Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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SUPPLEMENTARY APPENDIX

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SUPPLEMENTARY METHODS

Study Design

Patients received a boost immunization with trivalent inactivated poliovirus (IPOL™; Sanofi-Pasteur, Swiftwater, PA) at least one week prior to PVSRIPO administration. Neutralizing anti-poliovirus (type 1) antibody titers were monitored in all study subjects from the time of enrollment until at least 56 days post PVSRIPO; additionally, shedding of infectious PVSRIPO with stool was tested in all patients (see below section **Analyses of serum anti-poliovirus type 1 neutralizing antibodies and of virus shedding with stool**). For confirmation of viable malignant glioma and for genomic analyses of tumors, a stereotactic biopsy was performed prior to PVSRIPO infusion. Only a stereotactic biopsy was obtained with no additional tumor resection. Immediately after biopsy, a catheter [Vygon PIC-030 (Sophysa, Inc.; Crown Point, IN)] was implanted into the tumor. The location of the catheter implantation was selected by the operating neurosurgeon with the assistance of iPlan Flow (BrainLAB, Westchester, IL). To avoid PVSRIPO leakage into ventricles, the inserted catheter tip was at least 1 cm away from ventricles.

PVSRIPO was manufactured by the Biopharmaceutical Development Program/SAIC at the National Cancer Institute (NCI)-Frederick. Patients were infused with PVSRIPO by CED over 6.5 hours at a rate of 500 µL/hr using a Medfusion 3500 or 3010 (Smiths Medical ASD, Inc, Minneapolis, MN) infusion pump along with the infusion catheter and infusion tubing (PIT 400, Sophysa, Inc). The volume of the delivered inoculum was 3.25 mL. Patients underwent MRI imaging within 4 hours of completion of infusion to define the shape and dissemination of the infusate relative to the patients' brain anatomy. To this end, gadolinium-diethylene triamine pentaacetic acid (Gd-DTPA) contrast agent was co-infused with PVSRIPO.

Statistical Design

Continual Reassessment Method Model

In this trial, the starting dose of PVSRIPO was 1 x 10^8 tissue culture infectious dose (TCID50), which is 1/10^{th} of the highest non-toxic dose in non-human primates (NHPs) in the definitive, IND-directed toxicology study and 1/50^{th} of the highest non-toxic dose in NHPs in the dose-range-finding toxicology study[1].

Dose escalation followed a two-step continual reassessment method (CRM) design that included an escalation step and a model-guided step[2,3]. In the escalation period, dose levels

(DL) were rapidly escalated as preclinical data suggested that dose-limiting toxicity (DLT) would not occur at any of the five DLs evaluated[4]. Decisions concerning dose escalation for subsequent patients were based upon the occurrence of DLT during the first 4 weeks after treatment administration.

Beginning at DL 1 (1.0×10^8 TCID50), one patient was treated at each DL, up to DL 5 (1.0×10^{10} TCID50). Plan was for a total of 21 patients to be treated on DL 5. The DLs were as follows, DL 1 - 1.0×10^8 TCID50, DL 2 - 3.3×10^8 TCID50, DL 3 - 1.0×10^9 TCID50, DL 4 - 3.3×10^9 TCID50, and DL 5 - 1.0×10^{10} TCID50. The escalation step was halted if a patient assigned to one of the first 4 DLs or more than 20% of patients treated at DL 5 experienced a DLT. In that case, further dose escalation or de-escalation was to be guided by the likelihood-based implementation of the CRM, in which the one-parameter hyperbolic tangent model was used to estimate the probability of DLT at each of the 5 dose levels based upon available data[5]. The highest DL for which this estimated probability is less than 20% was to be identified. If this optimal dose was less than or the same as the current dose, subsequent patients were to be treated at that DL. If greater than the current dose, subsequent patients were to be treated at one DL higher than the current dose. The optimal dose was to be recomputed whenever the status of DLT has been determined for a patient.

Statistical Modification and Dose Expansion

The fourth patient treated on DL 5 experienced a DLT that prompted a dose reduction to DL 4 for subsequent patients. After one additional patient was treated on DL 4, the dose escalation portion of the study was terminated and a dose expansion portion was initiated at DL 2. This dose reduction was not due to concern for DLT, but due to the observation that some patients treated on the higher DLs experienced localized tumor inflammation requiring prolonged steroid use. Six additional patients were treated on DL 2. The dose was further reduced to DL -1, 5.0×10^7 TCID50, with the goal of further reducing difficulties with steroid tapering. Twenty-four patients were treated at that DL before it was further reduced to DL -2, 1.0×10^7 TCID50. Fifteen patients were treated on DL -2 before the study team re-escalated to DL -1. Seven additional patients were treated on this study at DL -1, for a total of 31 patients treated on DL -1. A total of 61 patients were treated with PVSRIPO across all DLs.

In describing the adverse events for PVSRIPO patients, the highest-grade event of each type of event experienced was summarized. Overall survival was defined as the time of PVSRIPO

infusion until death. For patients alive at the time of analysis, survival time was censored at the date of last follow-up. No PVSRIPO patients were lost to follow-up. For the PVSRIPO patients and the historical controls, the Kaplan-Meier estimator was used to describe the distribution of survival time. SAS 9.4 was used for all analyses.

Historical Control Group

We identified a historical control group of patients previously treated at our institution who would have been eligible for the PVSRIPO study if the study had been available at the time of their disease progression. We felt that the identification of a historical control group was the best compromise to evaluate survival, given that treating patients with a sham saline CED infusion would be unethical and given concerns for patient dropout if randomized to the control arm. We felt that the identification of a historical control was necessary because a comparison to recently reported phase III study results of an approved modality, like NovoTTF[6], would be confounded by the fact that enrollment on such studies was not based on the enrollment criteria specific to the PVSRIPO study (e.g. limitation in tumor volume and steroid dosage). Since recently reported studies of effective immunotherapeutic agents showed 1) delayed antitumor effects and, hence, delayed separation of survival curves, and 2) a percentage of patients who survived for extended periods, we focused on evaluating long-term survivorship at a specific time point (i.e. 24 months). Within the historical control group, the 24-month survival probability was 13.5%. Assuming 27 patients treated at DL -1, simulation studies showed that a two-tailed chi-square test (α=0.2) conducted to compare the proportion of patients alive at 24 months at the 0.2 level has 88.7% power to detect an increase to 35% in 24-month survival. The Kaplan-Meier estimator was used to describe the distribution of survival time.

The historical cohort used for the evaluation of PVSRIPO efficacy among WHO grade IV malignant glioma patients was derived from the Primary and Recurrent Glioma Registry (PRoGREss; Duke IRB# Pro00027120). The PRoGREss registry contains medical information of all patients diagnosed after 12/31/2004 with a primary CNS tumor who were seen at Duke University Medical Center's Preston Robert Tisch Brain Tumor Center (PRTBTC). Specifically, that database includes: (1) retrospective data from all patients diagnosed after 12/31/2004, but deceased as of 11/3/2011, and (2) data collected retrospectively and prospectively from patients who were alive as of 11/3/2011 and provided registry consent. Both demographic and clinical information was extracted from clinical and research records, which includes such information

as diagnosis, gender, medical history, medications, disease progression, types of treatment, and survival.

To create the historical control cohort, data available from the PRoGREss registry as of 12/15/2014 were reviewed. All recurrent WHO grade IV malignant glioma patients treated at Duke from 1/01/2007 to 12/15/2014 were reviewed to determine potential eligibility on the phase I PVSRIPO trial, had the trial been available during that time period. All radiologic records were reviewed by a study investigator (A.D.) without knowledge of the patient's survival outcome.

Patients deemed eligible from an MRI standpoint were then selected for inclusion in the historical control group based on the following criteria: patients must have a diagnosis of recurrent supratentorial WHO Grade IV malignant glioma based on imaging studies with measurable disease (≥ 1 cm and ≤ 5.5 cm of contrast-enhancing tumor), age ≥ 18 years, must not have taken part in the PVSRIPO study, KPS ≥ 70 at the time the patient could have been enrolled in the PVSRIPO study, absence of rapid clinical decline, and a steroid limit of ≤ 4 mg per day of dexamethasone (or equivalent) within 2 weeks prior to being eligible for PVSRIPO infusion. Survival time was computed as the time between the date of the first MRI at which time the patient would have been eligible to receive PVSRIPO and the date of death. If the patient remained alive at the time of analysis, survival time was censored at the date of last follow-up.

Toxicity Evaluation

Events associated with the biopsy procedure or catheter placement (unless grade 3 or higher), seizures, new neurologic deficits ascribed to histopathology-proven tumor progression, thromboembolism, Syndrome of Inappropriate Antidiuretic Hormone (SIADH), complications from corticosteroids (steroid myopathy, weight gain, etc.) and tumor progression were not considered DLTs.

Imaging Analysis

An exploratory objective of this study was to describe changes visualized on imaging after PVSRIPO inoculation to assist with developing imaging response classification criteria specific to PVSRIPO for future studies. Potent proinflammatory responses to PVSRIPO tumor infection in immunodeficient[7] or immunocompetent[8] rodent tumor models, indicated that patient imaging would reveal an inflammatory reaction. Magnetic resonance imaging (MRI) was obtained at screening, within 4 hours after completion of infusion, 4 and 8 weeks after the completion of the

infusion, followed by every 8 weeks for one year, and afterward at an interval selected by the treating physician. Radiographic Assessment Forms were filled out and reviewed by a study investigator for each MRI. This included enhancing tumor measurements, change in size from baseline and nadir, extent of FLAIR signal abnormalities, presence of new disease, steroid dose, and clinical status, among other information.

IDH1(R132H) and CD155 immunohistochemistry, and MGMT promoter methylation assay

CD155 immunohistochemistry (IHC) was not an enrollment criterion for PVSRIPO for practical and scientific reasons. Such a test would unduly delay virus infusion after catheter installation. We recently reported positive CD155 IHC in 62 out of 63 cases of glioblastoma[9]. Immunohistochemistry of CD155 on formalin-fixed, paraffin-embedded tissue specimens was performed as reported recently[9]. Immunoperoxidase staining and detection of IDH1 was performed on formalin-fixed, paraffin-embedded tissue specimens. Sections were deparaffinized in xylene, rehydrated, and placed in the Dako PT module where they were heated at 100°C in Envision Flex Target Retrieval Solution (low pH; 20 min). The sections were washed in Dako wash buffer (5 min) and incubated with the mIDH1R132H (Dianova, Hamburg, Germany) monoclonal antibody (mouse IgG, diluted 1:60) that specifically recognizes IDH1(R132H) using the Dako LINKS platform. The DAKO Link Kit was used for the post-primary antibody blocker, Dako (HRP) Labeled Envision Flex Mouse Plus as the detection, Dako DAB+ as the chromogen, and Hematoxylin as the counterstain. The expression of IDH1-R132H was determined by semi-quantitatively assessing the proportion of positively stained tumor cells. MGMT promoter methylation was analyzed by quantitative methylation-specific polymerase chain reaction using sample DNA extracted from formalin-fixed, paraffin-embedded (FFPE) specimens (LabCorp, Burlington, NC).

Analyses of serum anti-poliovirus type 1 neutralizing antibodies and of virus shedding with stool

Anti-poliovirus type 1 neutralizing antibody titers were determined in serum samples from all patients at defined intervals: at the time of enrollment, the day prior to PVSRIPO infusion (day -1), and days 7, 14, 28, 56 post PVSRIPO infusion. We used the enhanced plaque neutralization method described by Boone et al.[10] for our analyses. Virus shedding with stool was tested at days 7, 14, 28 and 56 post PVSRIPO infusion for patients 1-7, and at day 56 post PVSRIPO infusion for all remaining patients treated with PVSRIPO. We used previously published methods for detecting infectious PVSRIPO in stool samples from non-human primates[1].

Table S1. Inclusion and exclusion criteria for NCT01491893.

