnosed glioblastoma with methylated MGMT promoter. *Neuro Oncol.* **24**, 1935–1949 (2022).
5. Larkin, J. et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N. Engl. J. Med.* **373**, 23–34 (2015).
6. Garon, E. B. et al. Pembrolizumab for the treatment of non–small-cell lung cancer. *N. Engl. J. Med.* **372**, 2018–2028 (2015).
7. Jackson, C. M., Choi, J. & Lim, M. Mechanisms of immunotherapy resistance: lessons from glioblastoma. *Nat. Immunol.* **20**, 1100–1109 (2019).
8. Lim, M., Xia, Y., Bettegowda, C. & Weller, M. Current state of immunotherapy for glioblastoma. *Nat. Rev. Clin. Oncol.* **15**, 422–442 (2018).
9. Liu, Y., Zhou, F., Ali, H., Lathia, J. D. & Chen, P. Immunotherapy for glioblastoma: current state, challenges, and future perspectives. *Cell. Mol. Immunol.* **21**, 1354–1375 (2024).
10. Harris-Bookman, S. et al. Expression of LAG-3 and efficacy of combination treatment with anti-LAG-3 and anti-PD-1 monoclonal antibodies in glioblastoma. *Int. J. Cancer* **143**, 3201–3208 (2018).
11. Huo, J.-L., Wang, Y.-T., Fu, W.-J., Lu, N. & Liu, Z.-S. The promising immune checkpoint LAG-3 in cancer immunotherapy: from basic research to clinical application. *Front. Immunol.* **13**, 956090 (2022).
12. Shan, C., Li, X. & Zhang, J. Progress of immune checkpoint LAG-3 in immunotherapy. *Oncol. Lett.* **20**, 207 (2020).
13. Tawbi, H. A. et al. Relatlimab and nivolumab versus nivolumab in untreated advanced melanoma. *N. Engl. J. Med.* **386**, 24–34 (2022).
14. Ellingson, B. M., Wen, P. Y. & Cloughesy, T. F. Modified criteria for radiographic response assessment in glioblastoma clinical trials. *Neurotherapeutics* **14**, 307–320 (2017).
15. Panda, A., Rosenfeld, J. A., Singer, E. A., Bhanot, G. & Ganesan, S. Genomic and immunologic correlates of LAG-3 expression in cancer. *Oncoimmunology* **9**, 1756116 (2020).
16. Kaech, S. M. & Ahmed, R. Memory $CD8^+$ T cell differentiation: initial antigen encounter triggers a developmental program in naïve cells. *Nat. Immunol.* **2**, 415–422 (2001).
17. Hou, D. et al. B-cells drive response to PD-1 blockade in glioblastoma upon neutralization of TGFβ-mediated immunosuppression. Preprint at *Research Square* https://doi.org/10.21203/rs.3.rs-2399170/v1 (2023).
18. Mair, M. J. et al. LAG-3 expression in the inflammatory microenvironment of glioma. *J. Neurooncol.* **152**, 533–539 (2021).
19. Woroniecka, K. et al. T-cell exhaustion signatures vary with tumor type and are severe in glioblastoma. *Clin. Cancer Res.* **24**, 4175–4186 (2018).
20. Forde, P. M. et al. Neoadjuvant PD-1 blockade in resectable lung cancer. *N. Engl. J. Med.* **378**, 1976–1986 (2018).
21. Huang, A. C. et al. A single dose of neoadjuvant PD-1 blockade predicts clinical outcomes in resectable melanoma. *Nat. Med.* **25**, 454–461 (2019).
22. Schalper, K. A. et al. Neoadjuvant nivolumab modifies the tumor immune microenvironment in resectable glioblastoma. *Nat. Med.* **25**, 470–476 (2019).
23. Benguigui, M. et al. Interferon-stimulated neutrophils as a predictor of immunotherapy response. *Cancer Cell* **42**, 253–265 (2024).
24. Deng, H. et al. An IFN-γ-related signature predicts prognosis and immunotherapy response in bladder cancer: results from real-world cohorts. *Front. Genet.* **13**, 1100317 (2023).
25. Li, S. et al. A high interferon gamma signature of $CD8^+$ T cells predicts response to neoadjuvant immunotherapy plus chemotherapy in gastric cancer. *Front. Immunol.* **13**, 1056144 (2022).
26. Reijers, I. L. M. et al. IFN-γ signature enables selection of neoadjuvant treatment in patients with stage III melanoma. *J. Exp. Med.* **220**, e20221952 (2023).
27. Jackson, C. et al. Distinct myeloid-derived suppressor cell populations in human glioblastoma. *Science* **387**, eabm5214 (2025).
28. Sloan, L. et al. Radiation immunodynamics in patients with glioblastoma receiving chemoradiation. *Front. Immunol.* **15**, 1438044 (2024).
29. Long, G. V. et al. Neoadjuvant triplet immune checkpoint blockade in newly diagnosed glioblastoma. *Nat. Med.* **31**, 1557–1566 (2025).

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Methods

Clinical trial registration and information

This trial is registered under NCT02658981 with preregistration date of 24 August 2016. The primary completion date was 30 April 2022. The study protocol and all related procedures were reviewed and accepted by the NCI Cancer Therapy Evaluation Program of the Division of Cancer Treatment and Diagnosis.

Ethical approval and consent

This study was approved by the Johns Hopkins Office of Human Subjects Research institutional review board (IRB), specifically IRB-2 with Committee Chair D. Cornblath. All regulatory documents were supported by the Cancer Trials Support Unit, a service of the NCI. All patients enrolled in the trial provided informed consent for tissue collection and trial enrollment as stated in the central IRB. The full list of IRB members is provided in Supplementary Information.

Patient selection

Potential participants were identified during chart review in advance of a routine clinic visit or during a routine clinic visit with a provider. Individuals were approached by the study team if willing to learn more about a study for which they may be eligible. Discussions regarding trial participation took place privately, and individuals were provided with the IRB-approved consent form. To our knowledge, there were no clear examples of self-selected bias.

Patients with histologically confirmed recurrent GBM or gliosarcoma after radiation therapy and temozolomide were recruited. Disease recurrence was defined as tumor progression despite standard-of-care treatment with the Stupp et al. protocol[1]. Eligibility criteria included measurable contrast-enhancing disease (≥1 cm × 1 cm on magnetic resoance imaging (MRI)) within 21 days of treatment initiation, and all patients had to be off steroids (at the equivalent dose of 7.5 mg per day of prednisone) for at least 2 weeks prior to enrollment. Last treatment of systemic chemotherapy was at least 6 weeks prior to trial initiation, and radiation or local chemotherapy with carmustine wafers was at least 12 weeks prior.

