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Clinical Trial Results

Hypofractionated Stereotactic Re-irradiation and Anti-PDL1 Durvalumab Combination in Recurrent Glioblastoma: STERIMGLI Phase I Results

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Abstract

Background: Hypofractionated stereotactic radiotherapy (hFSRT) is a salvage option for recurrent glioblastoma (GB) which may synergize anti-PDL1 treatment. This phase I study evaluated the safety and the recommended phase II dose of anti-PDL1 durvalumab combined with hFSRT in patients with recurrent GB.

Methods: Patients were treated with 24 Gy, 8 Gy per fraction on days 1, 3, and 5 combined with the first 1500 mg Durvalumab dose on day 5, followed by infusions q4weeks until progression or for a maximum of 12 months. A standard 3 + 3 Durvalumab dose de-escalation design was used. Longitudinal lymphocytes count, cytokines analyses on plasma samples, and magnetic resonance imaging (MRI) were collected.

Results: Six patients were included. One dose limiting toxicity, an immune-related grade 3 vestibular neuritis related to Durvalumab, was reported. Median progression-free interval (PFI) and overall survival (OS) were 2.3 and 16.7 months, respectively. Multi-modal deep learning-based analysis including MRI, cytokines, and lymphocytes/neutrophil ratio isolated the patients presenting pseudoprogression, the longest PFI and those with the longest OS, but statistical significance cannot be established considering phase I data only.

Conclusion: Combination of hFSRT and Durvalumab in recurrent GB was well tolerated in this phase I study. These encouraging results led to an ongoing randomized phase II. (ClinicalTrials.gov Identifier: NCT02866747).

Key words: recurrent glioblastoma; hypofractionated stereotactic re-irradiation; Durvalumab; deep learning; phase I clinical trial.

Lessons Learned

- The combination of hypofractionated stereotactic re-irradiation with Durvalumab for patients with recurrent gliobalstoma shows good tolerance with encouraging clinical response.
- Patients with the best responses were identified by multimodal deep learning-based analysis.
- These promising results led to the development of a translational randomized phase II trial that is currently recruiting with overall survival as primary objective.

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Discussion

Glioblastoma (GB) is the most common and aggressive primary brain tumor in adults with very few effective treatment options at the systematic relapse. Temozolomide, lomustine, and/or bevacizumab have marginal efficacy, with 6-month progression-free survival (PFS) ranging from 18% to 40%.[1] Re-irradiation is a salvage option. Hypofractionated stereotactic radiotherapy (hFSRT) has proven to be safe with schedules from 36 Gy in 5 fractions,[1,2] to a single-dose radiosurgery (SRS) of 15-20 Gy[3,4] resulting in PFS ranging from 3.4 to 5 months with RT alone and more recently to 7 months when associated with bevacizumab.[5] In addition to its direct cell death effects, radiotherapy can also cause immune-mediated tumor cell death,[6] the 3 × 8 Gy hFSRT being the optimal dose for inducing immune-mediated cell death and an abscopal effect.[7,8] However, radiotherapy can also induce immunosuppressive effects via upregulation of PD-L1 on tumor cells and of PD-1 on CD8+ tumor infiltrating lymphocytes.[9] Combining an immune checkpoint inhibitor (ICI) with hFSRT may improve salvage treatment efficacy. We designed and conducted the STERIMGLI phase I/II clinical trial to study the combination of 3 × 8 Gy hFSRT (days 1, 2, and 5) in combination with the anti-PDL1 Durvalumab and then monthly until relapse or up to 12 months. We report here the results of the phase I trial and the results of an independent deep learning analysis from pooled biological and MRI data.

Six patients were enrolled in the study at dose level 1. During the dose limiting toxicity (DLT) period, all patients received the 3 fractions of hFSRT as scheduled. All patients were assessed for DLT and were followed until death. Three patients received 4 cycles of Durvalumab; the 3 remaining patients received 3, 6, and 7 cycles, respectively. Five patients stopped immunotherapy due to disease progression, and one patient discontinued after 3 cycles due to toxicity.

During the DLT period, all patients but one experienced at least one adverse event (AE). One patient presented an immune-related AE as DLT corresponding to a grade 3 vestibular neuritis related to Durvalumab from which he recovered without sequelae. During the DLT period, no other severe AEs occurred.

As shown in Fig. 1A, 1 patient had a partial response (PR), 2 patients had stable disease (SD) after a pseudoprogression on the first MRI, and 3 patients had a progression disease (PD) on the first MRI. Interestingly, the 2 patients, who presented a pseudoprogression and the longest PFI, had a normal initial lymphocyte counts which remained stable during follow-up (Fig. 1B). The patient who presented the DLT was the patient with the longest PFI and who presented an abscopal effect.

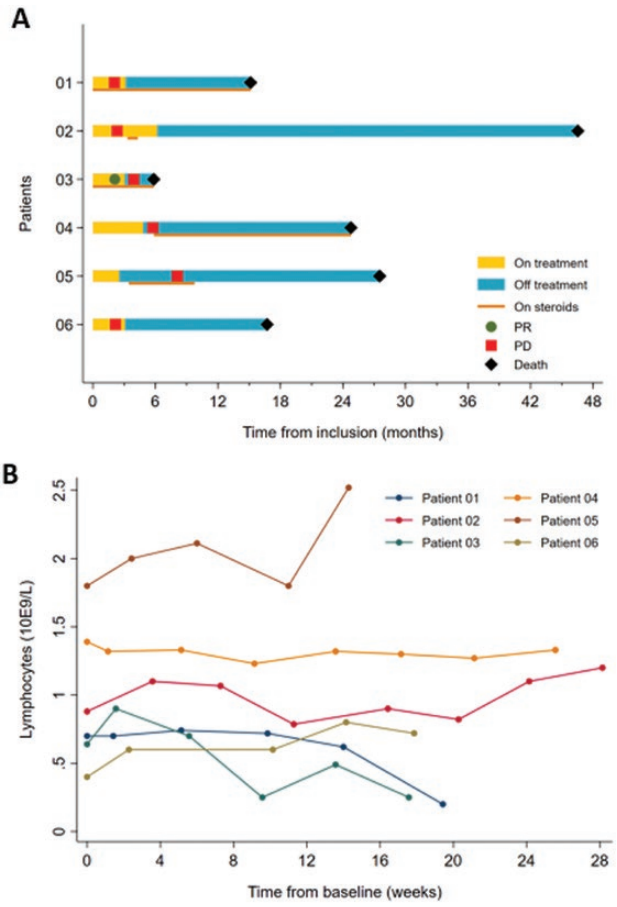

Figure 1. (**A**) Swimmer plot describing the follow-up of each patient. Duration of treatment (hFSRT and Durvalumab infusion), duration of steroid treatment, and clinical outcome (PR: partial response; PD: progression disease). (B) Lymphocytes count follow-up for each patient.