Inclusion Criteria	Exclusion Criteria
• Recurrent supratentorial WHO Grade IV Malignant Glioma based on imaging studies with measurable disease (≥ 1 cm and ≤ 5.5 cm of contrast-enhancing tumor). Prior histopathology consistent with a WHO Grade IV Malignant Glioma. • Adults ≥ 18 years of age. • Karnofsky Performance Score (KPS) of ≥ 70%. **Lab values prior to biopsy:** • Neutrophil count ≥ 1000. • Hemoglobin ≥ 9. • Prothrombin and Partial Thromboplastin Times ≤ 1.2 x normal. • Creatinine ≤ 1.2 x normal. • Total bilirubin, SGOT, SGPT, alkaline phosphatase ≤ 2.5 x normal. **Requirements:** • Platelet count ≥ 125,000/μL prior to tumor biopsy. • Platelets ≥ 100,000/μL prior to PVSRIPO infusion. • Positive serum anti-poliovirus titer prior to biopsy. • Received a boost immunization with trivalent inactivated IPOL™ (Sanofi-Pasteur) at least 1 week prior to administration of the study agent. • At the time of biopsy, prior to administration of virus, the presence of recurrent tumor must have been confirmed by histopathological analysis. • Able to undergo brain MRI with and without contrast. • A signed informed consent form approved by the Duke University Institutional Review Board (IRB) will be required for patient enrollment into the study. Able to read and understand the informed consent document and must sign the informed consent indicating that they are aware of the investigational nature of this study.	• Females who are pregnant or breast-feeding during the study period. • Adults of reproductive potential not employing an effective method of birth control will be excluded. • Prior, unrelated malignancy that required active treatment with the exception of cervical carcinoma in situ and adequately treated basal cell or squamous cell carcinoma of the skin. • An impending, life-threatening cerebral herniation syndrome, based on the assessment of the study neurosurgeons or their designate. **Because the potential toxicities from the agent being studied may be similar to some known diseases or may be more dangerous in the context of certain known diseases, patients with the following were excluded to avoid confounding the study results:** • An active infection requiring intravenous treatment or having an unexplained febrile illness (Tmax > 99.5 F/37.5 C) • Known immunosuppressive disease or known human immunodeficiency virus infection. • An unstable or severe intercurrent medical condition such as severe heart (New York Heart Association Class 3 or 4) or known lung (FEV1 < 50%) disease, uncontrolled diabetes mellitus. • A known albumin allergy. • A known Gadolinium allergy. • History of neurological complications due to poliovirus infection. **Patients who had not recovered from the toxic effects of prior chemotherapy and/or radiation therapy were excluded according to the following criteria:** • Chemotherapy or bevacizumab < 4 weeks [except for nitrosourea (6 weeks) or metronomic dosed chemotherapy such as daily etoposide or cyclophosphamide (1 week)] prior to starting the study drug unless recovered from side effects of such therapy. • Immunotherapy < 4 weeks prior to starting the study drug unless patient has recovered from side effects of such therapy.

	• Less than 12 weeks from radiation therapy, unless progressive disease outside of the radiation field or 2 progressive scans at least 4 weeks apart or histopathologic confirmation. **Patients who had not completed all standard of care treatments including surgical procedure, and radiation therapy (at least 59 Gy):** • If MGMT promoter unmethylated, patients did not have to have received chemotherapy prior to participating in this trial. • If MGMT promoter methylated or MGMT promotor methylation status was unknown at the time of screening, patients must have received at least one chemotherapy regimen prior to participating in this trial. • Radiological evidence of active (growing) multifocal disease, tumors extending into or crossing the corpus callosum or leptomeningeal disease. • Undetectable anti-tetanus toxoid IgG. • Known history of agammaglobulinemia. • On greater than 4 mg per day of dexamethasone within the 2 weeks prior to admission for PVSRIPO infusion. • Worsening steroid myopathy (history of gradual progression of bilateral proximal muscle weakness, and atrophy of proximal muscle groups).

Table S2. PVSRIPO dose expansion patients (dose levels -2, -1, 2) with adverse events attributable to bevacizumab among the patients who received bevacizumab (N=34).

Adverse Event	Grade of Adverse Event				
	1	2	3	4	5
Blood and lymphatic system disorders					
Anemia	5 (15%)	-	-	-	-
Gastrointestinal (GI) disorders					
Colitis	-	1 (3%)	-	-	-
Gingivitis	1 (3%)	-	-	-	-
Hemorrhoids	-	1 (3%)	-	-	-
Lower GI hemorrhage	1 (3%)	-	-	-	-
Nausea	2 (6%)	-	-	-	-
Vomiting	1 (3%)	-	-	-	-
General disorders and administration site conditions					
Infusion related reaction	-	1 (3%)	-	-	-
Infections and infestations					
Upper respiratory infection	1 (3%)	-	-	-	-
Investigations					
Creatinine increased	6 (18%)	-	-	-	-
Leukopenia	4 (12%)	2 (6%)	-	-	-
Lymphopenia	-	1 (3%)	-	-	-
Neutropenia	1 (3%)	1 (3%)	1 (3%)	-	-
Thrombocytopenia	3 (9%)	-	-	-	-
Musculoskeletal and connective tissue disorders					
Arthralgia	4 (12%)	-	-	-	-
Nervous system disorders					
Headache	-	2 (6%)	-	-	-
Intracranial hemorrhage	-	1 (3%)	-	-	2 (6%)
Renal and urinary disorders					
Hematuria	1 (3%)	-	-	-	-
Proteinuria	3 (9%)	-	1 (3%)	-	-
Respiratory, thoracic and mediastinal disorders					
Epistaxis	1 (3%)	-	-	-	-
Nasal congestion	2 (6%)	-	-	-	-
Postnasal drip	3 (9%)	-	-	-	-
Vascular disorders					
Hypertension	2 (6%)	7 (21%)	1 (3%)	-	-
Thromboembolic event	-	1 (3%)	3 (9%)	-	-
Total Patients with an Event*	11 (32%)	9 (26%)	3 (9%)	0 (0%)	2 (6%)

*Each patient is included only once in the Total row under the grade level representing their highest-grade event.

Table S3. PVSRIPO patients experiencing an adverse event during dose-escalation (Dose Levels 1-5) and dose expansion (Dose Exp)*.

Body System	Adverse Event	Grade	PVSRIPO Dose Escalation Dose Level					Dose Exp
			1 (N=1)	2 (N=1)	3 (N=1)	4 (N=2)	5 (N=4)	(N=52)
Blood and lymphatic system disorders	Anemia	Grade 1	1	-	1	2	3	32
		Grade 2	-	-	-	-	-	2
Cardiac disorders	Cardiac arrhythmia	Grade 1	-	-	-	-	-	1
		Grade 2	-	-	-	-	-	1
Ear and labyrinth disorders	Ear and labyrinth disorders - Other	Grade 1	-	-	-	-	-	2
	Hearing impaired	Grade 1	-	-	-	-	-	2
	Tinnitus	Grade 1	-	-	-	-	-	1
	Vertigo	Grade 1	-	-	-	-	-	3
Endocrine disorders	Cushingoid	Grade 1	-	-	-	-	1	2
		Grade 2	-	-	-	1	-	1
	Diabetes	Grade 2	-	1	-	-	-	-
Eye disorders	Blurred vision	Grade 1	-	-	-	-	1	5
	Diplopia	Grade 1	-	-	-	-	-	3
	Eye disorders - Other	Grade 1	-	-	-	-	-	2
	Farsighted	Grade 1	-	-	-	-	-	2
	Focusing difficulty	Grade 1	-	-	-	-	-	1
	Strabismus	Grade 1	-	-	-	-	-	1
	Visual field cut - hemianopia	Grade 1	-	-	-	-	-	10
		Grade 2	-	-	-	-	-	2
Gastrointestinal disorders	Abdominal pain	Grade 1	-	-	-	-	-	1
	Cholelithiasis	Grade 1	-	-	-	-	-	1
	Colitis	Grade 2	-	-	-	-	-	1
	Constipation	Grade 1	-	-	-	-	-	12
		Grade 2	-	-	-	-	-	1
	Diarrhea	Grade 1	-	1	-	-	-	8
		Grade 2	-	-	1	-	-	1
	Dry mouth	Grade 1	-	-	-	1	-	1
	Dysphagia	Grade 1	-	-	-	1	-	-
	Enterocolitis	Grade 1	-	-	-	-	-	2
		Grade 2	-	-	-	-	-	1
	Fecal incontinence	Grade 1	-	-	1	-	-	2
	Gastroesophageal reflux/disease	Grade 1	1	-	-	-	-	1
		Grade 2	-	-	-	-	-	1
	Gingivitis	Grade 1	-	-	-	-	-	2
	Hemorrhoids	Grade 1	-	-	-	-	1	-
		Grade 2	-	-	-	-	-	1
	Lower gastrointes-tinal hemorrhage	Grade 1	-	-	-	-	-	2

Body System	Adverse Event	Grade	PVSRIPO Dose Escalation Dose Level					Dose Exp
			1 (N=1)	2 (N=1)	3 (N=1)	4 (N=2)	5 (N=4)	(N=52)
	Mucositis oral	Grade 1	-	-	-	-	-	2
		Grade 2	-	-	-	1	-	1
	Nausea	Grade 1	1	-	-	1	-	20
	Pancreatic hemorrhage	Grade 2	-	-	-	-	1	-
	Taste disorder	Grade 1	-	-	-	-	-	1
	Vomiting	Grade 1	1	-	1	-	-	11
General disorders and administration site conditions	Chills	Grade 1	-	-	-	-	-	1
	Edema face	Grade 1	-	-	-	-	-	1
	Fatigue	Grade 1	1	-	-	1	1	13
		Grade 2	-	-	1	-	1	5
		Grade 3	-	-	-	1	-	-
	Fever	Grade 1	-	-	-	-	1	-
		Grade 2	-	-	-	-	-	1
	Gait disturbance	Grade 1	-	-	1	-	-	5
		Grade 3	-	-	-	-	-	1
	Infusion related reaction	Grade 2	-	-	-	-	-	1
	Irritability	Grade 1	-	-	-	-	-	3
		Grade 2	-	-	-	-	-	1
	Malaise	Grade 2	-	-	-	-	-	1
	Non-cardiac chest pain	Grade 1	-	-	-	-	-	1
	Pain	Grade 1	-	1	-	-	-	20
		Grade 2	-	-	-	-	-	1
	Steroid myopathy	Grade 1	-	-	-	1	-	3
		Grade 2	-	-	1	1	-	1
		Grade 3	-	-	-	-	-	1
Immune system disorders	Allergic reaction	Grade 1	-	-	-	-	-	2
Infections and infestations	Bronchial infection	Grade 2	-	-	-	-	-	1
	Conjunctivitis infective	Grade 2	-	-	-	1	-	-
	Enterocolitis infectious	Grade 1	-	-	-	-	-	1
		Grade 3	-	-	-	-	-	1
	Infections and infestations - Other	Grade 1	-	1	-	-	-	1
		Grade 2	-	-	1	-	-	1
	Lung infection	Grade 2	-	-	-	-	-	1
		Grade 3	-	-	-	1	-	-
	Mucosal infection	Grade 1	-	-	-	-	-	4
		Grade 2	-	-	-	-	-	1
	Phlebitis infective	Grade 2	-	1	-	-	-	-
	Skin infection	Grade 1	-	-	-	-	-	3