Participants had to be at least 18 years of age, have a KPS of at least 60%, have preserved organ/marrow function (including absolute lymphocyte count ≥1,000 per µl) and have no concurrent malignancies (except curatively treated basal or squamous cell carcinoma of the skin or carcinoma in situ of the cervix, breast or bladder). Prior malignancies must have been disease free on whole-body surveillance for at least 5 years. Women of childbearing potential required a negative pregnancy test and contraception use during and after treatment given risks of possible teratogenic effects with therapy. Males were also expected to use anticonception measures during treatment periods.

Exclusion criteria included any other investigational agents, active/recent autoimmune disease (except type 1 diabetes, hypothyroidism requiring only hormone replacement or non-systemic skin disorders), immunosuppressive therapy within 14 days of treatment start or >1 mg per day dexamethasone for ≥1 week before treatment. HIV-positive individuals on antiretroviral therapy were ineligible.

Study design, treatment schedule and sample collection

As part of the NCI ABTC, ABTC 1501 was a multicenter, phase 1 open-label platform design in the United States that allowed for multi-arm approaches with the opportunity to add on or drop off checkpoint agent/agents during the trial (Table 1). The initial study agents were anti-LAG-3 and anti-CD137 mAb (BMS-663513) administered as a single agent or in combination with nivolumab antibody for patients with recurrent GBM. Any patients deemed surgical candidates underwent maximally safe surgical resection prior to initiation of therapy. At time of surgery, patient samples were collected and stored in either saline or formalin for pathology processing per standard of care and downstream immunophenotyping. For the purposes of this paper, we focused on the relatlimab portion of the study and will report findings involving anti-CD137 antibodies in a subsequent report. The study was designed in three parts for safety evaluation with a target DLT rate of less than 30%.

Part A determined the MTD of relatlimab monotherapy in patients who were assigned either relatlimab or anti-CD137 monotherapy by sequential allocation as designed by our statistical team. A modified rule-based dose escalation with a total of seven patients was required per dosage cohort to determine the MTD. There were three prespecified doses of relatlimab at 80 mg, 240 mg and 800 mg. These dosages were based on studies in other cancers treated with the same investigational agents[30].

Part B aimed to identify a safe combination regimen of relatlimab when administered in combination with nivolumab. Treatment with the combination of relatlimab and nivolumab was initiated after the MTD of relatlimab was confirmed in part A. Nivolumab dosages followed historical safe dosages (240 mg). Given increased rates of immune-related adverse events with combination immunotherapy[31], the plan was to originally dose relatlimab one step down from the MTD that was determined in part A. However, due to reports of increased rates of myocarditis with combination nivolumab and relatlimab in another trial[13], the decision was made to start relatlimab dosage at 80 mg and escalate to 160 mg for combination therapy with 240 mg nivolumab. Patients again were assigned by sequential allocation.

Part C examined the safety and impact of neoadjuvant relatlimab monotherapy and combination relatlimab/nivolumab therapy on the immune microenvironment for recurrent GBM. Patients received one preoperative (neoadjuvant) dose 10 days (±3 days) prior to planned surgery. After surgery, patients underwent treatment with either relatlimab alone (800 mg) or combination relatlimab (160 mg every 2 weeks) and nivolumab (240 mg every 2 weeks). Sample sizes for parts A, B and C were determined based off of previous trials for GBM (Extended Data Table 1) and published FDA guidance for expansion cohorts[32]. All procedures were approved by the Cancer Therapy Evaluation Program with a central IRB and Brain Malignancy Steering Committee as above, with all patients enrolled in the clinical trial consented for tissue specimen and blood collection for research. Researchers involved with tissue processing and immune assays were blinded to patient demographics.

Clinical assessments

The safety evaluation period for dose-escalation decisions was 4 weeks from the initial dose (cycle 1). Version 5.0 of the NCI CTCAE was used for scoring toxicity and adverse events. Safety assessments involved monitoring and recording all adverse events, pregnancy testing (in women with childbearing potential), serial monitoring of hematology and blood chemistry, regular measurement of vital signs and physical/neurological examinations; electrocardiograms and other cardiac monitoring were performed as necessary after clinical evaluation. All adverse events were evaluated and recorded during the trial period. Patients with measurable disease were assessed by MRI prior to every odd cycle.

mRANO criteria

Patients with measurable (1 cm × 1 cm) contrast-enhancing disease at baseline were assessed by MRI prior to every odd cycle of immune checkpoint inhibitor treatment under mRANO criteria[14] to mitigate potential immunotherapy-related pseudoprogression by requiring confirmation of radiographic progression within a 6-month window of starting treatment in non-surgical patients. Complete response was defined as disappearance of all enhancing and non-enhancing disease for at least 4 weeks, no new lesions, discontinuation of corticosteroids (except physiologic doses), stable or improved T2/FLAIR lesions and stable or improved clinical status.

Partial response required at least 50% reduction in the sum of perpendicular diameters of measurable lesions sustained for 4 weeks, no progression of non-measurable disease, no new lesions, corticosteroid dose no greater than baseline and stable or improved T2/FLAIR lesions and clinical status; again, non-measurable disease precludes partial response. Stable disease represents maintenance of status that does not qualify for complete response, partial response or progression, requiring 4-week duration with stable imaging findings on same or lower corticosteroid doses.

Progressive disease was defined by at least 25% increase in tumor measurements, significant increase in T2/FLAIR lesions not due to other causes, any new lesion, clear clinical deterioration not attributable to other factors, death or clear progression of non-measurable disease. For the first 6 months after initiation of treatment, radiographic progression required confirmation of progression as defined by increased contrast-enhancing tumor growth more than 2 months after initial progressive enhancement to be considered progressive disease. Pseudoprogression was defined as initial progressive enhancement within the first 6 months of treatment followed by disease stability or regression on the confirmation scan. If radiographic progression occurred after 6 months from the start of treatment, no confirmation was required for progressive disease.

Immune correlation studies

Immunofluorescence. An automated multiplex immunofluorescence (mIF) panel was developed for use in FFPE GBM human samples using methods previously described[33]. The final 3-plex panel was validated on archival GBM to show equivalence with serial sections of individual immunohistochemistry (IHC) stained slides.