Trial information	
Disease	Glioblastoma
Stage of disease/treatment	Recurrent
Prior therapy	Standard radiochemotherapy Stupp protocol
Type of study	Phase I, 3 + 3
Primary endpoint	Toxicity, safety
Secondary endpoints	Recommended dose of Durvalumab in combination with Hypofractionated stereotactic re-irradiation for the phase II trial, exploratory efficacy analysis, best objective response, ancillary biologic studies, multi-modal deep learning-based analysis
Investigator's analysis	Active and should be pursued further

Additional Details of Endpoints or Study Design

Study Design and Participants

Prospective single-arm, open-label phase I study was conducted in two French Comprehensive Cancer Centers with a "3 + 3" Durvalumab dose de-escalation design. This study was registered at clinicaltrials.gov (NCT02866747). Appropriate approvals were obtained from the relevant ethics committees and the French Competent Authority. All patients participating in the study gave their written informed consent. Durvalumab was provided by AstraZeneca.

The first Durvalumab infusion was a 1500 mg flat dose IV at level 1. In case of de-escalation according to DLTs rules, a first 750 mg flat dose IV (level -1), Durvalumab infusion was envisaged. Independently of the level, 1500 mg Durvalumab infusions were subsequently administered every 4 weeks (Q4W).

The inclusion criteria were (i) a recurrent nodule of ≤35 mm as evaluated from the T1 weighted postgadolinium (Gd-T1) MRI of a histologically confirmed GB, (ii) a recurrence occurring within or external to the previous irradiation field, (iii) age ≥18 years, (iv) Karnofsky performance status ≥70%, (v) any line of treatment for recurrence defined by the modified Radiologic Assessment in Neuro-Oncology (RANO) criteria,[10] and (vi) any patient for whom a hFSRT re-irradiation was considered a suitable treatment option. Patients must have received a prior treatment with at least a standard RT dose (conventionally 60 Gy) and temozolomide; prior RT must have ended at least 12 weeks before the hFSRT. In case of a prior anti-VEGF/VEGFR-targeted therapy, the interval between the last dose of antiangiogenic therapy and the first fraction of hFSRT had to be ≥28 days. Adequate bone marrow, pulmonary, kidney, and liver function were required. The exclusion criteria were (i) a multifocal recurrence, (ii) a distance between tumor and brainstem or optic ways including chiasma <1 cm, (iii) prior re-irradiation, (iv) prior exposure to ICI, (v) current or prior use of immunosuppressive medication within 28 days before inclusion into the study (with the exception of systemic corticosteroids, ie, prednisone or equivalent, at doses ≤10 mg/day), (vi) suspected active or previously diagnosed autoimmune disease, and (vii) diffuse leptomeningeal disease or extracranial disease.

Treatments

The study treatment (hFSRT + Durvalumab) started with three days of hFSRT delivered on days 1, 3, and 5 (Fig. 2). The first dose of Durvalumab was administered on week 0 (W0) D5, the same day as the last hFSRT fraction and continued on a Q4W schedule for a maximum of 12 months (last infusion, week 50). Study treatment was discontinued prior to 12 months upon (i) diagnosis of progressive disease (PD), (ii) initiation of alternative cancer therapy, (iii) unacceptable toxicity, (iv) withdrawal of consent, or (v) for any other reason to discontinue the study treatment. Dose reductions were not permitted. Patients who missed one dose due to toxicity could resume treatment and complete the 12-month treatment period.

Radiotherapy Technique

The treatment preparation required a CT-Scan of 1-mm slice thickness, head mask fixation, and registration with MRI Gd-T1 images (with high resolution of 1 mm^3). Organs at risk (OAR) were the brain, eyes, lens, optic nerves, optic chiasm, pituitary gland, brainstem, cochlea, and cervical cord.[11] Gross tumor volume (GTV) was defined as contrast enhancing tumor recurrence on Gd-T1. Margins of 1-2 mm around the GTV defined the planning target volume (PTV). hFSRT delivered 24 Gy, 8 Gy per fraction prescribed to the 60% to 90% isodose line (preferentially 80%) on the PTV, in 3 fractions on days 1, 3, and 5.

Patient Evaluations

Baseline evaluations were performed within 28 days of the start of treatment. Patients were assessed for AEs and toxicity on a monthly basis after hFSRT and from W0. Imaging follow-up with MRI was performed before hFSRT (W0) then every 2 months until local progression, patient withdrawal from the study or lost to sight. In case of regional progression (outside of the irradiated field), MRI follow-up continued until local progression.

Safety and Efficacy Assessments

AEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 (CTC-AE V4.03). Dose-limiting toxicities (DLTs) were evaluated from the first administration of Durvalumab until 1 month after the last hFSRT fraction. A DLT was defined as any grade 3 or higher toxicity that occurred during the DLT period. Toxicity causally related to the GB or another etiology was excluded from this definition. The following were considered drug-related DLTs: any grade 4 immune-related AE (irAE), any ≥grade 3 colitis, any grade 3 or 4 non-infectious pneumonitis irrespective of duration, any grade 2 pneumonitis that did not resolve to ≤grade 1 within 3days of the initiation of maximal supportive care, any grade 3 irAE—excluding colitis or pneumonitis that did not downgrade to grade 2 within 3 days after onset of the event despite optimal medical management including systemic corticosteroids or

did not downgrade to ≤grade 1 or baseline within 14 days, elevated liver transaminase of >8 × ULN or total bilirubin of >5 × ULN, any ≥grade 3 non-irAE, grade 3 thrombocytopenia lasting >5 days, grade 3 neutropenia >7 days, grade 4 neutropenia or thrombocytopenia of any duration, and febrile neutropenia.