Body System	Adverse Event	Grade	PVSRIPO Dose Escalation Dose Level					Dose Exp
			1 (N=1)	2 (N=1)	3 (N=1)	4 (N=2)	5 (N=4)	(N=52)
		Grade 2	-	-	-	1	-	-
	Tooth infection	Grade 1	-	-	-	-	-	2
	Upper respiratory infection	Grade 1	-	-	-	-	-	9
		Grade 2	-	-	-	1	-	7
	Urinary tract infection	Grade 2	-	-	-	-	-	5
	Vaginal infection	Grade 2	-	-	-	-	-	2
Injury, poisoning and procedural complications	Bruising	Grade 1	-	-	-	1	-	3
	Burn	Grade 1	-	-	-	-	-	1
	Fall	Grade 1	-	-	-	1	-	4
	Vascular access complication	Grade 2	-	-	-	-	-	1
Investigations	Alanine aminotrans-ferase increased	Grade 1	-	1	-	1	-	8
		Grade 2	-	-	-	-	-	1
	Alkaline phospha-tase increased	Grade 1	-	-	-	1	-	5
	Aspartate amino-transferase increased	Grade 1	1	1	-	2	-	12
		Grade 3	-	-	-	-	-	1
	Blood bilirubin increased	Grade 1	-	1	-	-	-	4
		Grade 2	-	-	-	1	-	-
	Creatinine increased	Grade 1	-	-	-	1	-	9
	Leukopenia	Grade 1	-	-	-	-	-	5
		Grade 2	-	-	-	-	1	5
	Lymphopenia	Grade 1	-	-	-	-	-	4
		Grade 2	-	-	-	-	2	7
		Grade 3	-	-	1	1	-	8
		Grade 4	-	-	-	-	-	1
	Neutropenia	Grade 1	-	-	-	-	1	5
		Grade 2	-	1	-	-	-	2
		Grade 3	-	-	-	-	-	1
	Thrombocytopenia	Grade 1	-	-	1	1	1	18
		Grade 2	-	1	-	1	-	-
Metabolism and nutrition disorders	Anorexia	Grade 1	-	-	-	-	-	4
	Dehydration	Grade 1	-	-	-	-	-	1
		Grade 2	-	-	-	1	-	2
	Hyperglycemia	Grade 1	1	-	-	-	-	17
		Grade 2	-	1	1	1	1	11
		Grade 3	-	-	-	-	1	4
	Hyperkalemia	Grade 1	-	-	-	-	-	1
		Grade 2	-	-	-	-	-	1
	Hypernatremia	Grade 1	-	-	-	-	-	2

Body System	Adverse Event	Grade	PVSRIPO Dose Escalation Dose Level					Dose Exp
			1 (N=1)	2 (N=1)	3 (N=1)	4 (N=2)	5 (N=4)	(N=52)
	Hypoalbuminemia	Grade 1	1	-	1	1	2	23
		Grade 2	-	-	-	-	1	7
	Hypocalcemia	Grade 1	1	-	1	1	1	20
		Grade 2	-	-	-	-	1	3
		Grade 3	-	-	-	-	-	1
	Hypoglycemia	Grade 1	-	-	-	-	-	10
	Hypokalemia	Grade 1	-	-	1	1	1	17
		Grade 2	-	-	-	1	-	-
	Hypomagnesemia	Grade 1	1	-	-	1	-	3
	Hyponatremia	Grade 1	-	1	-	-	2	12
		Grade 3	-	-	-	-	-	1
	Hypophosphatemia	Grade 1	-	-	-	-	-	1
		Grade 3	-	-	-	-	1	-
Musculoskeletal and connective tissue disorders	Arthralgia	Grade 1	-	-	-	-	-	6
		Grade 2	-	-	-	-	-	1
	Arthritis	Grade 1	-	1	-	-	-	3
	Generalized muscle weakness	Grade 1	-	-	-	-	-	1
		Grade 3	-	-	-	1	-	1
	Muscle cramp	Grade 1	-	-	-	-	-	3
Nervous system disorders	Cognitive disturbance	Grade 1	1	-	-	-	-	13
		Grade 2	-	-	1	-	-	2
	Dysgeusia	Grade 1	-	-	-	-	-	1
	Dysphasia	Grade 1	-	-	-	-	1	8
		Grade 2	-	-	1	-	1	10
	Dystonia	Grade 3	-	-	-	-	-	1
	Edema cerebral	Grade 4	-	-	-	-	-	1
	Facial muscle weakness	Grade 1	-	-	-	-	-	1
	Headache	Grade 1	1	-	-	2	1	21
		Grade 2	-	-	-	-	-	15
		Grade 3	-	-	-	-	-	1
	Hydrocephalus	Grade 2	-	-	-	-	-	1
	Intracranial hemorrhage	Grade 1	-	-	-	-	-	1
		Grade 2	-	-	-	-	-	1
		Grade 4	-	-	-	-	1	-
		Grade 5	-	-	-	-	-	3
	Myokymia	Grade 1	-	-	-	-	-	1
	Nervous system disorders - Other	Grade 1	-	-	-	-	-	2
	Paresthesia	Grade 1	-	1	-	-	-	10
		Grade 2	-	-	-	1	1	1
	Peripheral sensory neuropathy	Grade 1	-	-	-	-	-	2

Body System	Adverse Event	Grade	PVSRIPO Dose Escalation Dose Level					Dose Exp
			1 (N=1)	2 (N=1)	3 (N=1)	4 (N=2)	5 (N=4)	(N=52)
	Presyncope	Grade 2	-	1	-	-	-	-
	Pyramidal tract syndrome (hemiparesis)	Grade 1	-	-	-	-	-	13
		Grade 2	-	-	-	-	1	11
		Grade 3	-	-	-	1	2	4
	Restless leg syndrome	Grade 1	-	-	-	-	-	1
	Seizure	Grade 1	-	1	-	-	4	24
		Grade 2	-	-	1	-	-	4
		Grade 3	1	-	-	-	-	2
		Grade 5	-	-	-	-	-	1
	Tremor	Grade 1	-	-	-	-	-	2
Psychiatric disorders	Agitation	Grade 1	-	-	-	-	-	1
	Anxiety	Grade 1	-	-	-	-	-	5
		Grade 3	-	-	-	-	-	1
	Confusion	Grade 1	-	-	-	-	1	5
		Grade 2	-	-	-	-	-	6
		Grade 3	-	-	-	-	-	1
	Delusions	Grade 3	-	-	-	-	-	1
	Depression	Grade 1	-	-	-	-	-	3
		Grade 2	-	-	-	-	-	2
	Hallucinations	Grade 1	-	-	-	-	-	2
	Insomnia	Grade 1	-	-	-	-	1	13
		Grade 2	-	-	-	-	-	1
	Suicidal ideation	Grade 2	-	-	-	-	-	2
Renal and urinary disorders	Hematuria	Grade 1	-	-	-	-	-	4
	Proteinuria	Grade 1	-	-	-	1	-	5
		Grade 3	-	-	-	-	-	1
	Renal and urinary disorders - Other	Grade 1	-	-	-	-	-	1
	Renal calculi	Grade 2	-	-	-	-	-	1
	Urinary frequency	Grade 1	-	-	1	-	-	2
	Urinary incontinence	Grade 1	-	-	1	-	-	5
		Grade 2	-	-	-	-	-	1
	Urinary retention	Grade 1	-	-	-	-	-	1
Reproductive system and breast disorders	Irregular menstruation	Grade 1	-	-	-	-	-	2
	Vaginal hemorrhage	Grade 1	-	-	-	-	-	1
Respiratory, thoracic and mediastinal disorders	Allergic rhinitis	Grade 2	-	-	-	-	-	4
	Cough	Grade 1	-	-	1	1	-	-
		Grade 2	-	-	-	-	-	1
	Dry nose	Grade 1	-	-	-	-	-	1
	Dyspnea	Grade 1	-	-	1	-	-	6
	Epistaxis	Grade 1	-	-	-	-	-	4

Body System	Adverse Event	Grade	PVSRIPO Dose Escalation Dose Level					Dose Exp
			1 (N=1)	2 (N=1)	3 (N=1)	4 (N=2)	5 (N=4)	(N=52)
	Hiccups	Grade 1	-	-	-	-	-	1
	Hoarseness	Grade 1	-	-	-	-	1	3
	Nasal congestion	Grade 1	1	-	1	1	-	4
	Postnasal drip	Grade 1	-	-	-	-	-	5
	Pulmonary Nodule	Grade 1	-	-	1	-	-	-
	Respiratory, thoracic and mediastinal disorders - Other	Grade 1	-	-	-	-	-	1
	Sleep apnea	Grade 2	-	-	-	-	-	2
Skin and subcutaneous tissue disorders	Dry skin	Grade 1	-	-	-	-	-	7
	Pruritus	Grade 1	-	-	-	-	-	1
	Rash	Grade 1	-	-	-	-	1	9
		Grade 2	-	-	-	-	-	1
	Skin and subcutaneous tissue disorders - Other	Grade 1	-	-	-	-	-	1
	Skin trauma	Grade 1	-	-	-	-	-	4
		Grade 2	-	-	-	-	-	2
Social circumstances	Menopause	Grade 1	-	-	-	-	-	1
Surgical and medical procedures	Incisional hernia repair	Grade 3	-	1	-	-	-	-
Vascular disorders	Hot flashes	Grade 1	-	-	-	-	-	2
	Hypertension	Grade 1	-	-	-	-	-	2
		Grade 2	-	-	-	-	1	8
		Grade 3	-	-	-	-	-	2
	Thromboembolic event	Grade 2	-	-	1	-	1	2
		Grade 3	-	1	-	-	-	3
Total Patients with an Event			1	1	1	2	4	52

*Only includes the highest-grade event for each type of adverse event experienced by a patient.

Table S4. PVSRIPO patient prognostic, treatment and survival information*.

Pat.	Age at tx	PVSRIPO Dose Level	Prior Recurr. (#)	Prior BEV failure	KPS at tx	BEV post-PVSRIPO (mg/kg)	Chemo +/- BEV post-PVSRIPO	OS (months)	IDH1 Status at dx	MGMT Status at dx	MGMT Status at tx
1	20	1	1	Yes	90	None	None	70.4+	Mutated	Unknown	Unmethylated
2	69	2	1	Naïve	90	None	None	69.2+	Wildtype	Unknown	Methylated
3	62	3	1	Yes	90	10.0	None	5.8	Wildtype	Unknown	Unmethylated
4	70	4	1	Yes	70	None	None	5.6	Wildtype	Unknown	Unmethylated
5	60	5	3	Naïve	80	None	None	19.8	Wildtype	Unmethylated	Methylated
6	61	5	1	Naïve	80	10.0	Lomustine/BEV, VP-16/ BEV	12.5	Wildtype	Unknown	Unmethylated
7	55	5	1	Naïve	90	10.0	Lomustine	15.2	Wildtype	Unknown	Unmethylated
8	40	5	1	No	90	None	Lomustine	57.5+	Wildtype	Unknown	Methylated
9	58	4	1	Yes	90	5.0	TMZ/BEV, CPT-11/BEV	16.5	Wildtype	Unknown	Unmethylated
10	68	2	1	Naïve	90	10.0	None	7.0	Wildtype	Unknown	Methylated
11	50	2	2	Yes	80	7.5	TMZ/BEV	9.3	Wildtype	Unknown	Unmethylated
12	38	2	2	Naïve	90	10.0	None	6.3	Wildtype	Unknown	Unmethylated
13	37	2	1	Yes	90	10.0	TMZ/BEV, Lomustine/BEV	12.6	Wildtype	Unknown	Unmethylated
14	60	2	1	Naïve	80	7.5-10.0	None	8.6	Wildtype	Unknown	Unmethylated
15	56	2	1	Naïve	100	7.5	CPT-11/BEV, Lomustine/BEV	15.9	Wildtype	Unknown	Unmethylated
16	57	-1	1	Naïve	80	None	None	41.1+	Wildtype	Methylated	Methylated

Pat.	Age at tx	PVSRIPO Dose Level	Prior Recurr. (#)	Prior BEV failure	KPS at tx	BEV post-PVSRIPO (mg/kg)	Chemo +/- BEV post-PVSRIPO	OS (months)	IDH1 Status at dx	MGMT Status at dx	MGMT Status at tx
17	58	-1	1	Naïve	90	7.5	TMZ/BEV	17.3	Unknown	Methylated	Methylated
18	69	-1	1	Naïve	80	10.0	None	3.1	Unknown	Methylated	Unmethylated
19	69	-1	2	Yes	80	7.5	None	6.5	Wildtype	Unmethylated	Unmethylated
20	28	-1	1	Yes	90	7.5	TMZ	15.0	Wildtype	Unmethylated	Methylated
21	59	-1	3	Yes	90	7.5	Lomustine/BEV	8.3	Wildtype	Methylated	Methylated
22	35	-1	1	Naïve	90	None	TMZ	36.1+	Wildtype	Methylated	Unmethylated
23	39	-1	1	Naïve	90	7.5	Lomustine/Optune/BEV /Valcyte, Nivo/BEV	17.8	Wildtype	Unmethylated	Methylated
24	54	-1	1	Naïve	90	7.5	Lomustine, CPT-11/BEV, Carboplatin/BEV	14.3	Wildtype	Methylated	Unmethylated
25	59	-1	1	Naïve	100	7.5	None	34.1+	Wildtype	Unmethylated	Unmethylated
26	24	-1	4	Yes	90	None	CPT-11/BEV, Carboplatin	6.7	Mutated	Unmethylated	Unknown
27	60	-1	1	Naïve	90	7.5	TMZ/BEV	9.4	Wildtype	Methylated	Methylated
28	53	-1	2	Naïve	90	7.5	TMZ/BEV, Lomustine/BEV	12.3	Wildtype	Unmethylated	Unmethylated
29	55	-1	2	Yes	80	7.5	TMZ/BEV	5.6	Wildtype	Unmethylated	Unmethylated
30	55	-1	1	Naïve	80	7.5	TMZ/BEV, Lomustine/BEV	9.6	Wildtype	Unmethylated	Unmethylated
31	54	-1	2	Naïve	90	7.5	None	10.4	Wildtype	Unmethylated	Unmethylated
32	48	-1	1	Naïve	90	7.5	Lomustine/BEV	9.8	Wildtype	Unmethylated	Unmethylated
33	64	-1	1	Naïve	90	7.5	Lomustine/BEV	27.6+	Wildtype	Methylated	Unknown
34	73	-1	1	Naïve	90	7.5	None	27.1+	Wildtype	Methylated	Methylated