In brief, samples were baked offline for 3 h at 65 °C and loaded onto a Leica BOND RX automated research stainer (Leica Biosystems). Samples were baked online at 60 °C for 30 min, and residual paraffin was removed (Dewax; Leica Biosystems). Initial antigen retrieval was performed using a pH 9 EDTA buffer (ER2; Leica Biosystems) for 40 min at 100 °C, followed by another round of antigen retrieval using pH 6 sodium citrate buffer (ER1; Leica Biosystems) at 95 °C for 20 min. After initial blocking for endogenous peroxidases (BLOXALL; Vector Labs), non-specific antibody binding was blocked (Protein Block; Agilent Technologies). All primary antibodies were diluted in antibody diluent with background-reducing components (Agilent Technologies). Primary antibodies, polymers and opals were applied for Position 1 (see table), and then antibody stripping was performed using a pH 6 sodium citrate buffer (ER1; Leica Biosystems) for 20 min at 95 °C. This process was repeated for each position, after which slides were counterstained (Spectral DAPI; Akoya Biosciences) and coverslipped (ProLong Diamond; Invitrogen).

Whole-slide scans of the mIF-stained slides were acquired using PhenoImager HT (Akoya Biosciences) with the standard set of multispectral slide scan filters. Regions of interest were selected using Phenochart version 1.1.0 (Akoya Biosciences) and then unmixed using a microscope-specific library consisting of the matching fluorophores using inForm version 2.5.1 (Akoya Biosciences). Individual unmixed TIFF files were stitched together using HALO version 6.8 (Indica Labs). Using this software, we quantified the percentages of cells positive for CD8, PD-1 and/or LAG-3. Due to substantial intratumoral heterogeneity of GBM, $CD8^+$ T cells, $CD8^+PD\text{-}1^+$, $CD8^+LAG\text{-}3^+$ and $CD8^+PD\text{-}1^+LAG\text{-}3^+$, subsets were quantified across entire whole-slide images rather than within predefined regions of interest. All measurements were obtained from a single experimental replicate ($n = 1$) and a single experimental run to minimize batch effects and ensure analytical consistency. Each dot represents whole-slide data from a single patient.

RNA isolation and gene expression profiling. Total RNA was extracted from 5-µm-thick FFPE sections obtained from surgical resection of patients with recurrent GBM using an RNeasy FFPE Kit (Qiagen) following the manufacturer's protocol. RNA was quantified using Qubit and NanoDrop. Then, 100 ng of total RNA was run on an nCounter SPRINT Profiler system for gene expression profiling using the commercial human pan-cancer immune profiling panel with 770 immune-related genes, including 14 internal reference controls (XT-PGX-Huv1, NanoString; Bruker Spatial Biology). Data were analyzed using nSolver 4.0 software with an nCounter Advanced Analysis 2.0 add-on (NanoString; Bruker Spatial Biology).

T cell clonality. Genomic DNA from FFPE samples were extracted (Qiagen AllPrep DNA/RNA Kit) and amplified by polymerase chain reaction (PCR) with primers (Adaptive Biotechnologies) that span Vβ and Jβ regions to capture TCRβ gene segments. Sequencing adapters were ligated using a library preparation kit and performed on an Illumina platform (MiSeq), and raw sequencing reads were processed (MiXCR) to identify clonotypes and their relative frequencies. Simpson clonality was calculated to assess the TCR repertoire with visualization and statistical analysis performed using GraphPad Prism.

Gene expression profiling. FFPE samples from surgical resection of patients with recurrent GBM were deparaffinized for RNA extraction. Reporter and capture probes for genes of interest were mixed in hybridization buffer with the sample at 65 °C for 16–20 h with hybridized samples loaded into nCounter cartridge and nCounter Digital Analyzer for barcode detection to measure gene expression (NanoString; Bruker Spatial Biology). Results were analyzed using nSolver (NanoString; Bruker Spatial Biology). *P* values for differential expression were computed using a negative binomial generalized linear model with Wald test statistics in the NanoString nSolver Advanced Analysis module. In this exploratory analysis, significance was defined as a nominal $P < 0.05$.

MIBI-TOF. MIBI-TOF was performed using a custom human panel targeting immune (CD45, CD4, CD8, Foxp3, P2RY12, CD68, CD14, CD20, HLA1, CD163, CD206), tumor (Sox9, Olig2), exhaustion (PD-1) and metabolic (ASCT2, CD36) markers, obtained in a preformulated, lyophilized format from IONpath, Inc. Antibodies were resuspended in TBS-IHC Tween with 3% donkey serum and filtered before staining. Tissue preparation followed standard protocols, including deparaffinization, antigen retrieval, blocking, overnight antibody incubation, fixation and dehydration. Hematoxylin and eosin (H&E)-stained sections were reviewed for quality control, and background staining was manually assessed.

MIBI data were acquired using a uniform field of view (800 μm^2, when possible), an 8.5-nA beam current and a 0.58-ms dwell time. Background correction and noise removal settings were turned off. To correct for signal contamination, we applied Rosetta Matrix Compensation, a method that subtracts known overlapping markers using an empirically determined matrix[34]. Intensity normalization was performed using median pulse height (MPH), fitting a polynomial function to mass-specific data across runs and adjusting signal drift based on a predefined normalization curve[34].

Cells were segmented using the deep-learning-based Mesmer model, using HH3/dsDNA for nuclear segmentation and a composite of CD14, CD45, CD8 and HLA1 for membrane segmentation[34]. Cell phenotyping was conducted using Pixie, an unsupervised pixel clustering algorithm that overclustered pixels and cells before hierarchical consensus clustering into 20 metaclusters[35–37]. Assignments were validated through manual inspection in Adobe Photoshop, and systematic errors were corrected by refining clustering parameters.

Statistical analysis

Baseline patient and disease characteristics were summarized using descriptive statistics. Safety data were evaluated based on NCI CTCAE version 5, and the incidence rate of adverse events was presented using a proportion of the total number of treated patients for each

group. Tumor responses were initially classified by investigator assessment and subsequently followed by a central review. PFS and OS were measured from date of treatment initiation to database locking, with exceptions for clinical event occurrence or censorship. Survival probability was estimated using the Kaplan–Meier method. The confidence interval of median survival time was constructed by the Brookmeyer–Crowley method. Immune correlative studies were exploratory and mechanistic in nature, and the data were presented either descriptively or through two-tailed Student's *t*-test, with $P < 0.05$ considered statistically significant using R and GraphPad Prism. The analysis of clinical data was performed using SAS version 9.4 (SAS Institute).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The full study protocol with the statistical plan is available in Supplementary Information. Any requests for additional clinical data can be sent by email to M.L. and will be reviewed by the Johns Hopkins University IRB prior to being shared. Immune correlative data that will be used for further scientific investigation as stated by email are also available by request to M.L. and should have a response within 2 weeks.