Grade 3 headache associated with grade 3 nausea for >7 days despite corticosteroid treatment, grade 4 seizure despite antiepileptic treatment, grade 3 confusion lasting >2 days despite corticoids treatment, worsening of neurological status despite best supportive care for >7 days were considered DLTs due to radiosensitization by Durvalumab. AEs and DLTs were assessed as either being related or not related to the study treatment by a study investigator.

Tumor response was defined according to the iRANO criteria: transient appearance of new enhancing lesions at either local or distant sites that might occur in patients with neuro-oncological malignancies receiving immunotherapy. These pseudoprogression radiographic findings typically manifest within 6 six months of starting immunotherapy and may arise more frequently with hFSRT. During the 6-month period after starting Durvalumab, patients with early progressive MR imaging, including new lesions but without substantial clinical decline, continued on Durvalumab until further radiographic confirmation of progression. If progression was confirmed by subsequent imaging, the date of progression corresponded to the date of the last MRI before the progression was detected. Because hFSRT associated with Durvalumab may increase the risk of cerebral edema and pseudoprogression, increased doses of corticosteroids were permitted without considering the patient as having a progression. An independent expert performed a retrospective centralized neuroradiological analysis for all patients.

Tumor Sample Analyses

The immunohistochemistry for PD-L1 expression (total percent of positive tumor cells and positive infiltrating immune cells) was centralized and performed on representative formalin fixed paraffin embedded (FFPE) tissue sections from archived primary GB tumor tissue. PD-L1 staining was performed on the Ventana BenchMark Ultra using the Ventana PD-L1 (SP263) clone and validated assay. The O6-methylguanine DNA methyltransferase (MGMT) promoter methylation status and the isocitrate dehydrogenase (IDH) mutation status were known for all patients.

Blood Sample Analyses

Blood samples for the cytokine analysis were collected at the screening phase, during the study treatment, and at the time of progression. All blood samples were collected in EDTA coated tubes with plasma immediately prepared by centrifugation at 1500*g* for 10 minutes, and then aliquoted and stored at −80 °C until transferred for analysis.

Analysis of Cytokines From Patient Plasma

Cytokines and chemokines present in patient plasma (350 µL plasma samples per membrane) throughout the treatment were analyzed using the Human XL Proteome Profiler Array (R&D Systems, Minneapolis, MN, USA) that detects 105 human cytokines simultaneously.

Magnetic Resonance Imaging Acquisition

Longitudinal MR images for the patients' follow-up were acquired on a Siemens Magnetom Aera 1.5T. Among the MRI modalities, T1-weighted MR images after Gadolium-based contrast agent (Gd-T1) and T2-weighted FLuid Attenuated Inversion Recovery (FLAIR) MR images were considered for the Deep Learning analysis.

Deep Learning Data Preparation and Modeling

A convolutional neural network was trained to extract relevant feature vectors from 2D slices of FLAIR and Gd-T1 MRI scans (Fig. 3A). Considering the limited amount of available training data in this phase I trial, the algorithm was first trained on the publicly available MICCAI dataset and fine-tuned on phase I data and on available data from the ongoing phase II (19 patients in total). Contrastive unsupervised training was conducted using a bimodal autoencoder and a triplet loss. Positive pairs were composed of a patient's FLAIR and Gd-T1 at a given date. Negative pairs were composed of a patient's FLAIR at a given date and either another patient's FLAIR or the same patient's FLAIR at a different date. Training was performed over a total of 2400 samples (32 × 32 pixel patches). The goal of this training scheme was to capture each scan's particularities at any given date, regardless of the patient's outcome. The MRI feature extraction training procedure is summarized in Fig. 3B.

The 22 cytokines, measured in patient plasma, were also considered as an additional modality at each visit. Principal component analysis was applied to the normalized data in order to eliminate cross-correlation and reduce dimensionality. The neutrophil/lymphocyte ratio was added as another modality.

Outcomes and Statistical Analysis

The primary objective was to evaluate the safety and tolerability of the combination of hFSRT and Durvalumab and to determine the recommended phase II dose of Durvalumab. The studied treatment was defined as safe if a maximum of one patient (ie, 0 or 1) out of 6 presented with a DLT. Exploratory efficacy analyses consisted of assessing the tumor response, intracranial progression free interval (PFI) both distant (outside of the re-irradiated volume) and local intracranial progression, and the overall survival (OS). PFI and OS were defined as the time from inclusion to local and/or distant progression and death from any cause, respectively. Survival data were assessed with the Kaplan-Meier method.

Drug information	
Generic/working name	Durvalumab
Company name	AstraZeneca
Drug type	Anti-PDL1
Drug class	Human immunoglobulin G1 kappa (IgG1κ) monoclonal antibody
Dose	1500 mg flat dose
Unit	mg
Route	IV
Schedule of administration	monthly

Dose de-escalation table			
Dose level	**Dose of drug: Durvalumab during radiotherapy, mg**	**Number enrolled**	**Number evaluated for toxicity**
1	1500	6	6
−1	750	0	0

Patient characteristics	
Histology	Glioblastoma
Number of patients, male	3
Number of patients, female	3
Stage	IV
Age: median (range)	65.5 [48.0-72.0]
Number of prior systemic therapies: median (range)	**1 (1-2)**
Karnofsky performance status	
100%	2 (33.3)
80%	2 (33.3)
70%	**2 (33.3)**
Steroid use at day 1 of hFSRT	
No	4 (66.7)
Yes	2 (33.3)
MGMT promoter methylation status	
Methylated	3 (50)
Unmethylated	3 (50)
IDH mutation status	
Wildtype	6 (100)
PD-L1 tumor expression levels	
<1%	3 (50)
≥1%	3 (50)
≥10%	3 (50)
≥50%	1 (16.7)
PD-L1 stroma infiltration expression levels	
<1%	3 (50)
≥1%	3 (50)
Glioblastoma location	
Left hemisphere	2 (33.3)
Right hemisphere	4 (66.7)
Location of primary tumor	
Temporal	3 (50)
Parietal	1 (16.7)
Frontoparietal	1 (16.7)
Frontal	1 (16.7)
Previous treatments	
Surgery	6 (100)
Complete resection	3 (50)
Subtotal resection	3 (50)
Radiotherapy	6 (100)
Chemotherapy	6 (100)
Trageted therapy (Pazopanib)	1 (16.7)
Lymphocytes count Durvalumab start day	
Low	4 (66.7)
Normal	2 (33.3)
Tumor volume (cm^3)	
Median (range)	7.15 (46.7-0.3)