Pat.	Age at tx	PVSRIPO Dose Level	Prior Recurr. (#)	Prior BEV failure	KPS at tx	BEV post-PVSRIPO (mg/kg)	Chemo +/- BEV post-PVSRIPO	OS (months)	IDH1 Status at dx	MGMT Status at dx	MGMT Status at tx
35	51	-1	1	Naïve	90	7.5	CPT-11/BEV	15.3	Wildtype	Unmethylated	Unmethylated
36	52	-1	1	Naïve	90	None	Lomustine/BEV	6.7	Unknown	Unmethylated	Unmethylated
37	40	-1	1	Naïve	80	7.5	Lomustine/BEV, CPT-11/BEV	13.1	Wildtype	Unmethylated	Unmethylated
38	58	-1	2	Naïve	90	7.5	None	10.7	Unknown	Unknown	Methylated
39	54	-1	1	Naïve	90	7.5	Lomustine/BEV, TMZ/BEV	9.0	Wildtype	Unmethylated	Unmethylated
40	52	-2	1	Naïve	90	7.5	Lomustine/BEV Carboplatin/BEV	20.5+	Wildtype	Unmethylated	Unknown
41	54	-2	1	Naïve	90	7.5	None	17.1	Wildtype	Methylated	Unmethylated
42	55	-2	1	Naïve	80	7.5	Lomustine/BEV, CPT-11/BEV	10.0	Wildtype	Unmethylated	Unmethylated
43	40	-2	2	Naïve	90	None	None	4.8	Unknown	Unknown	Unmethylated
44	51	-2	1	Naïve	90	7.5	TMZ	8.0	Wildtype	Unmethylated	Unmethylated
45	73	-2	1	Naïve	90	7.5-10.0	None	8.9	Wildtype	Methylated	Methylated
46	32	-2	2	Naïve	90	None	CPT-11/BEV	13.2	Mutated	Unmethylated	Unmethylated
47	51	-2	1	Naïve	80	5.0-10.0	None	7.2	Unknown	Unknown	Unmethylated
48	54	-2	1	Naïve	90	7.5	Lomustine/BEV, CPT-11/Carboplatin/BEV	12.5	Wildtype	Unmethylated	Unmethylated
49	75	-2	1	Naïve	80	7.5	None	15.4+	Wildtype	Methylated	Unknown
50	47	-2	1	Naïve	90	None	None	15.1+	Mutated	Methylated	Methylated
51	43	-2	2	Yes	90	7.5	Lomustine/BEV	13.9+	Mutated	Unmethylated	Unknown
52	63	-2	1	Naïve	80	None	Marizomib, BEV	4.9	Wildtype	Unmethylated	Unmethylated

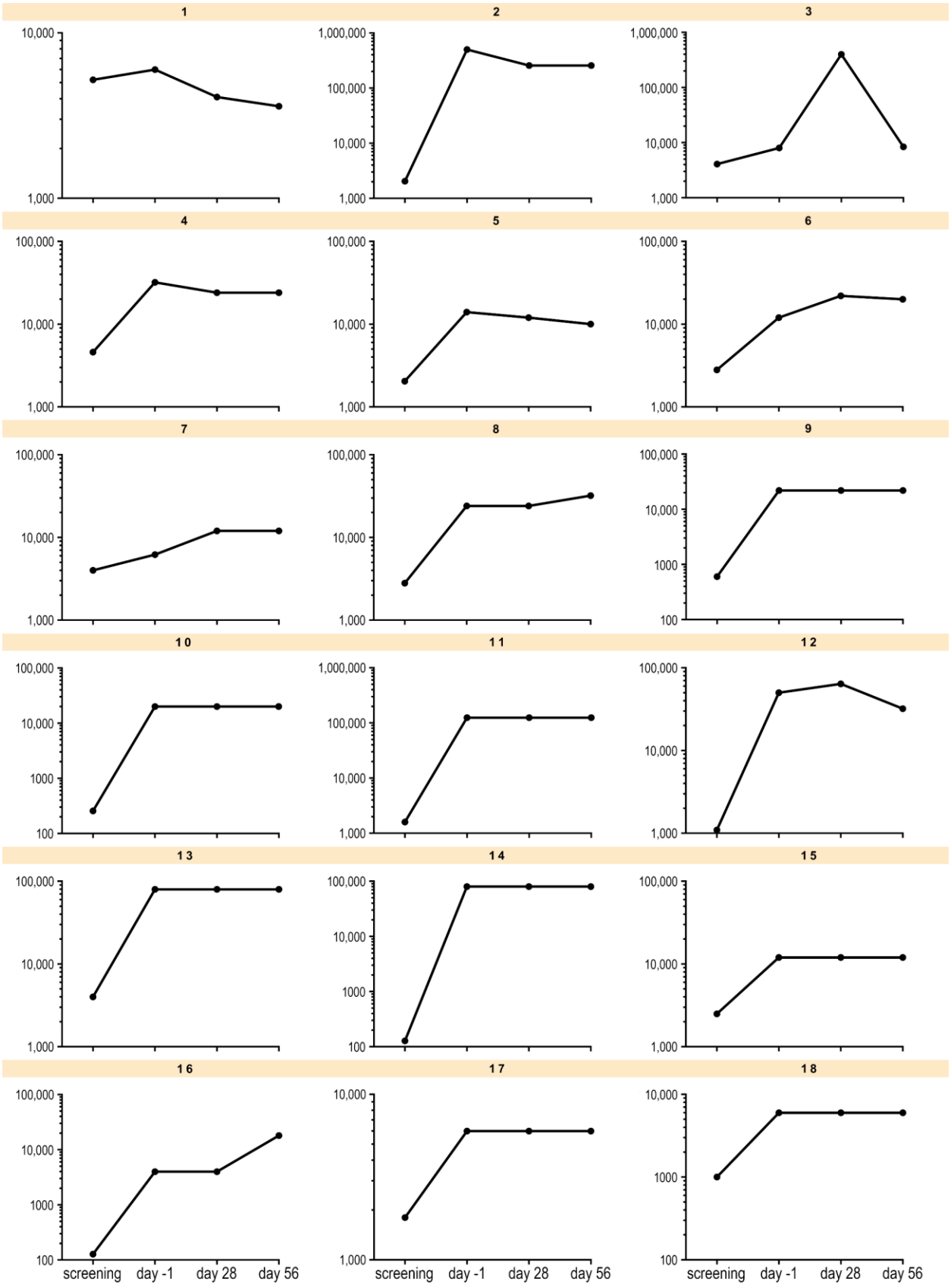
Pat.	Age at tx	PVSRIPO Dose Level	Prior Recurr. (#)	Prior BEV failure	KPS at tx	BEV post-PVSRIPO (mg/kg)	Chemo +/- BEV post-PVSRIPO	OS (months)	IDH1 Status at dx	MGMT Status at dx	MGMT Status at tx
53	45	-2	2	Naïve	90	None	None	13.5+	Mutated	Methylated	Unknown
54	56	-2	1	Naïve	90	7.5-10.0	Lomustine/BEV	11.0	Unknown	Unknown	Unknown
55	61	-1	2	Yes	90	7.5	None	7.9	Wildtype	Methylated	Unknown
56	29	-1	1	Naïve	90	7.5	Lomustine/BEV	11.9+	Unknown	Unknown	Unknown
57	39	-1	1	Naïve	90	7.5	Lomustine/BEV	10.2	Mutated	Unmethylated	Unmethylated
58	60	-1	4	Yes	90	7.5	None	11.4+	Wildtype	Methylated	Unknown
59	56	-1	1	Naïve	90	7.5	Lomustine/BEV	9.9	Wildtype	Methylated	Unknown
60	63	-1	1	Naïve	80	7.5	Lomustine/BEV, CPT-11/BEV	10.5+	Wildtype	Unmethylated	Unmethylated
61	57	-1	1	Naïve	80	7.5	CPT-11/BEV	9.8+	Unknown	Unknown	Unmethylated

*'Bev post-PVSRIPO' represents treatment with bevacizumab alone after administration of PVSRIPO and prior to any other treatments. 'Chemo +/- BEV post-PVSRIPO' represents treatment with chemotherapy alone or in combination with bevacizumab after going off the PVSRIPO study. For OS, '+' indicates the patient was alive as of 03/20/18.

Patient nr.	CD155 cytopl./membr. at dx			
	3+	2+	1+	-
1	80	20		
2		90	10	
3	10	90		
4				
5				
6		90	10	
7		30	20	
8		90		
9				
10		100		
11	70	30		
12				
13				
14				
15				
16				
17		75	25	
18		100		
19				
20		20	80	
21		80		
22	10	70	10	
23		80	20	
24		30	70	
25		80	10	
26				
27	100			
28				
29		20	80	
30		100		
31				

Patient nr.	CD155 cytopl./membr. at dx			
	3+	2+	1+	-
32		70	30	
33		100		
34		90	10	
35		70	30	
36		80	20	
37		80	20	
38				
39	90	10		
40		100		
41		60		
42		100		
43				
44		80		
45	20	80		
46		60	30	10
47				
48		100		
49		100		
50	100			
51		80		
52		70		
53		80		
54				
55		90		
56				
57		80		
58	80			
59	90			
60		90		
61				

Figure S1. CD155 immunohistochemistry in tumor tissues resected prior to enrollment of 43 out of 61 patients in the PVSRIPO clinical trial. Validation of the assay and the reagents used, and scoring for CD155 expression levels have been described previously[9]. Blue shading indicates that tissue was not available for analysis.