References

30. Ascierto, P. A. et al. Nivolumab and relatlimab in patients with advanced melanoma that had progressed on anti-programmed death-1/programmed death ligand 1 therapy: results from the phase I/IIa RELATIVITY-020 trial. *J. Clin. Oncol.* **41**, 2724–2735 (2023).
31. Zhang, B. et al. Immune-related adverse events from combination immunotherapy in cancer patients: a comprehensive meta-analysis of randomized controlled trials. *Int. Immunopharmacol.* **63**, 292–298 (2018).
32. *Expansion Cohorts: Use in First-in-Human Clinical Trials to Expedite Development of Oncology Drugs and Biologics: Guidance for Industry* (US Food & Drug Administration, 2022); https://www.fda.gov/regulatory-information/search-fda-guidance-documents/expansion-cohorts-use-first-human-clinical-trials-expedite-development-oncology-drugs-and-biologics
33. Van Gassen, S. et al. FlowSOM: using self-organizing maps for visualization and interpretation of cytometry data. *Cytometry A* **87**, 636–645 (2015).
34. Berry, S. et al. Analysis of multispectral imaging with the AstroPath platform informs efficacy of PD-1 blockade. *Science* **372**, eaba2609 (2021).
35. Greenwald, N. F. et al. Whole-cell segmentation of tissue images with human-level performance using large-scale data annotation and deep learning. *Nat. Biotechnol.* **40**, 555–565 (2022).
36. Liu, C. C. et al. Robust phenotyping of highly multiplexed tissue imaging data using pixel-level clustering. *Nat. Commun.* **14**, 4618 (2023).
37. Monti, S., Tamayo, P., Mesirov, J. & Golub, T. Consensus clustering: a resampling-based method for class discovery and visualization of gene expression microarray data. *Mach. Learn.* **52**, 91–118 (2003).

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Author contributions

M.L. and X.Y. contributed to the conception, design and execution of the study. All authors contributed to the acquisition and analysis of data and contributed to the final revision of the paper. The final version of the paper was approved by all authors.

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Competing interests

M.L. is a consultant for Biohaven, the Global Coalition for Adpative Research, CraniUS, Hemispherian, Hoth, Insightec, MediFlix, Novocure, Noxxon, Sanianoia, Stryker, VBI Vaccines, Pyramid Bio, Century Therapuetics, InCando, InCephalo Therapeutics, Merck, Bristol Myers Squibb and XSense and has grant funding from Arbor, Accuray, Biohaven, Kyrin-Kyowa, Urogen and Bristol Myers Squibb. M.L. has honoraria from Insightec and Tocagen and ownership interest with Egret Therapeutics. M.L. has stock/stock options in Pyramid Bio and Egret Therapeutics. M.L. was previously on the data safety monitoring board for Cellularity. C.B. is a consultant for Depuy-Synthes, Bionaut Labs and Haystack Oncology. C.B. is a co-founder of Belay Diagnostics and OrisDx. P.Y.W. has received research support from AstraZeneca, Black Diamond, Bristol Myers Squibb, Chimerix, Eli Lily, the Global Coalition For Adaptive Research, Kazia, MediciNova, Merck, Novartis, Quadriga, Servier and VBI Vaccines and honoraria for consultation from AstraZeneca, Chimerix, Day One Bio, Fore Biotherapeutics, Genenta, GlaxoSmithKline, Merck, Mundipharma, Nerviano, Nuvation Bio, Medical Sciences, Novartis, Novocure, Rigel, Sapience, Servier and Telix. M.A. has grants from Pfizer and is a consultant for Bayer, Xoft, Nuvation Bio, SDP Oncology, Apollomics, Prelude, Janssen Pharmaceuticals, Viewray, Caris Lifesciences, Pyramid Biosciences, Varian Medical Systems, Anheart Therapeutics, Theraguix, Menarini Ricerche, Sumitomo Pharma Oncology, Autem Therapeutics, GT Medical Techhnologies, Modifi Biosciences, Bugworks, Allovir, EquilliumBio, VBI Vaccines, Servier Pharmaceuticals, Incyte and Recordati. B.M.E. is on the advisory board and is a paid consultant for Medicenna, MedQIA, Servier Pharmaceuticals, Siemens, Janssen Pharmaceuticals, Imaging Endpoints, Kazia, Chimerix, Sumitomo Pharma Oncology, ImmunoGenesis, Ellipses Pharma, Monteris, Neosoma, Alpheus Medical, Sagimet Biosciences, Sapience Therapeutics, Orbus Therapeutics and the Global Coalition for Adaptive Research. The other authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41591-026-04475-7.