Primary assessment method	
Title	**Safety**
Number of patients screened	6
Number of patients enrolled	6
Number of patients evaluable for toxicity	6
Evaluation method	National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 (CTC-AE V4.03)

Adverse events	
Any grade adverse events during DLT period	***N*** **(%)**
Patients with adverse events (any grade)	4 (66.7%)
Nervous system disorders	2 (33.3%)
Grade 3 vestibular neuritis	1 (16.7%)
Grade 2 seizure	1 (16.7%)
Grade 1 ideomotor slowing	1 (16.7%)
Cardiac disorders	1 (16.7%)
Grade 1 bradycardia	1 (16.7%)
Grade 1 other AEs	
Dizziness	1 (16.7%)
Vomiting	1 (16.7%)
Oral mucositis	1 (16.7%)
Fatigue	1 (16.7%)
Infection (bronchitis)	1 (16.7%)
Muscle weakness	1 (16.7%)
Cough	1 (16.7%)
Weight loss	1 (16.7%)
Neutropenia	1 (16.7%)

Any grade of treatment-related adverse events (TRAE) during the entire treatment period: Patients with at least one TRAE (any grade) 6 (100%)			
Type of adverse events	**Grade 1**	**Grade 2**	**Grade 3**
Vestibular neuritis	—	—	1
Cerebellar syndrome	—	—	1
Seizure	—	1	—
Visual field amputation	—	1	—
Intracranial hypertension	—	—	1
Acute anterior ischemic optic neuritis	—	1	—
Nystagmus	—	1	—
Eye papillitis	—	1	—
Fatigue	2	1	1
Lung tuberculosis	—	—	1
Aseptic lymphocytic meningitis	—	—	1
Muscular pain	1	—	—
Erectile dysfunction	—	1	—

Adverse events in the 6 patients			
Patients with at least one adverse event (any grade)	**6 (100%)**		
Type of adverse events	**Grade 1**	**Grade 2**	**Grade 3**
Fatigue	2	2	1
Gait disturbance	—	2	—
Muscle weakness	2	—	—
Neck pain	—	1	—
Dysarthria	1	1	—
Intracranial hypertension	—	—	1
Cerebellar neuritis	—	—	1

Adverse events in the 6 patients			
Patients with at least one adverse event (any grade)	6 (100%)		
Type of adverse events	**Grade 1**	**Grade 2**	**Grade 3**
Headache	1	—	—
Nystagmus	—	1	—
Seizure	—	2	—
Tinnitus	—	1	—
Vertigo	—	1	—
Vestibular syndrome	—	1	—
Vestibular neuritis	—	—	1
Acute anterior ischemic optic neuritis	—	1	—
Abdominal pain	1	—	—
Constipation	—	1	—
Dry mouth	1	—	—
Nausea	1	—	—
Vomiting	1	—	—
Weight loss	1	—	—
Bronchial infection	—	3	—
Cough	2	—	—
Tuberculosis	—	—	1
Mucositis	1	—	—
Hypertension	—	—	1
Hypotension	1	—	—
Ventricular tachycardia	1	—	—
Maculo-papular rash	1	—	—
Erectile dysfunction	—	1	—
Acute renal injury	—	—	1
Hypoalbuminemia	—	1	—
Creatinine increase	—	—	1
Lymphocyte count decrease	1	—	—
Neutrophil count decrease	1	—	—

Secondary assessment method	
Title	**Response**
Number of patients screened	6
Number of patients enrolled	6
Number of patients evaluable for toxicity	6
Number of patients evaluated for efficacy	6
Evaluation method	iRANO
Response assessment, CR	0 (0%)
Response assessment, PR	1 (16.7%)
Response assessment, SD	2 (33.3%)
Response assessment, PD	3 (50%)
Median duration assessment, PFI	2.33 months (95% CI, 2.07-NR)
Median duration assessment, TTP	2.33 months (95% CI, 2.07-NR)
Median duration assessment, OS	16.72 months (95% CI, 5.85-NR)
Mediandurationof treatment	2.9 months (range = 2.5-7 months)

Outcome Notes

Results

Patient Characteristics and Treatment

Between January and October 2017, 6 patients were enrolled into the study at dose level 1. Baseline clinical and pathological patient characteristics and previous treatments are detailed in the table "Patients characteristics."

Safety

Safety Data During the DLT Period

During the DLT period, all patients but one experienced at least one AE. One patient (P#05) presented an irAE as

DLT corresponding to a grade 3 vestibular neuritis related to Durvalumab from which he recovered without sequelae. Auditory nerve and cochlea only received 4 Gy. During the DLT period, no other severe AEs occurred. The most common AEs observed during this period are reported in the table "Any grade AEs during DLT period." Grade 2 seizure related to hFSRT was observed in one patient; grade 1 weight loss related to Durvalumab occurred in one patient. Grade 1 fatigue related to Durvalumab and/or hFSRT was reported in one patient.

Safety Data for the Whole Treatment Period

The most common treatment-related AE (TRAE) was fatigue (Table "Any grade of treatment-related adverse events (TRAE) during the entire treatment period." Grade 3 toxicities were reported in 2 patients: one patient presented intracranial hypertension related to hFSRT, and the second (P#05) presented with vestibular neuritis as a DLT and with fatigue, aseptic lymphocytic meningitis, tuberculosis, and cerebellar syndrome due to Durvalumab after the DLT period. This latter patient discontinued Durvalumab due to these neurological TRAEs and was treated with steroids. The same patient also presented with a grade 2 visual field amputation considered to be an irAE. No grade 4/5 TRAEs were reported. All AEs are reported in the table "Adverse events in the 6 patients."

Exploratory Efficacy Analysis

All patient responses were evaluated according to the RANO criteria: as best objective response, 1 patient (P#03) (16.7%) had a PR, 2 patients (P#04 and P#05) (33.3%) had SD after a pseudoprogression on the first MRI, and 3 patients (P#01, P#02, P#06) (50%) had a PD on the first MRI. Interestingly, the two patients who presented a pseudoprogression (P#4 and P#5) and the longest PFI had a normal initial lymphocyte counts which remained stable during follow-up. Moreover, P#05 achieved a complete response on the two distant punctiform non-irradiated lesions, initially considered as non-significant, 4 months after interruption of Durvalumab for toxicity. All patients had a local progression of the targeted lesion. At the time of analysis, all patients had died. The longest OS was 46.6 months. Median PFI and OS were 2.3 and 16.7 months, respectively. Table 1 summarizes pathological, biological, and outcome characteristics for each individual patient.