1
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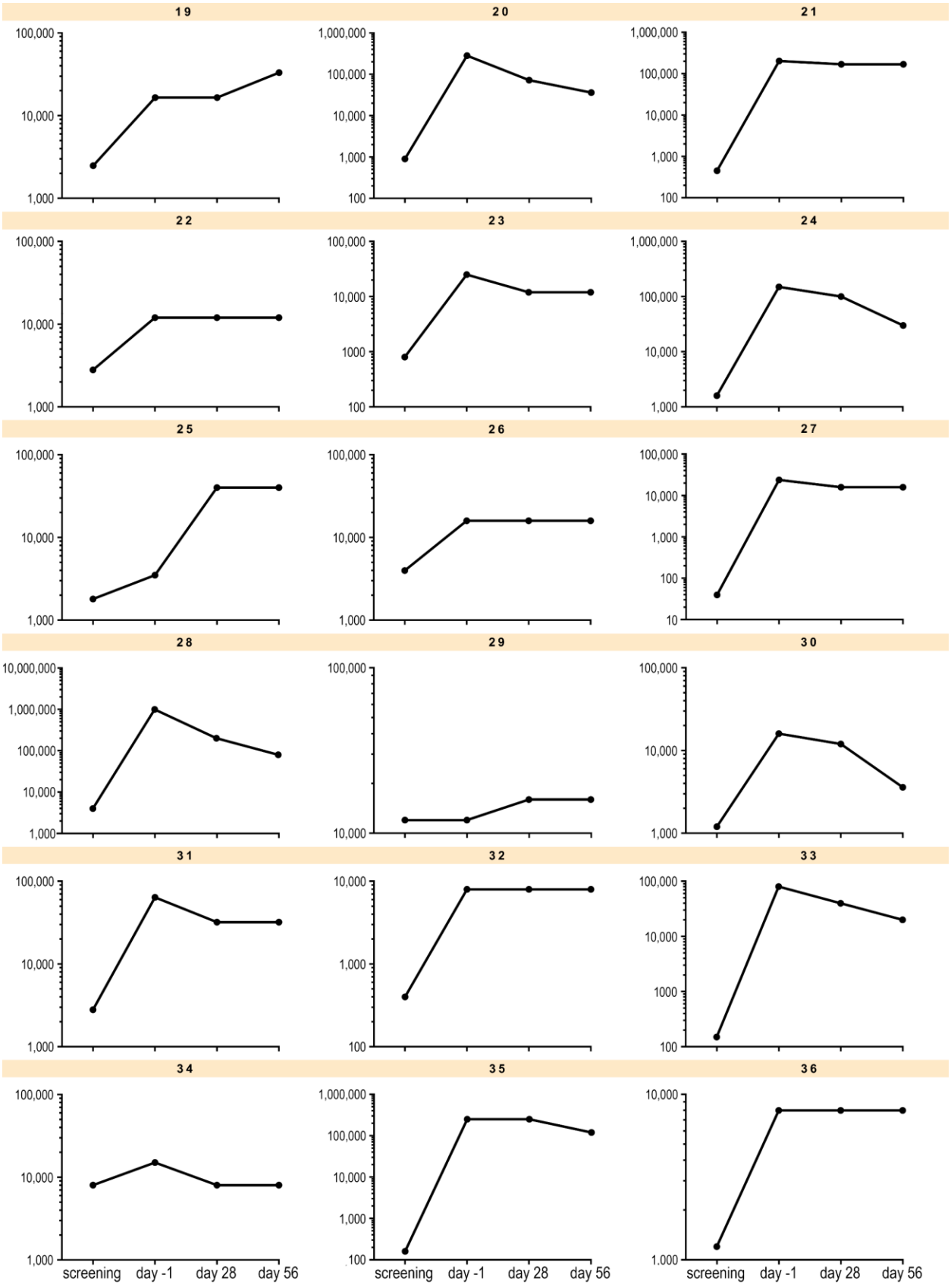
15
16
17
18
screening
day -1
day 28
day 56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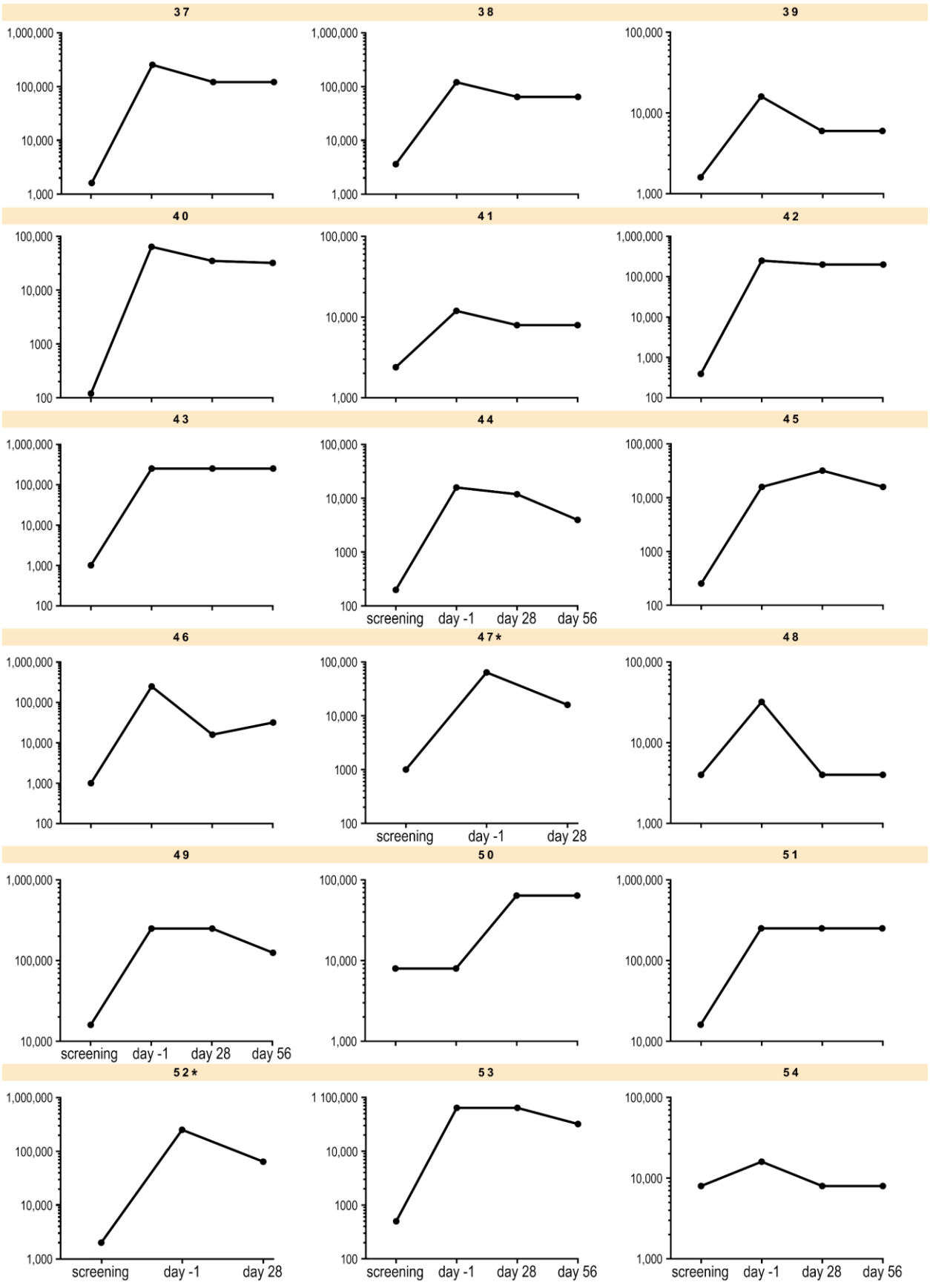
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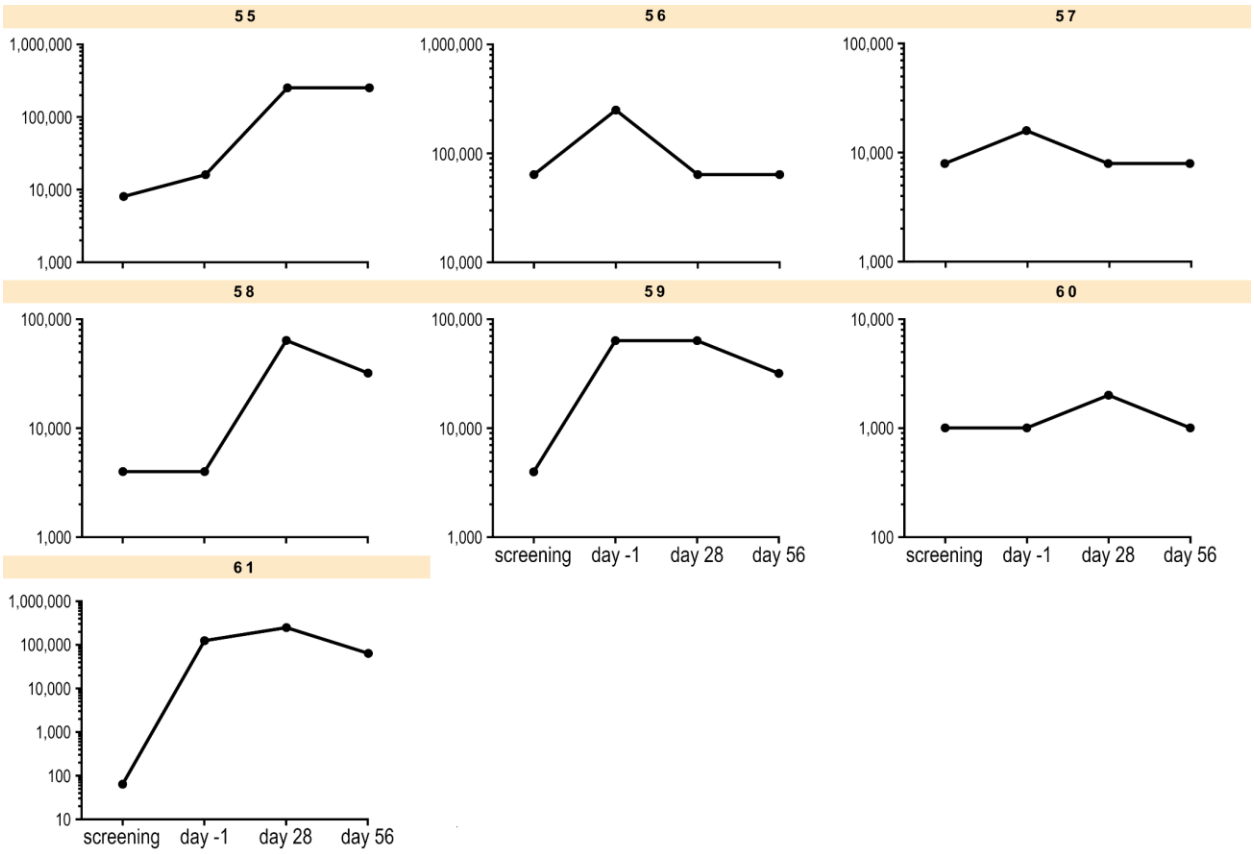


Figure S2. Serum anti-poliovirus (type 1) neutralizing antibody titers. Anti-poliovirus (type 1) neutralizing antibody titers in patients treated with PVSRIPO at the screening stage (pre-boost immunization, pre-PVSRIPO), day -1 (post-boost immunization, pre-PVSRIPO), and days 28/56 (post-boost immunization, post-PVSRIPO). Asterisks indicate two patients where a day 56 sample was not available for testing. The range of titers in all patient samples at the screening stage was 40-64,000; and 1,000-1,000,000 (day -1); 1,600-400,000 (day 28); 1,600-256,000 (day 56) thereafter.

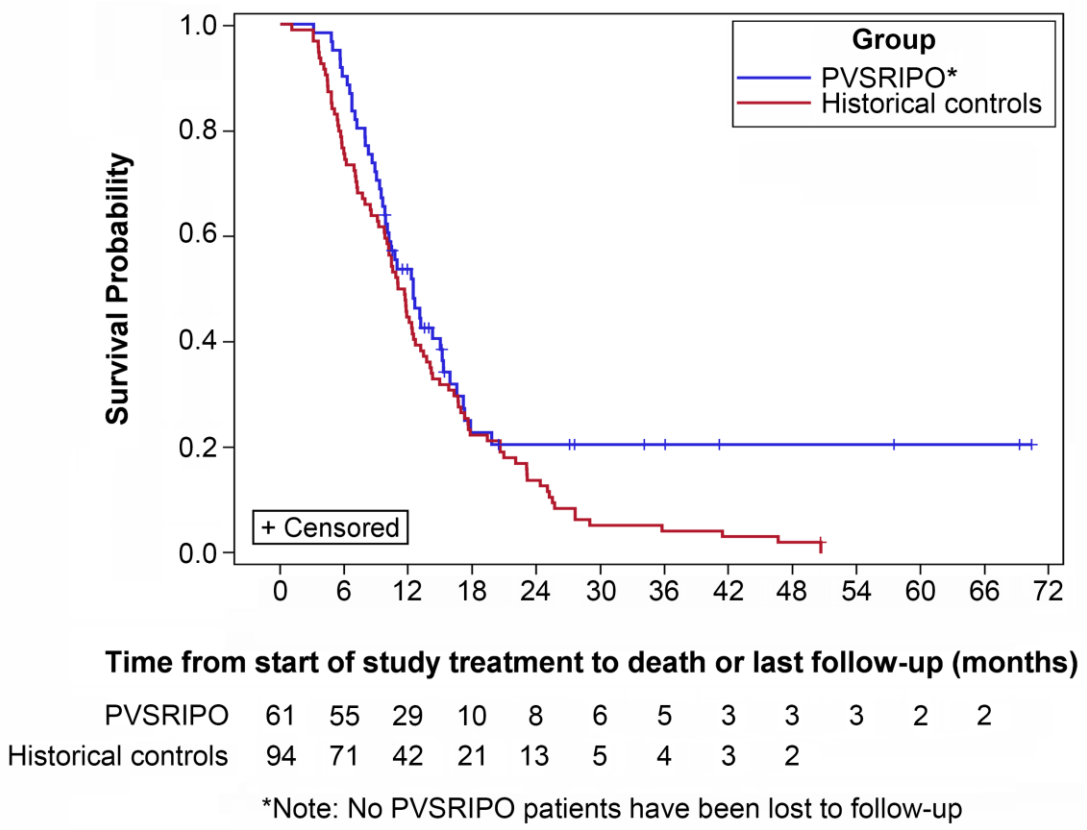


Figure S3. Survival of PVSRIPO patients and the historical control cohort excluding biopsy-only patients. Overall survival sensitivity analysis in which the historical control cohort (n=104) was reduced to 94, by removing 10 patients who had received only a biopsy at the time of diagnosis (see **Table 1**; compare **Figure 1**).