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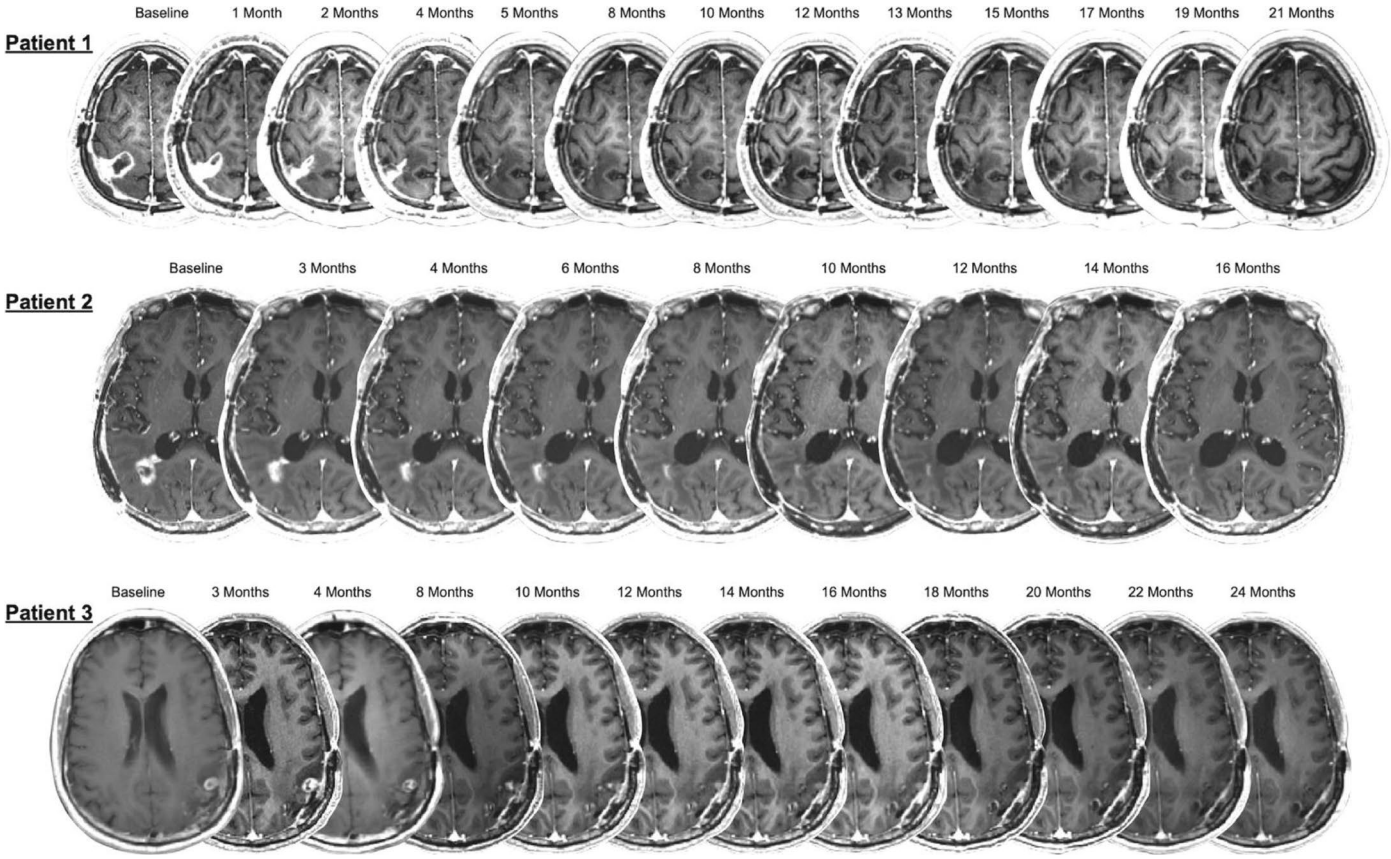
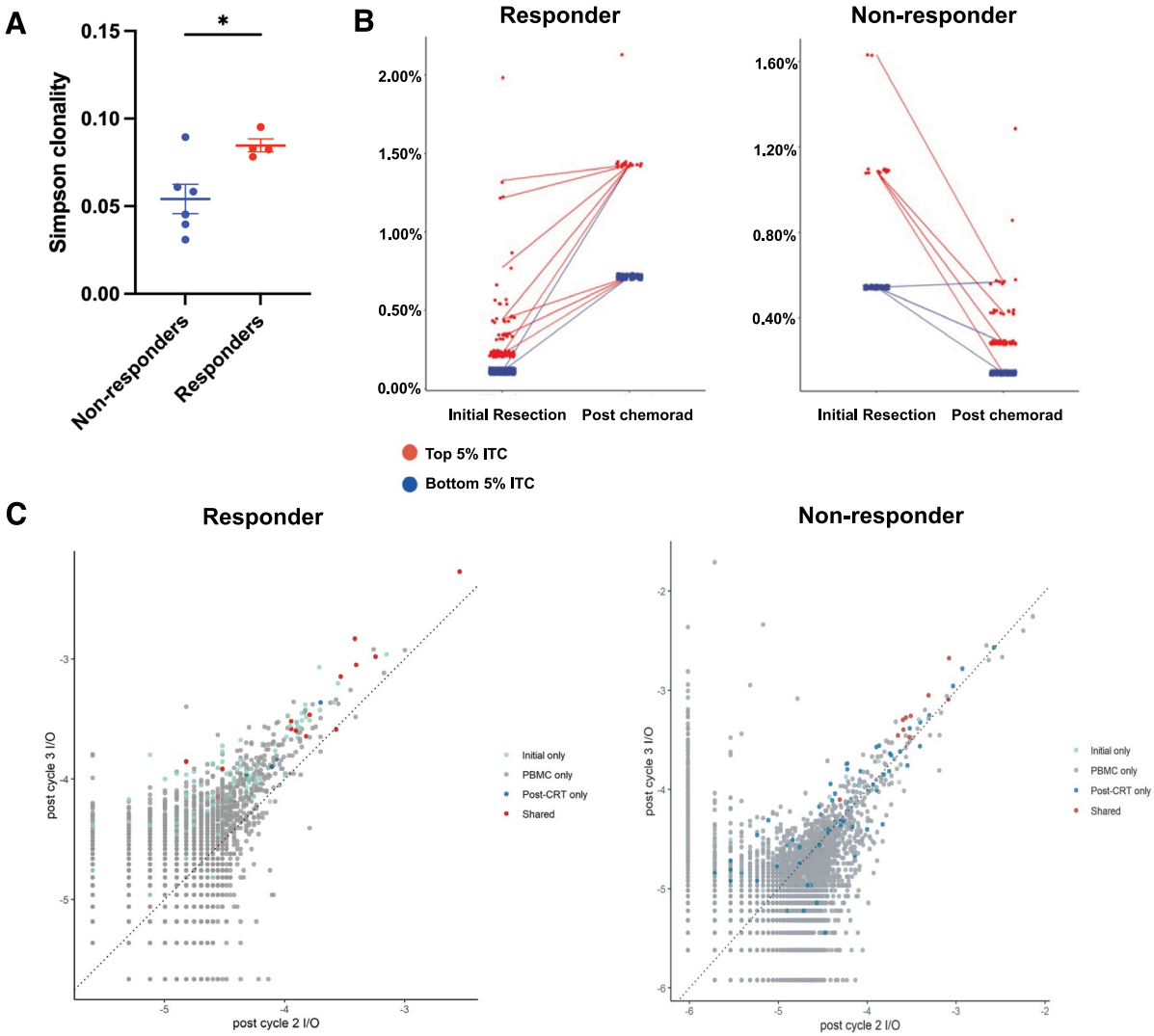


Treatment group	No. of Patients	No. censored	≥12-month OS N (%)	≥24-month OS N (%)	mOS	mOS 95% CI	No. pseudoprogression	No. disease progression	No. stable disease
LAG3 + Neoadjuvant LAG3	23	2	8 (34.8%)	1 (4.4%)	8.7	6.1 -15.3	13	11	5
(LAG3 + PD1) + (Neoadjuvant LAG3 + PD1)	23	1	12 (52.2%)	5 (21.7)	12.1	7.9 -17.0	12	5	6