Cytokine Analysis

Analysis of cytokine expression revealed that 3 cytokines, PDGF-AA (platelet-derived growth factor-AA), PDGF-BB, and CCL17 (C-C Motif Chemokine Ligand 17) were differentially expressed in P#04 and P#05 throughout the treatment. In P#04, PDFG-AA and PDGF-BB expression increased 2- and 1.5-fold, respectively, after RT treatment, compared to their level before treatment and then decreased after one month of Durvalumab. P#05 had a stronger increase of PDGF-AA, PDGF-BB, and CCL17 expression after RT that diminished after the first administration of Durvalumab and even more so after the second infusion. Unfortunately, a progression plasma sample was not obtained for this patient. This cytokine profile was observed in P#04 and P#05 who achieved the longest PFIs. For P#01 who had a shorter PFI, a different expression profile for PDGF-AA, PDGF-BB, and CCL17 was observed with decreased expression after RT.

Deep Learning-Based Analysis

Using the trained encoder for FLAIR MRI, the FLAIR scans for each of the six patients were encoded, and the corresponding feature vectors obtained. These were concatenated with the reduced cytokine profiles and the neutrophil/lymphocyte ratio. A k-means clustering algorithm was subsequently applied. On the 2D UMAP (Uniform Manifold Approximation and Projection) representations, a 2-cluster setting showed that P#05 was very different from the rest of the patients. Progressively increasing the number of clusters led to the separation of P#01 and P#03 from the rest of the cohort (Fig. 4). These patients incidentally also had the shortest PFIs. Finally, in a six-cluster configuration, P#04 (second visit) and P#05 (second and third visits) started separating, as depicted in Fig. 5B. P#04 and P#05 presented with a pseudoprogression and had the longest PFIs. When considering feature vectors encoded from FLAIR MRIs only, a 3-, 4-, or 5-cluster configuration puts P#02 and P#05 in the same cluster (Fig. 5A). These patients had the longest OS. The statistical significance of these results cannot be established with only six patients, but the same methodology can be applied on future phase II data and, therefore, a larger cohort.

Assessment, Analysis, and Discussion	
Completion	Study completed
Investigator's assessment	Active and should be pursued further

Salvage treatment for recurrent GB is a challenging issue. Re-irradiation is an option which efficacy needs to be improved. If immunotherapy has failed to improve OS of patients in first line treatment, the hFSRT scheme (3 × 8 Gy) used in our trial is known to induce immune cell death,[7,8] but also immunosuppressive effects via several mechanisms including upregulation of PD-L1 levels on tumor cells and of PD-1 on CD8+ tumor infiltrating lymphocytes,[9] suggesting the importance to combine such hFSRT with anti-PD-L1 treatment in re-irradiation situations as a new option for recurrent GB.

We report here the good tolerance of the combination of hypofractionated stereotactic re-irradiation and of the anti PDL1 Durvalumab in patients with recurrent GB already treated with radiochemotherapy Stupp protocol. If such combination have been performed in brain metastases with good tolerance, our study addresses the question of the tolerance of this combined treatment on an already irradiated brain area in patients with recurrent GB. In the current study, Durvalumab was discontinued due to disease progression in five patients and due to neurological toxicity in one patient. The combination of 3 × 8 Gy fractions of hFSRT with 1500 mg Durvalumab was well tolerated. The toxicity of hFSRT was not increased by Durvalumab. The only DLT observed was related to Durvalumab. To the best of our knowledge, Sahebjam et al[12] is the only published trial to date that evaluated immunotherapy combined with hFSRT in recurrent high-grade glioma, while the 2 other studies studying

Nivolumab in combination with normo-fractionated radiotherapy were performed in newly diagnosed GB.[13,14] However, in Sahebjam et al, patients received bevacizumab in addition to pembrolizumab and hFSRT, and grade 3 gliomas were also included, which makes the tolerance and efficacy results of this trial difficult to compare with our study. In the 32 patients treated with the triple treatment, TRAEs were related to bevacizumab or pembrolizumab. Seizure and intracranial hypertension appeared to be less frequent than in our study. Bevacizumab may decrease the risk of edema induced by the double treatment and may explain this difference in tolerance profile. However, patient numbers are lower in our phase I study making it impossible to draw any definitive conclusions.

Despite our small sample size and due to the good tolerance of the studied treatment in this phase I, efficacy results seem to be encouraging and of interest considering the median OS (mOS) of 16.7 months in comparison to the 8 to 10 months mOS mostly reported in the same population. Disease was initially controlled in 3 patients: one had PR and 2 had SD. The first MRI of the 2 patients with SD (P#04 and P#05) detected pseudoprogression. Their PFIs of 5.8 and 8.1 months were the longest of the study. Pseudoprogression is often observed with both RT and immunotherapy in patients treated for GB.[15] Observing pseudoprogression in patients receiving both treatments is not surprising: we assume their responses and outcomes to be correlated with pseudoprogressions. Moreover, 2 non-irradiated lesions in P#05 showed a complete response, which could be explained by the efficacy of Durvalumab or an abscopal effect. The hypofractionated 3 × 8 Gy regimen is known to induce such an effect.[7]

PD-L1 expression, lymphocyte count, neutrophil to lymphocyte ratio (NLR), and microbiome have been evaluated as predictive factors for efficacy in patients receiving immunotherapy.[16] Here, we explored the plasma expression of 105 cytokines before and during combined treatment. Compared to the other patients, P#04 and P#05 presented a similar cytokine expression profile. Preliminary results from a phase I study do not allow explaining this type of profile, but T cell and macrophage activation has recently been reported in the context of a radiation-induced abscopal response enhanced by anti-PDL1 immunotherapy.[17] Moreover, we observed a decrease in the expression of CCL17, a cytokine which inhibits cytotoxic T lymphocyte survival and recruits Tregs. It is interesting to note that these 2 patients did not have lymphopenia before combined treatment was initiated, contrary to the 4 other patients. This biomarker has been previously reported as a predictive factor for response to ICI,[18] particularly to predict abscopal response after ICI and RT.[19] Although, a single biomarker is unlikely to be able to predict response or survival, multiple biomarkers may provide a better assessment of outcome and identify patients who are most likely to have good responses. These observations, limited to a small size of patients, lack of statistical power, but will be further studied in all the patients of the phase II trial.