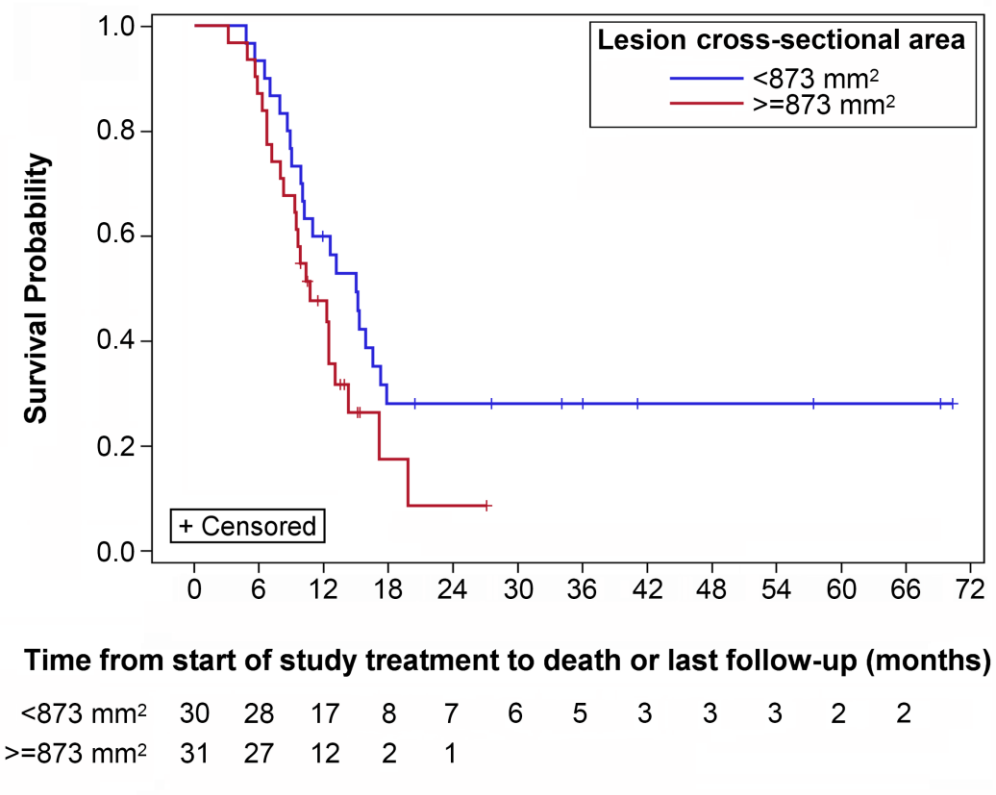


Figure S4. Survival according to lesion cross-sectional area.

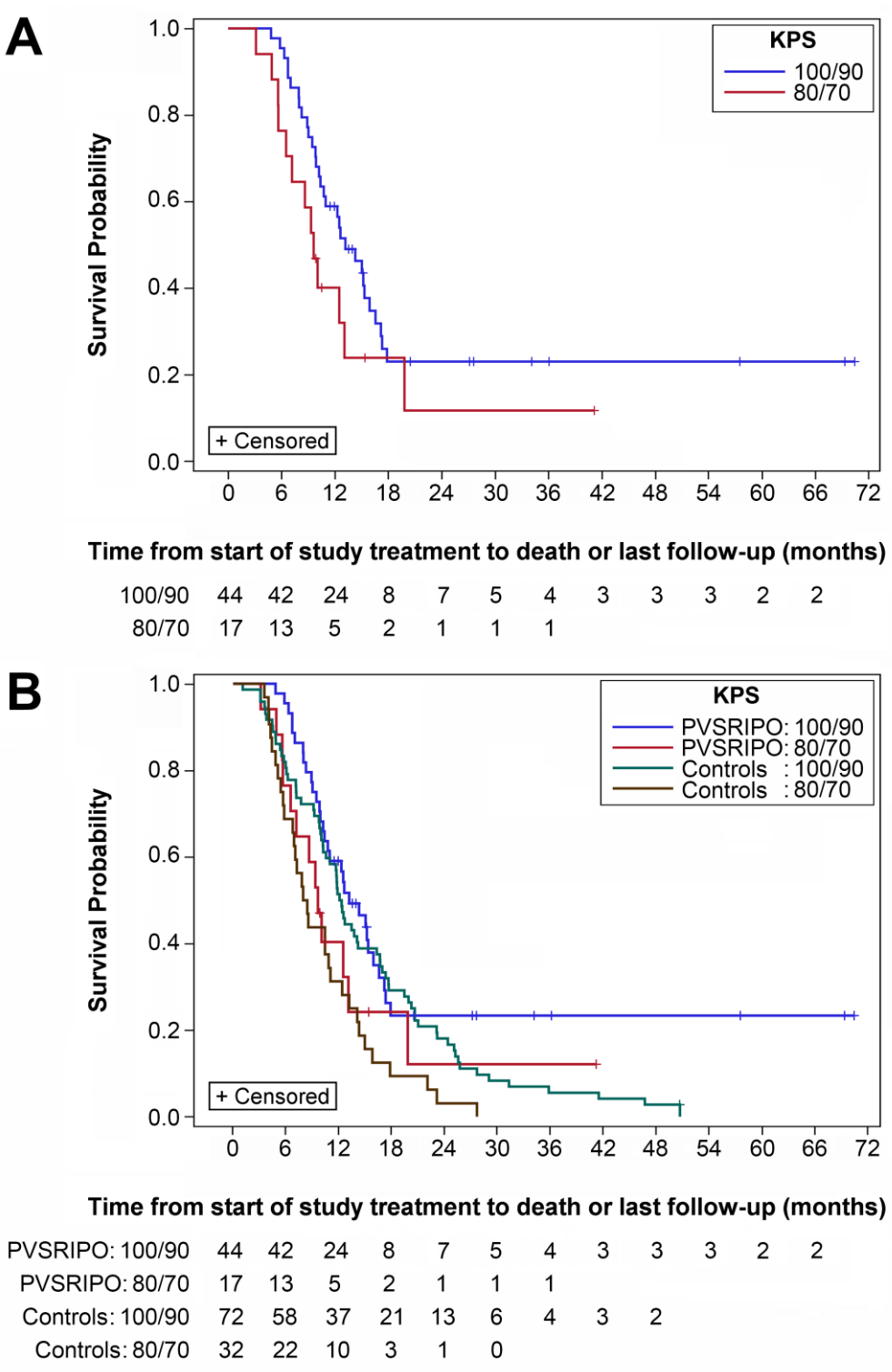


Figure S5. Survival according to KPS. A. PVSRIPO patients stratified by KPS level. **B.** PVSRIPO and historical control patients stratified by KPS level.

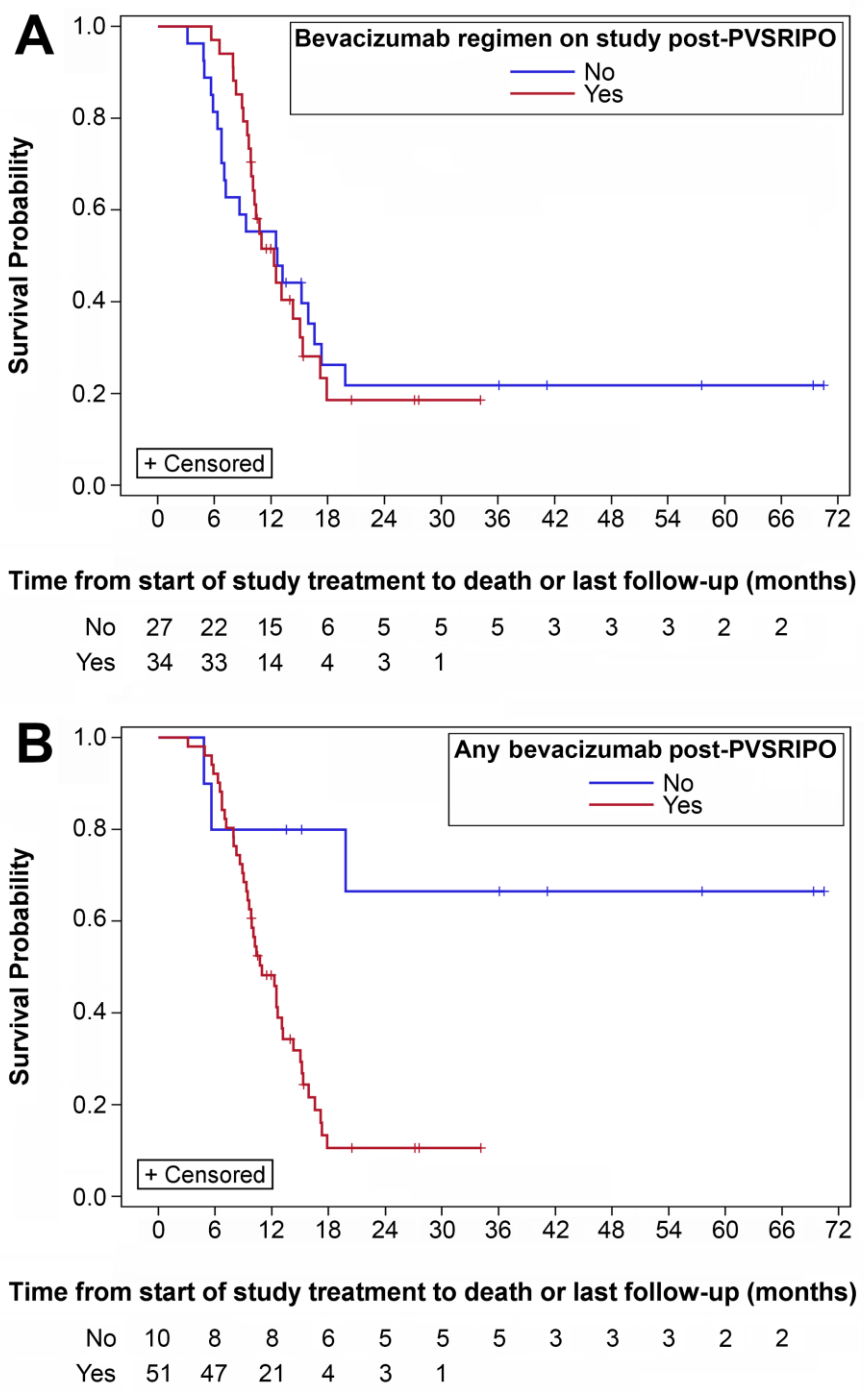


Figure S6. Survival according to bevacizumab use. A. PVSRIPO patients receiving bevacizumab on study at 7.5 mg/kg IV every 3 weeks. **B.** PVSRIPO patients receiving any bevacizumab-containing regimen on study or not.