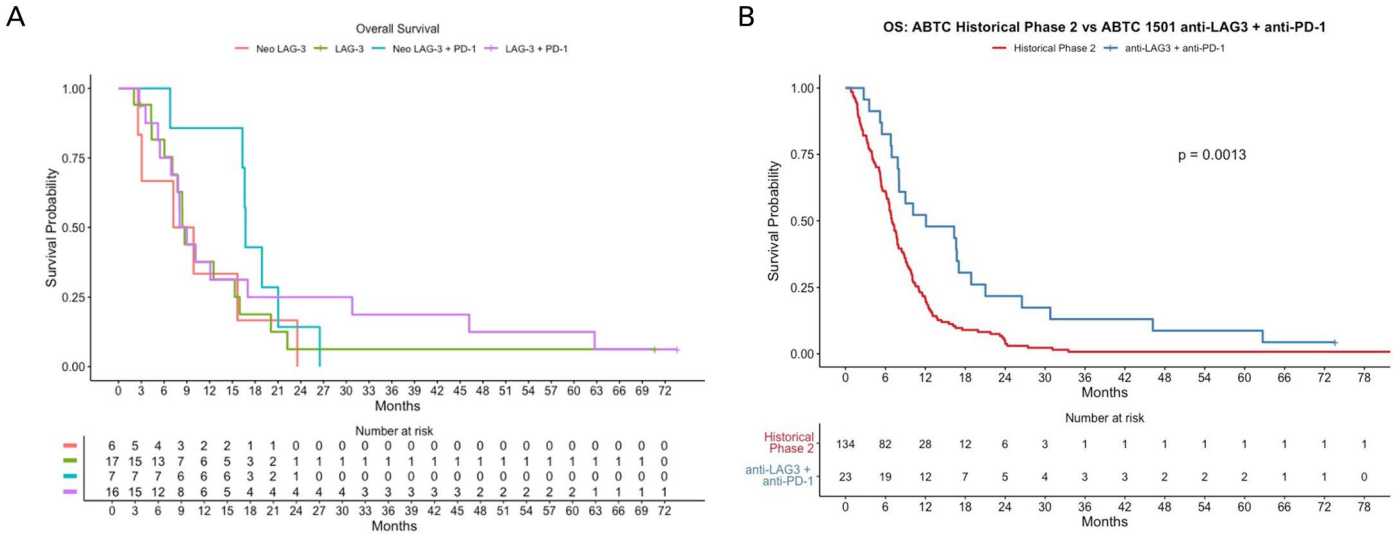
Extended Data Fig. 1 | Representative longitudinal MRI scans from three long-term survivors (OS > 24 months) treated with combination therapy, demonstrating delayed radiographic responses following initial post-treatment enhancement. Patient 1: 22-year-old male with MGMT-unmethylated recurrent GBM treated with adjuvant 80 mg anti-LAG-3/240 mg anti-PD-1 mAb demonstrating initial post-radiation enhancement followed by delayed radiographic improvement who received a total of 24 cycles and remains alive after completion of the trial. **Patient 2:** 64-year-old male with MGMT-methylated recurrent GBM treated with adjuvant 160 mg anti-LAG-3/240 mg anti-PD-1 mAb with gradual radiographic response and near-complete resolution by 16 months. **Patient 3:** 48-year-old male with MGMT-methylated recurrent GBM treated with adjuvant 160 mg anti-LAG-3/240 mg anti-PD-1 mAb, exhibiting progressive reduction in nodular enhancement and edema with near-complete radiographic response. Tabulated radiographic outcomes categorized as pseudoprogression, stable disease, or progressive disease according to mRANO criteria.

https://doi.org/10.1038/s41591-026-04475-7



Extended Data Fig. 2 | T cell clonality expansion after ICI treatment. (**A**) Simpson clonality indicates differential T cell receptor diversity across responders and non-responders. (**B**) Differences in T cell receptor diversity between initial resection and post-chemotherapy in one responder and non-responder patient. (**C**) Expansion of T cells in peripheral blood after immunotherapy initiation in one responder and one non-responder patient. Simpson clonality measurements were obtained from a single experimental replicate (N = 1) and each dot represents whole-slide data from a single patient.

https://doi.org/10.1038/s41591-026-04475-7



Extended Data Fig. 3 | Descriptive comparison of relatlimab/nivolumab in GBM and previous ABTC phase II trial with nivolumab alone. (**A**) Kaplan-Meier curves demonstrating combined relatlimab therapy across adjuvanat and neoadjuvant arms with overall survival at 12 months (OS12) of neoadjuvant combination relatlimab/nivolumab 86% compared to 38% in the adjuvant combination therapy arm. (**B**) Descriptive comparison of overall survival between patients treated with relatlimab/nivolumab in ABTC 1501 and patients treated with nivolumab alone in a prior ABTC phase II trial with historical control arm having 22% OS12. Shown for contextual reference only; not intended as a formal historical control.

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Extended Data Table 1 | Reference data for previous trials with objectives on patient survival

Consortium trials	Phase	Number of subjects	Baseine Age (median, range)	Baseline KPS (median, range)	Tumor	Specific mutation required	Year of the trial	Tested agent /agents	Median OS (month)
ABTC 0903	I/II	45	54 (23-80)	80 (60-100)	recurrent GBM	Noun	2010	Cediranib+Cilengitide	6.5
ABTC 0904	0/II	41	58 (33-79)	80 (60-100)	recurrent GBM	Noun	2009	GDC-0449	7.8
ABTC 0906	II	47	57 (35-75)	80 (60-100)	recurrent GBM	Noun	2010	RO4929097	7

The consortium used standardized protocols for the trial's development/execution with similar eligibility criteria for recurrent GBM. The historical trial information serves as a reference, not a formal historical control or formal trial comparator, since the ABTC 1501 was a phase I trial and not designed to formally conduct survival comparisons with a historical control.

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Extended Data Table 2 | Baseline characteristics, treatment exposure, and clinical outcomes of patients treated with anti-LAG-3-based therapy