We conducted artificial intelligence (AI)-based ancillary studies to identify additional parameters potentially associated with treatment responses.

The unsupervised deep learning-based analysis was able to identify P#04 and P#05, who had the longest PFIs, as part of the same cluster. When considering feature vectors encoded from FLAIR only, a 3-, 4-, or 5-cluster configuration places patients P#02 and P#05, who incidentally have the longest OS, in the same cluster. AI could be used to predict which patients would benefit the most from immunotherapy alone or from immunotherapy combined with hFSRT. In recent years, analysis with AI has opened up new perspectives for diagnosis, prediction of molecular markers, and evaluating treatment responses,[20] which have also been extended to gliomas.[21] Developing a multi-omics-based unified deep learning model to integrate multidimensional information including serial MRIs, laboratory data, and baseline clinical information may be a helpful approach. To the best of our knowledge, it is the first multimodal analysis combining imaging and biologic data in this context and constitutes a novel approach. Unsupervised contrastive learning was used to obtain numerically comparable patients (metric learning), regardless of outcome. Clustering applied to the 6 patients cohort showed groups that correspond to treatment response, thus isolating patients with the best response to treatment. The predictive potential of multimodal data (MRI/cytokine level/NLR) for PFI could be validated using the data of 100 patients included in the phase II trial randomizing re-irradiation alone versus re-irradiation and Durvalumab combination, as more patients are required to statistically validate the results of this analysis.

In this phase I study, hFSRT combined with a synchronous and then a monthly administration of Durvalumab as a re-irradiation salvage treatment for recurrent GB was well tolerated and will allow us to assess the efficacy of this treatment in the context of the phase II part of the trial. We observed encouraging efficacy results with deep learning-based models identifying patients with the longest PFI. The deep-learning model also identified patients with the longest OS. The randomized phase II evaluating OS of the combination of hFSRT and Durvalumab versus hFSRT alone is currently underway, with 100 patients expected to be randomized.

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Conflict of Interest

Damien Pouessel reported travel fees from AstraZeneca, Ipsen, scientific advisory board member for Pfizer, Merck, MSD, and Astellas, and honoraria from Pfizer, Merck, MSD, Janssen, and BMS. Marie Robert reported travel fees from Merck and Novartis and scientific advisory board member for Novartis and Eisai. Jean-Sebastien Frenel reported honoraria from Roche, AstraZeneca, Novartis, Daiichi, Lilly, Pfizer, Clovis, GSK, MSD, Gilead, Seagen, and Amgen. Elizabeth Cohen-Jonathan Moyal reported research funding from AstraZeneca, Novocure, Bayer, and Incyte, and scientific advisory board member and intellectual property with Novocure. The other authors indicated no financial relationships.

Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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Figures and Tables

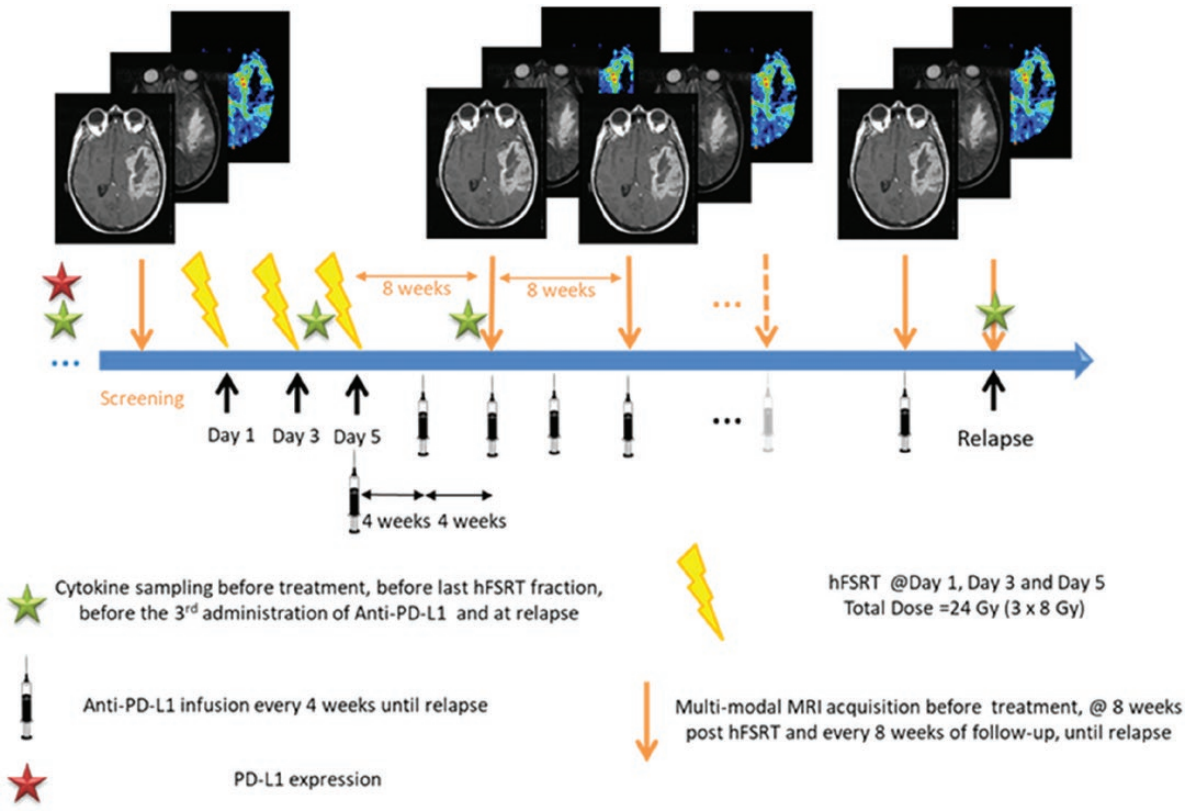

Figure 2. Study design. Patients began Durvalumab on day 5 of the hypofractionated stereotactic radiotherapy and then monthly for up to 12 months. hFSRT: hypofractionated stereotactic radiotherapy; PD-L1: programmed death ligand-1; MRI: magnetic resonance imaging.