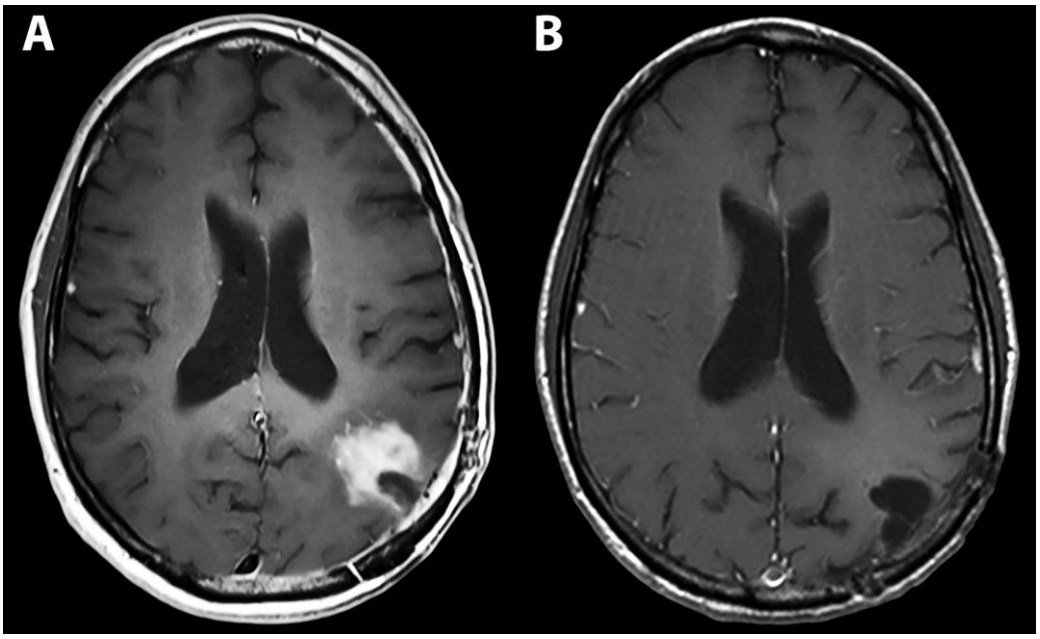


Figure S7. MRIs from patient 50 (dose level -2). Post-contrast axial T1 MRIs at baseline (**A**) and 9 months after PVSRIPO infusion (**B**), showing complete response.

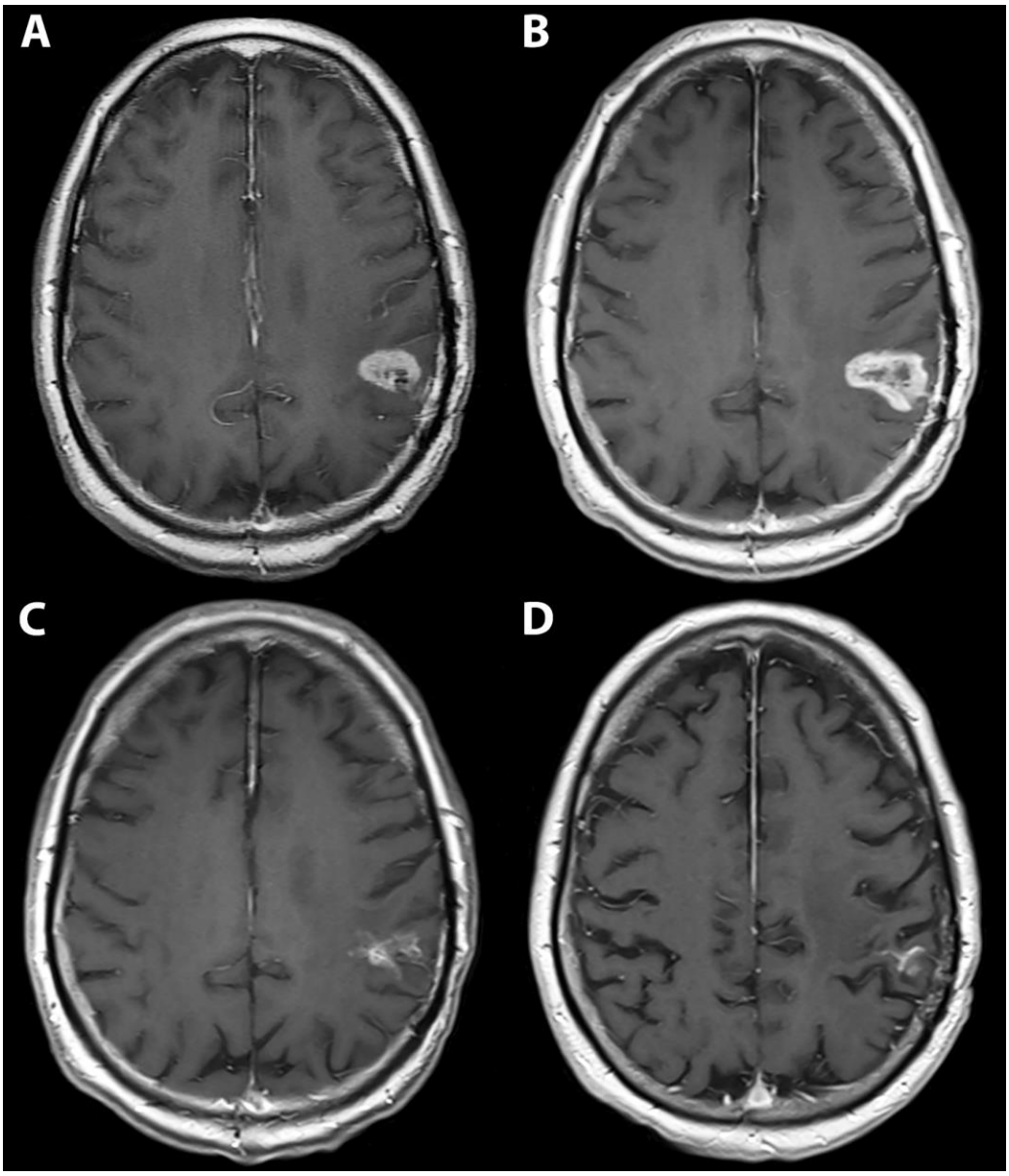


Figure S8. MRIs from patient 2 (dose level 2). A. Post-contrast axial T1 MRI at baseline. **B.** Increased tumor size and characteristic ‘soap-bubble’ appearance 4 months post PVSRIPO infusion. **C., D.** Tumor contraction 14 months (**C**) and 35 months (**D**) post infusion.

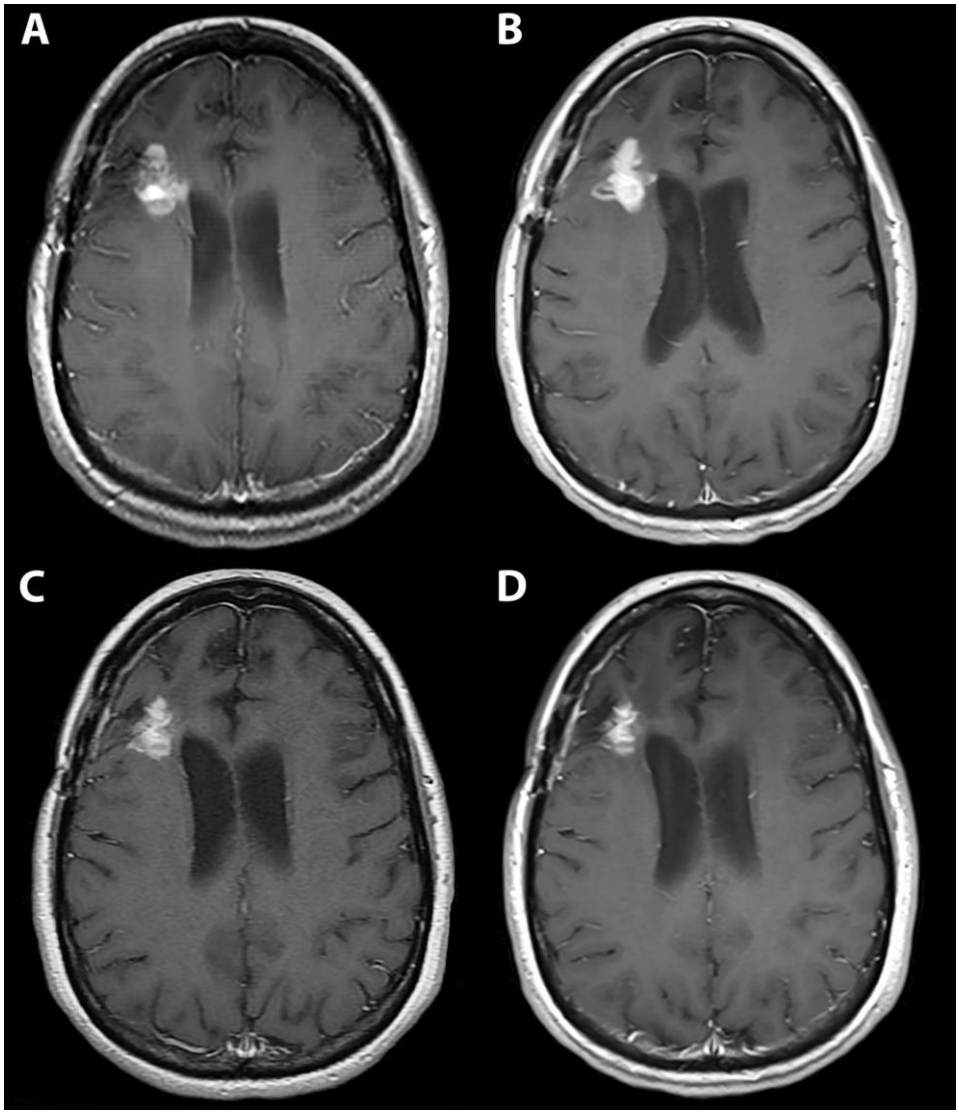


Figure S9. MRIs from patient 22 (dose level -1). A. Post-contrast axial T1 MRI at baseline. **B.** Enhanced tumor size and brightness 2 months post PVSRIPO infusion. **C., D.** Successive tumor contraction 12 months (**C**) and 22 months (**D**) post infusion.