De-Identified Labels	IDH status	Part/Arm	Dosage	Age (years)	Steroids	KPS base	Extent of surgery	MGMT status	PD (1=yes, 0=censored)	PFS (months)	New Therapy After PD	After Therapy	Death (1=yes, 0=censored)	OS (months)
N001	IDH WT	Group 1: Anti-LAG-3 Monotherapy	800mg	59		90	Gross Total Resection	Methylated	1	6.21	Yes	Temozolomide	1	23.56
N002	IDH WT	Group 1: Anti-LAG-3 Monotherapy	800mg	67	Dexamethasone	90	Subtotal Resection	Unmethylated	1	2.20	Yes	Bevacizumab	1	7.23
N003	IDH WT	Group 1: Anti-LAG-3 Monotherapy	800mg	61		70	Gross Total Resection	Unmethylated	1	1.77			1	2.56
N004	IDH WT	Group 1: Anti-LAG-3 Monotherapy	800mg	52		80	Gross Total Resection	Methylated	1	2.96			1	9.89
N005	IDH WT	Group 1: Anti-LAG-3 Monotherapy	800mg	63		80	Subtotal Resection	Methylated	1	2.76			1	3.06
N006	IDH WT	Group 1: Anti-LAG-3 Monotherapy	800mg	55		90	Gross Total Resection	Methylated	1	2.53			1	15.67
N007	IDH WT	Group 3: Anti-LAG-3 + Anti-PD-1	160 + 240mg	37	Dexamethasone	90	Subtotal Resection	Methylated	1	12.71	Yes	Lomustine + Procarbazine	1	21.03
N008	IDH WT	Group 3: Anti-LAG-3 + Anti-PD-1	160 + 240mg	43		100	Gross Total Resection	Methylated	1	3.06			1	18.89
N009	IDH WT	Group 3: Anti-LAG-3 + Anti-PD-1	160 + 240mg	39		90	Gross Total Resection	Unmethylated	1	2.53	Yes	Avastin	1	6.80
N010	IDH WT	Group 3: Anti-LAG-3 + Anti-PD-1	160 + 240mg	56		90	Gross Total Resection	Unmethylated	1	8.41	Yes		1	26.51
N011	IDH WT	Group 3: Anti-LAG-3 + Anti-PD-1	160 + 240mg	68	Dexamethasone	70	Gross Total Resection	Unmethylated	0	1.22			1	16.72
N012	IDH WT	Group 3: Anti-LAG-3 + Anti-PD-1	160 + 240mg	50		100	Gross Total Resection	Methylated	0	0.69			1	16.62
N013	IDH MT	Group 3: Anti-LAG-3 + Anti-PD-1	160 + 240mg	30		100	Gross Total Resection	Unmethylated	1	2.86	Yes	Bemcentinib	1	16.33
A001	IDH WT	Part A: Anti-LAG-3 Monotherapy	240mg	70		80	Gross Total Resection	Methylated	1	1.84	Yes	Capecitabine + Bevacizumab	1	8.41
A002	IDH MT	Part A: Anti-LAG-3 Monotherapy	800mg	29		90	Gross Total Resection	Methylated	1	5.59	Yes	Lomustine	0	70.67
A003	IDH WT	Part A: Anti-LAG-3 Monotherapy	800mg	64		80	Gross Total Resection	Unmethylated	1	7.00			1	10.09
A004	IDH WT	Part A: Anti-LAG-3 Monotherapy	80mg	58		70	Gross Total Resection	Unmethylated	1	2.46	Yes	Avastin (12/2/16; Pembrolizumab 12/15/16)	1	7.75
A005	IDH WT	Part A: Anti-LAG-3 Monotherapy	80mg	59		80	Subtotal Resection	Methylated	1	1.91			0	2.89
A006	IDH WT	Part A: Anti-LAG-3 Monotherapy	800mg	62		90	Subtotal Resection	Methylated	1	2.14	Yes	Avastin / Lomustine	1	15.31
A007	IDH WT	Part A: Anti-LAG-3 Monotherapy	800mg	47		80	Gross Total Resection	Unmethylated	1	2.04	Yes	Avastin	1	7.10
A008	IDH WT	Part A: Anti-LAG-3 Monotherapy	800mg	77		80	Gross Total Resection	Unmethylated	0	0.89			1	2.04
A009	IDH WT	Part A: Anti-LAG-3 Monotherapy	80mg	56		80	Gross Total Resection	Methylated	1	2.07	Yes	Radiation + Temozolimide	1	8.71
A010	IDH WT	Part A: Anti-LAG-3 Monotherapy	240mg	57		90	Gross Total Resection	Unmethylated	1	3.68	Yes	Radiation And Temozolomide	1	12.52
A011	IDH WT	Part A: Anti-LAG-3 Monotherapy	800mg	54		90	Subtotal Resection	Methylated	1	2.79	Yes	Bevacizumab	1	20.07
A012	IDH WT	Part A: Anti-LAG-3 Monotherapy	800mg	55		70	Gross Total Resection	Methylated	1	1.38	Yes	Avastin	1	8.38
A013	IDH WT	Part A: Anti-LAG-3 Monotherapy	800mg	60		100	Gross Total Resection	Unmethylated	1	2.27	Yes	Avastin	1	4.30
A014	IDH WT	Part A: Anti-LAG-3 Monotherapy	800mg	66		90	Gross Total Resection	Unmethylated	1	1.84	Yes	Avastin	1	6.05
A015	IDH WT	Part A: Anti-LAG-3 Monotherapy	800mg	65		90	Gross Total Resection	Methylated	1	1.91	Yes	Avastin	1	4.37
A016	IDH WT	Part A: Anti-LAG-3 Monotherapy	240mg	39		90	Gross Total Resection	Unmethylated	1	3.19	Yes	Pembrolizumab + Reirradiation	1	15.97
A017	IDH WT	Part A: Anti-LAG-3 Monotherapy	800mg	59		90	Gross Total Resection	Unmethylated	1	7.46	Yes	Lomustine	1	22.24
B001	IDH WT	Part B: Anti-LAG-3 + Anti-PD-1	160 + 240mg	47		90	Gross Total Resection	Unmethylated	1	1.64	Yes	Radiation Therapy	1	7.85
B002	IDH WT	Part B: Anti-LAG-3 + Anti-PD-1	160 + 240mg	64		90	Subtotal Resection	Methylated	1	14.03	Yes	AMG-232	1	30.78
B003	IDH WT	Part B: Anti-LAG-3 + Anti-PD-1	80 + 240mg	45		80	Subtotal Resection	Unmethylated	1	2.00	Yes	Avastin	1	6.93
B004	IDH WT	Part B: Anti-LAG-3 + Anti-PD-1	160 + 240mg	52		90	Subtotal Resection	Indeterminate	1	3.91			1	5.45
B005	IDH WT	Part B: Anti-LAG-3 + Anti-PD-1	80 + 240mg	22		90	Gross Total Resection	Unmethylated	0	23.26	Yes	Nivolumab	0	73.59
B006	IDH WT	Part B: Anti-LAG-3 + Anti-PD-1	160 + 240mg	52		70	Gross Total Resection	Unmethylated	1	1.87	Yes	BCNU	1	46.19
B007	IDH MT	Part B: Anti-LAG-3 + Anti-PD-1	160 + 240mg	48		90	Gross Total Resection	Methylated	0	28.62			1	62.72
B008	IDH WT	Part B: Anti-LAG-3 + Anti-PD-1	160 + 240mg	67		90	Gross Total Resection	Unmethylated	1	1.15	Yes	Avastin	1	5.19
B009	IDH WT	Part B: Anti-LAG-3 + Anti-PD-1	160 + 240mg	55	Dexamethasone	80	Gross Total Resection	Unmethylated	1	1.87	Yes	Avastin	1	8.02
B010	IDH WT	Part B: Anti-LAG-3 + Anti-PD-1	160 + 240mg	49		100	Gross Total Resection	Methylated	1	1.94	Yes	Avastin	1	9.00
B011	IDH WT	Part B: Anti-LAG-3 + Anti-PD-1	80 + 240mg	67		90	Gross Total Resection	Unmethylated	1	2.83	Yes	Bevacizumab	1	10.15
B012	IDH WT	Part B: Anti-LAG-3 + Anti-PD-1	160 + 240mg	60	Dexamethasone	80	Gross Total Resection	Unmethylated	0	3.55			1	3.55
B013	IDH MT	Part B: Anti-LAG-3 + Anti-PD-1	160 + 240mg	22		90	Gross Total Resection	Methylated	1	2.17	Yes	Radiation Therapy	1	17.02
B014	IDH MT	Part B: Anti-LAG-3 + Anti-PD-1	160 + 240mg	51	Dexamethasone	70	Gross Total Resection	Unmethylated	1	2.69	Yes	Bevacizumab 10 Mg/kg Iv Every 2 Weeks	1	12.09
B015	IDH WT	Part B: Anti-LAG-3 + Anti-PD-1	160 + 240mg	45		90	Gross Total Resection	Unmethylated	1	1.64	Yes	Bevacizumab 10 Mg/kg Iv Every 2 Weeks	1	8.05
B016	IDH MT	Part B: Anti-LAG-3 + Anti-PD-1	160 + 240mg	64		90	Gross Total Resection	Unmethylated	1	1.61			1	2.73