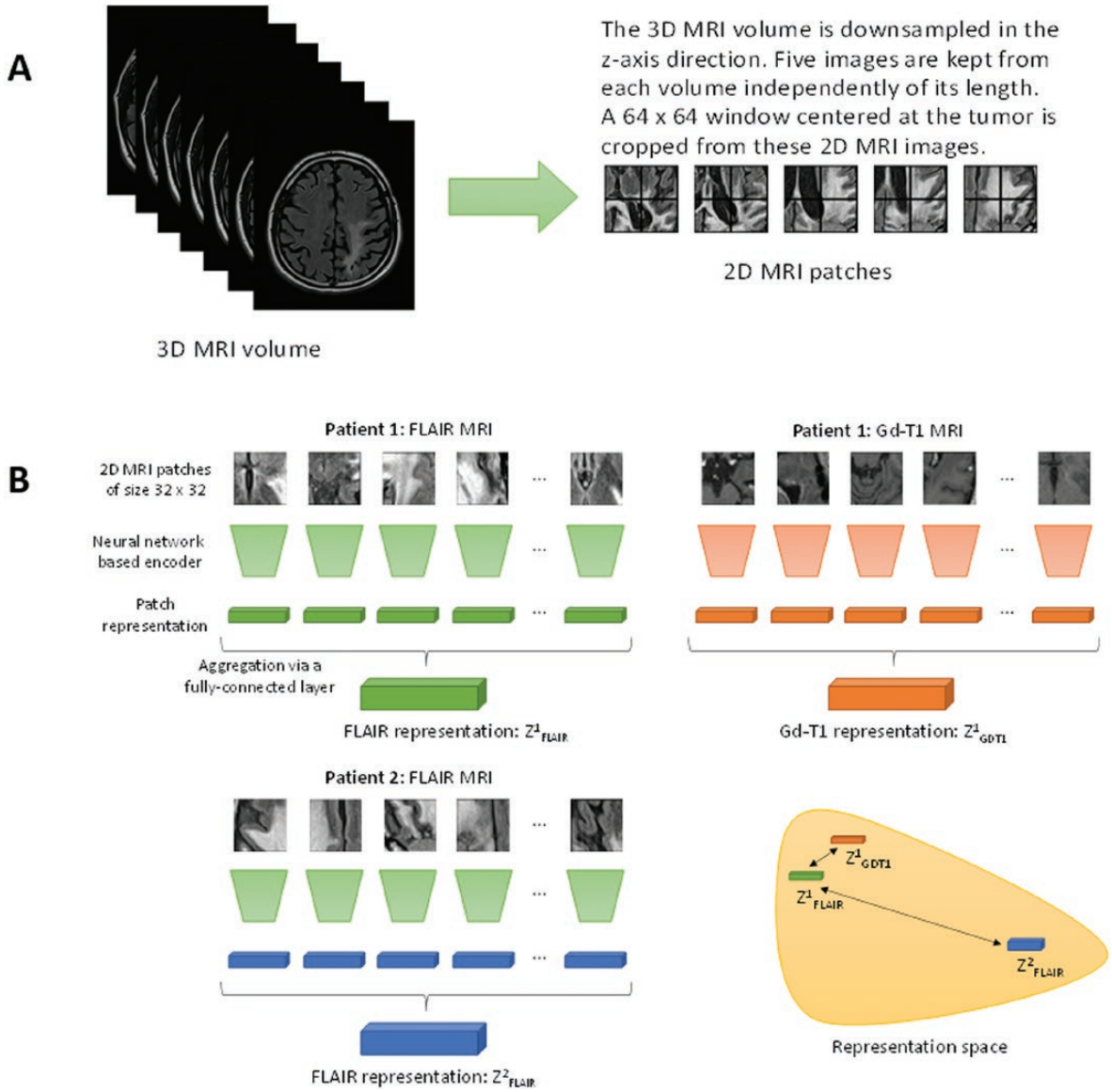

Figure 3. (**A**) Deep learning-based analysis data preparation. (**B**) Unsupervised contrastive learning using FLAIR and Gd-T1 MRI tumor slices. A triplet loss was used to compare the 64 features vectors Z1 FLAIR (anchor), Z1 Gd-T1 (positive sample), and Z2 FLAIR (negative sample). Note that Z2 FLAIR came either from another patient or from the same patient but at a different date.

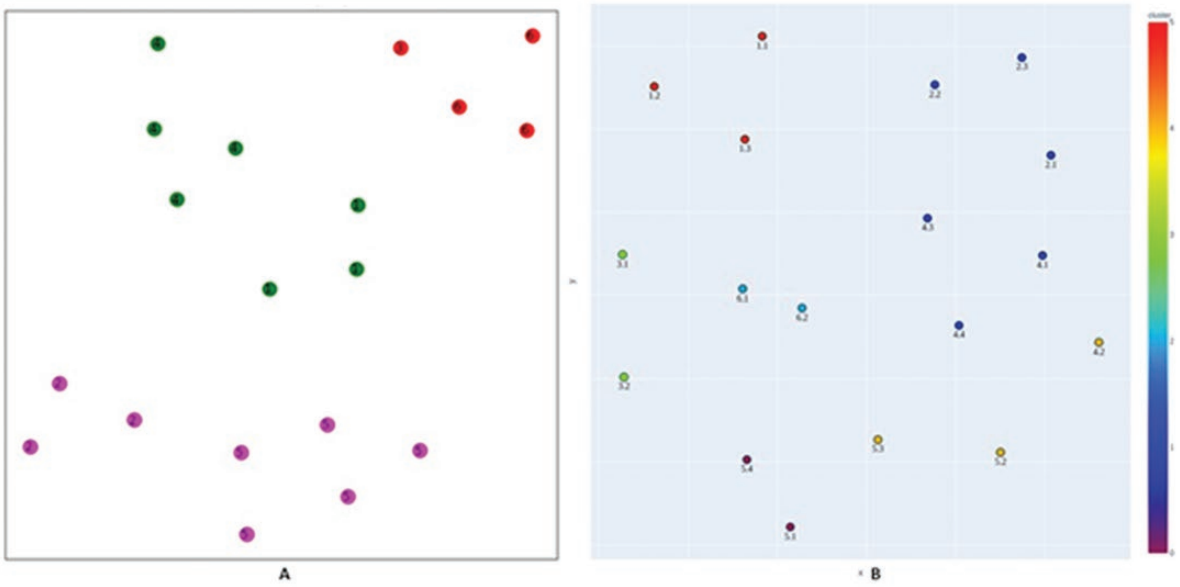

Figure 4. (**A**) MRI data only, diagram for 3 clusters. Each dot represents one patient (at different visits). Patients #02 and #05 with the longest overall survivals were clearly separated from the rest of the cohort. (**B**) Multimodal feature vectors (MRI; cytokine level; neutrophil/lymphocyte ratio) for all patients. Diagram for 6 clusters. Each dot represents one patient denoted by the first number and the visit number. Each color corresponds to a cluster as computed using k-means clustering. Patient #04's second visit was also clustered with patient #05's intermediate visits. Patient #04 also showed significant pseudoprogression.