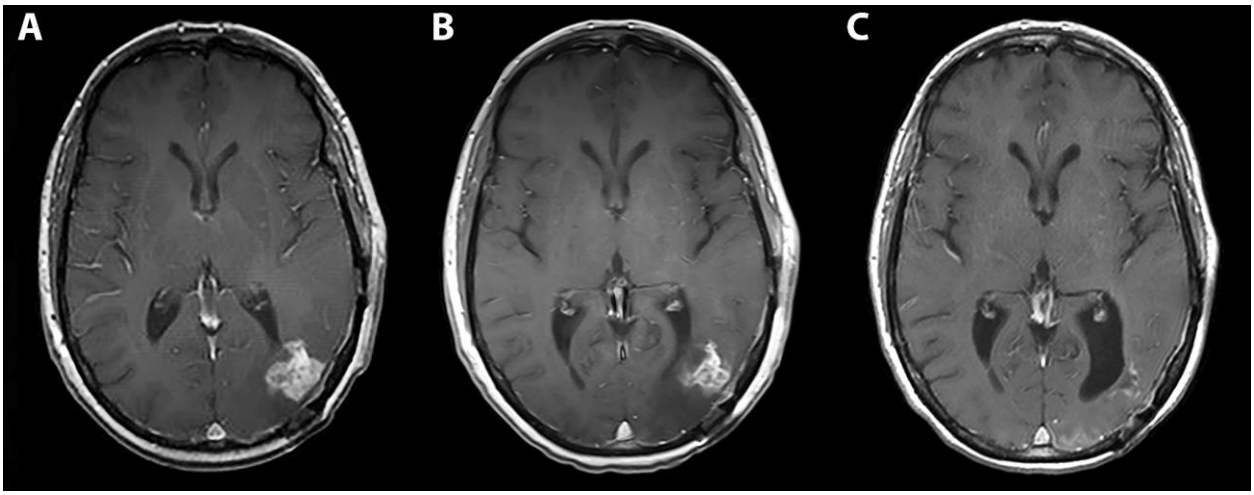
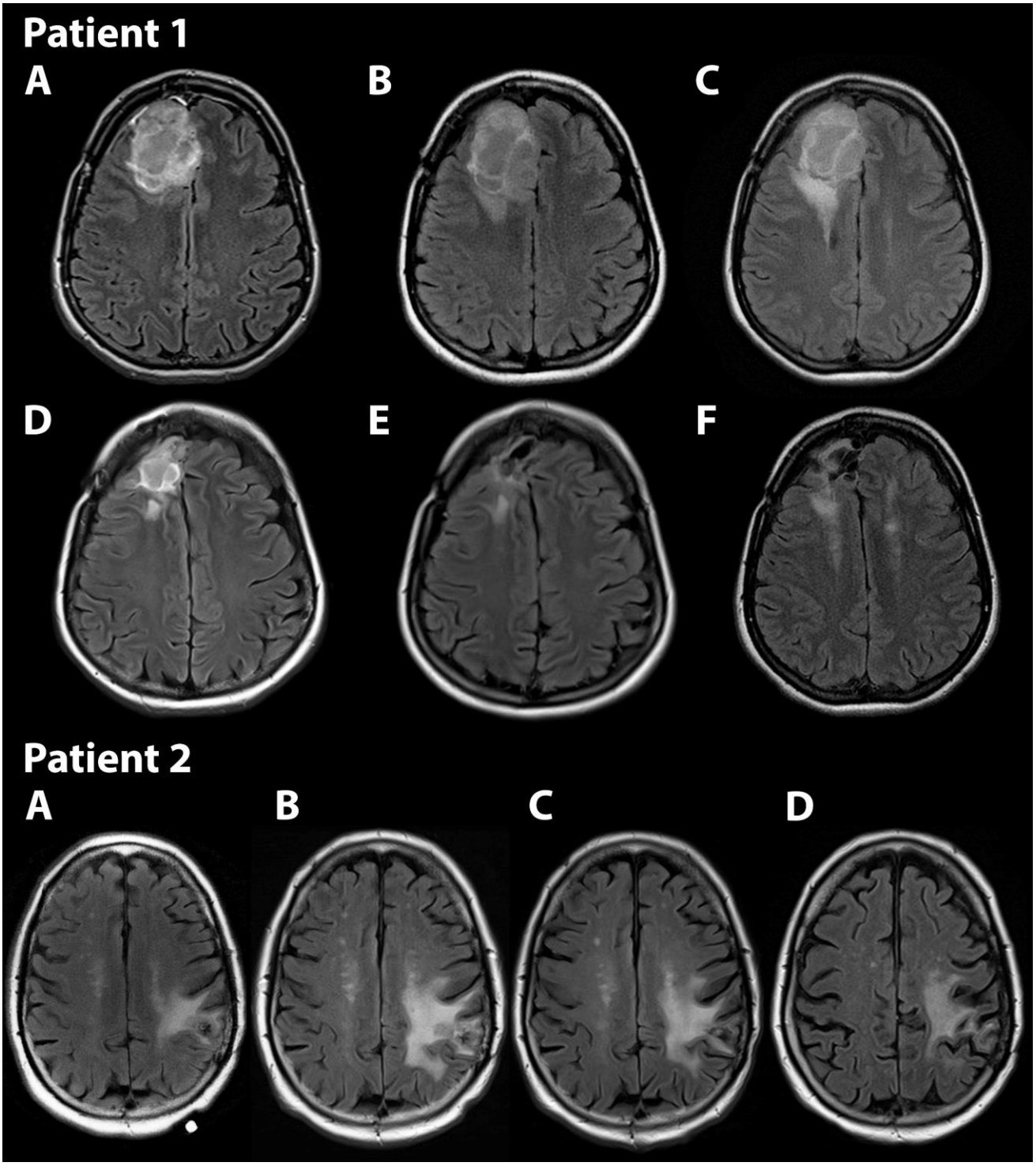
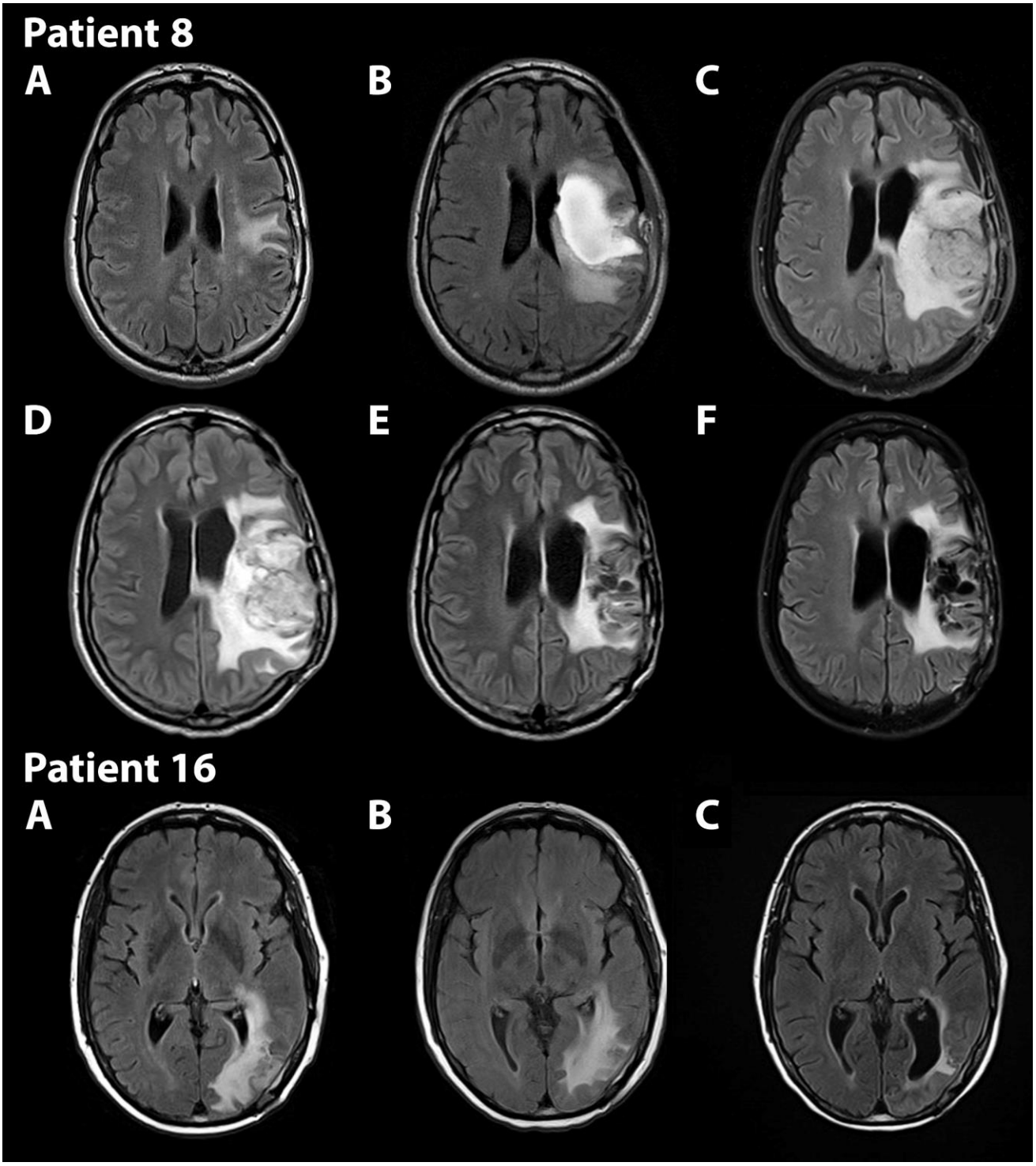


Figure S10. MRIs from patient 16 (dose level -1). A. Post-contrast axial T1 MRI at baseline. **B.** Initial tumor contraction and ‘soap-bubble’ appearance 1 month post infusion. **C.** Contraction of treated tumor 21 months post infusion.


Patient 1
A
B
C
D
E
F
Patient 2
A
B
C
D


Patient 8
A
B
C
D
E
F
Patient 16
A
B
C

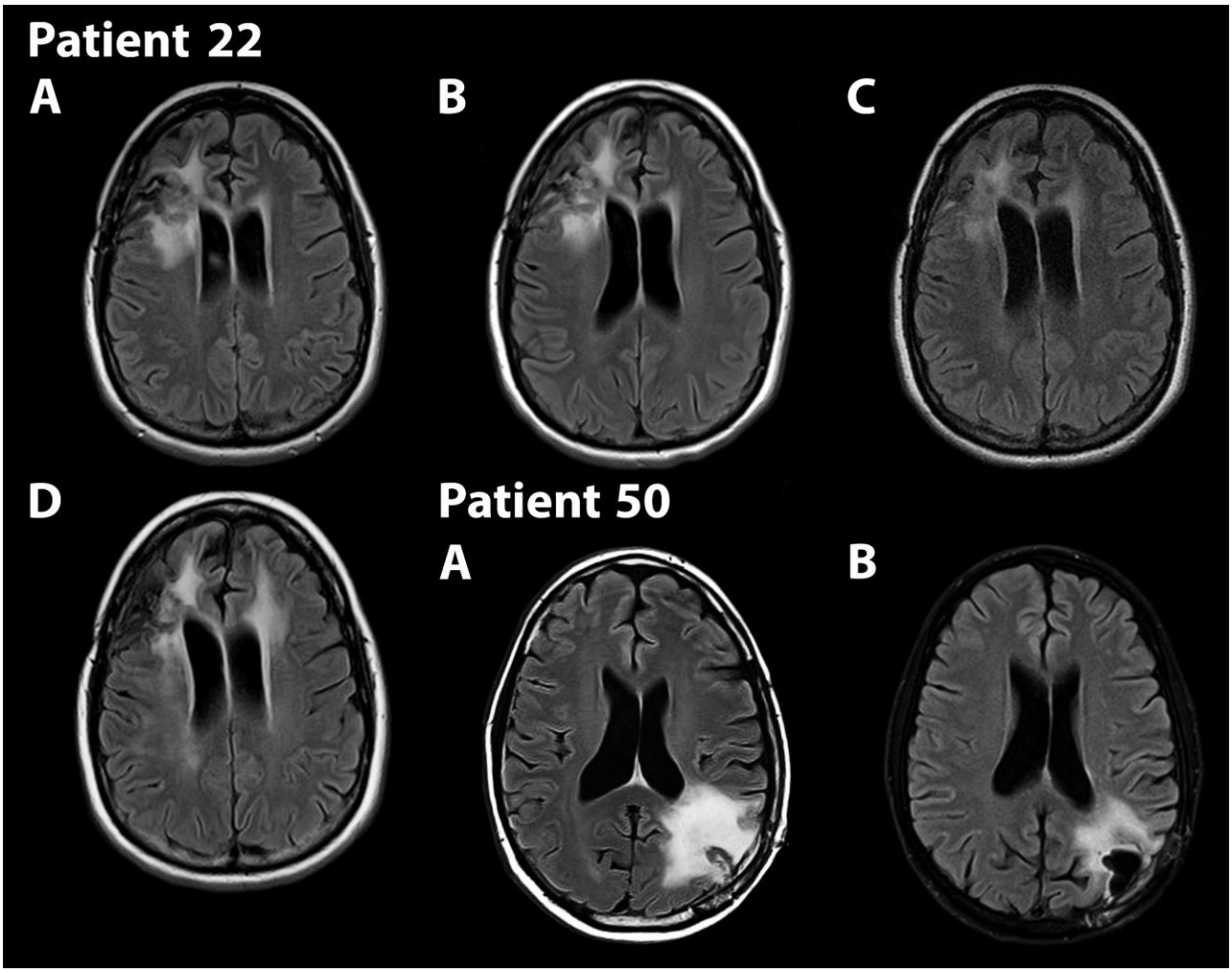


Figure S11. Flair MRIs from patient 1, 2, 8, 16, 22 and 50. Fluid attenuation inversion recovery (FLAIR) images from patients shown in Figure 2 (patient 1), Figure 3 (patient 8), Supplemental Figure S7 (patient 50), Supplemental Figure S8 (patient 2), Supplemental Figure S9 (patient 22), and Supplemental Figure S10 (patient 16). The labeled individual scans correspond to those in the post-contrast axial T1 MRIs shown in the referenced Figures/ Supplemental Figures.

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