Summary of individual patient-level data for adults with IDH-wildtype (IDH WT) or IDH-mutant (IDH MT) gliomas enrolled across study parts evaluating relatlimab monotherapy or relatlimab/nivolumab combination therapy. Shown are patient demographics (age), baseline performance status (KPS), history of steroid use, extent of surgery, and MGMT promoter methylation status, along with treatment assignment and dosing. Clinical outcomes include progression status (PD), progression-free survival (PFS, months), subsequent therapies administered after progression, and overall survival (OS, months), with censoring indicated where applicable. PD and death are coded as binary variables (1 = event, 0 = censored). **(KPS: Karnofsky Performance Status; MGMT: O6-methylguanine–DNA methyltransferase; PD: progressive disease; PFS: progression-free survival; OS: overall survival)**.

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No custom code was used
Data analysis	Statistical analysis done with SAS, version 9.4, Graphpad Prism 10, R version 4.4.3

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A de-identified dataset with information necessary to interpret, verify, and extend the research in the present study will be made available to the public upon request. This data could relate to immu ne correlative studies, clinical trial outcomes and demographics, and safety data. The contact for data request is Michael Lim. Requests will be honored within 4 weeks. No large database was required for accession codes.

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Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Demographics outlined in table 1 and are included in our clinical trial design document. In our study, we had 29 males (63%) and 17 females (37%) enrolled as defined by sex assignment at birth. This is assignment is similar to national statistics that show that the male-to-female incidence ratio is roughly 1.6:1 according to the 2011 study done by Dubrow and Darefsky that was published in BMC Cancer.
Reporting on race, ethnicity, or other socially relevant groupings	Demographics outlined in table 1 and are included in our clinical trial design document. In its original design, the study's demographics were limited to differentiating white (91%) and non-white patients. This is a limitation of the study and will be revised in the upcoming Alliance trial.
Population characteristics	Demographics outlined in table 1 and are included in our clinical trial design document. The pertinent population characteristics involved functional status (KPS), IDH status (which is the greatest prognostic indicator for gliomas), MGMT status (another prognostic indicator), and whether patients had steroids or anticonvulsants as part of their recurrence treatment.
Recruitment	Demographics outlined in table 1 and are included in our clinical trial design document with a full list of inclusion and exclusion criteria.
Ethics oversight	Demographics outlined in table 1 and are included in our clinical trial design document. This study and trial protocol were reviewed by Central Institutional Review, Cancer Therapy Evaluation Program, and Brain Malignancy Steering Committee.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	65; this was determined through preliminary statistical analysis for determining a maximum tolerated dosage.
Data exclusions	Two patients were excluded because they did not receive treatment
Replication	Immune correlative experiments were replicated in triplicate unless otherwise specified.
Randomization	Patients were sequentially allocated across anti-CD137, anti-LAG-3, and combination anti-LAG-3 and anti-PD-1 groups for both the adjuvant and neoadjuvant setting
Blinding	Immune correlative investigators were blinded to patient demographics.

Reporting for specific materials, systems and methods

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Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
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☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☐	☒ MRI-based neuroimaging

Antibodies

Antibodies used	anti-LAG-3 antibody (Relatlimab BMS-986016), anti-PD-1 antibody (Nivolumab, BMS-936558)
Validation	These antibodies were provided by BMS in the dosages described in the manuscript as pharmaceutical grade therapeutic monoclonal antibodies (anti-LAG-3: 20mg, 80mg, 240mg, 800mg; anti-PD-1: 240mg)

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	NCT02658981
Study protocol	Attached as a supplemental document
Data collection	The data were collected by the central operation office of the Adult Brain Tumor Consortium (ABTC) and is located at the Johns Hopkins Medical Institute (JHMI), USA. This trial is registered under NCT02658981 (https://clinicaltrials.gov/study/NCT02658981) with pre-registration date 2016-08-24. Primary completion date was 2022-04-30. The study protocol and all related procedures were reviewed and accepted by the Cancer Therapy Evaluation Program (CTEP) of the Division of Cancer Treatment and Diagnosis (DCTD), National Cancer Institute (NCI). It was approved by the Johns Hopkins Office of Human Subjects Research, Institutional Review Board (IRB), specifically IRB-2 with Committee Chair David Cornblath. All regulatory documents were supported by the Cancer Trials Support Unit (CTSU), a service of the NCI.
Outcomes	We defined our primary outcomes as the maximum tolerated dosage for anti-LAG-3 monotherapy and in combination with anti-PD-1. Secondary outcomes were exploratory and preliminary efficacy studies that were not statistically powered for clinical validation.

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Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*

Magnetic resonance imaging

Experimental design

Design type	Interval MRI surveillance for patient tumor recurrence
Design specifications	Baseline images with subsequent 1-2 month follow-up and 1-6 months surveillance scans thereafter
Behavioral performance measures	No functional studies

Acquisition

Imaging type(s)	T1 sequences with and without contrast, T2 flair sequences
Field strength	3T
Sequence & imaging parameters	T1 with and without contrast 1mm slice thickness, T2 flair 2mm slice thickness
Area of acquisition	Whole brain

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software	n/a

Normalization: n/a

Normalization template: n/a

Noise and artifact removal: n/a

Volume censoring: n/a

Statistical modeling & inference

Model type and settings: n/a

Effect(s) tested: n/a

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016): *Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.*

Correction: *Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).*

Models & analysis

n/a	Involved in the study
☒	☐ Functional and/or effective connectivity
☐	☒ Graph analysis
☒	☐ Multivariate modeling or predictive analysis

Graph analysis: Kaplan-Meier, column, and swimmer plots were used to represent and analyze clinical data.