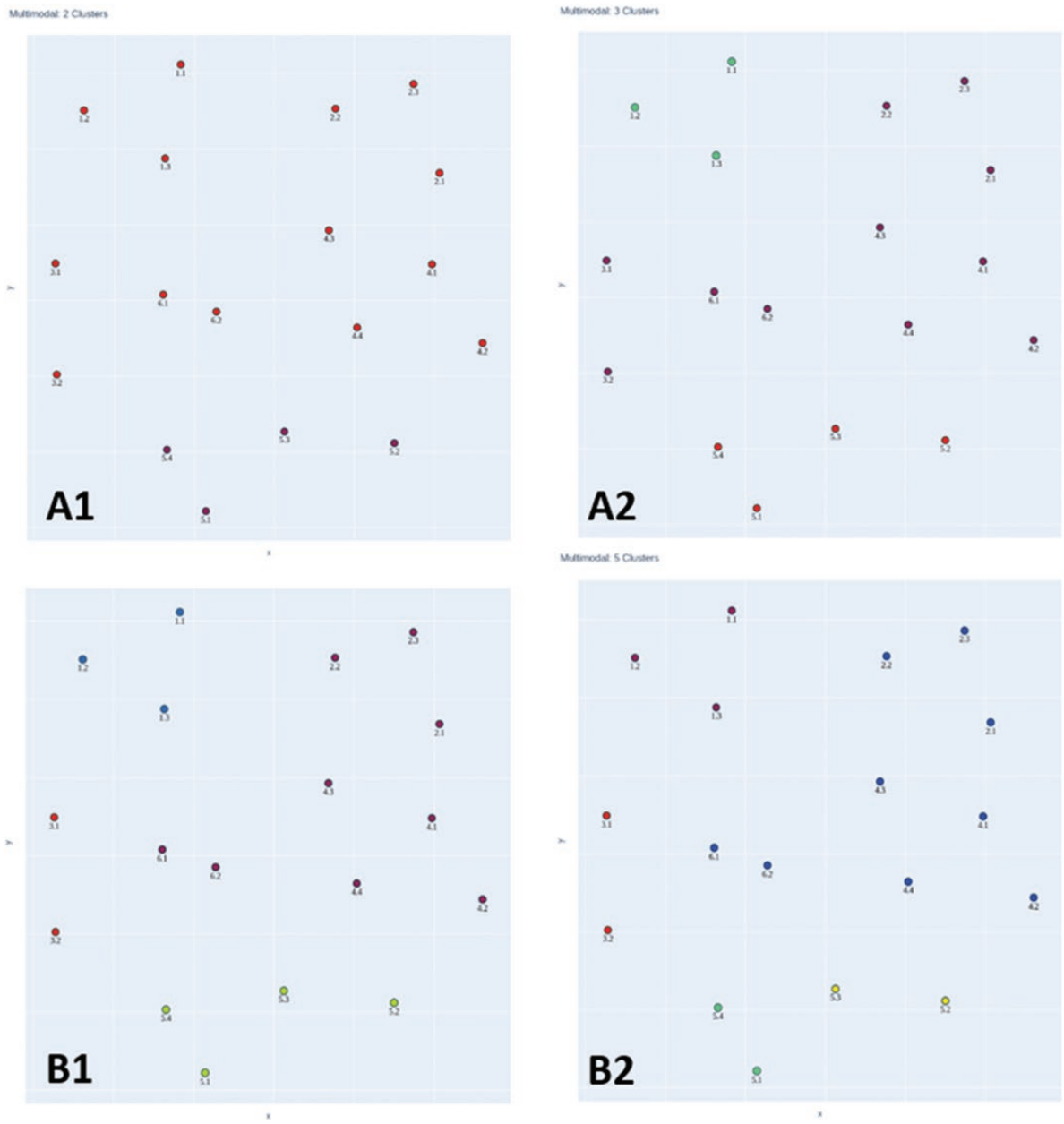

Figure 5. 2D UMAP representations of multimodal feature vectors (MRI; cytokine level; neutrophil/lymphocyte ratio) for all patients. Each dot represents one patient denoted by the first number and the visit number. Each color corresponds to a cluster as computed using k-means clustering. Patient #05's (longest PFI) visits were all clustered (A1). Increasing the number of clusters to 3 leads to a clustering of patient #01's visits (A2). Patients #01, #03, and #05's visits separated into distinct clusters (B1), ranging from the shortest to the longest PFI. At 5 clusters (B2), patient #05's intermediate visits (5.2; 5.3), during which clinical pseudoprogressions were identified, segregated into a distinct cluster.

Table 1. Pathological, biological, treatment and outcome characteristics, and summary for each patient

Patient	MGMT promoter methylation status	PD-L1 tumor expression (%)	PD-L1 expression in stroma infiltrate (%)	Lymphocyte count at Week 0 Day 5 (10^9/L)	Durvalumab cycle number	Best objective response	Progression free interval (months)	Survival in STERIMGLI trial (months)	Treatment at recurrence	Survival from initial diagnosis (years)
#01	Met	20	2	0.7	4	PD	2.1	15.2	Bevacizumab	3.6
#02	Met	70	1	1.1	7	PD	2.3	46.6	Bevacizumab	7.15
#03	Unm	15	0	0.9	4	PR	3.9	5.8	Best support-ive care	1.5
#04	Met	0.1	0.1	1.32	6	SD	5.8	24.8	Bevacizum-ab+Irinotecan	3.0
#05	Unm	0	1	2.0	3	SD	8.1	27.5	Surgery + te-mozolomide	7.7
#06	Unm	0	0	0.6	4	PD	2.2	16.7	Surgery	2.6