Supplemental appendix

The authors have provided this appendix to give readers additional information about their work.

Supplement to: Rixe, et al. *Clin Cancer Res.* 2024

Supplementary methods

Dose-limiting toxicity definitions

Dose-limiting toxicities (DLTs) were defined as any of the following treatment-related AEs or laboratory abnormalities:

- Any grade febrile neutropenia or neutropenic infection
- Grade 4 neutropenia lasting > 7 days
- Grade 4 thrombocytopenia
- Grade 3 thrombocytopenia with clinically significant bleeding or Grade 3 thrombocytopenia requiring a platelet transfusion
- Grade 4 anemia
- Grade ≥3 non-hematological toxicity (excluding alopecia, lymphopenia and Grade 3 nausea, vomiting, diarrhea, and electrolyte imbalances lasting less than 48 hours)
- Grade 4 nausea, vomiting, diarrhea, or electrolyte imbalances or Grade 3 nausea, vomiting, diarrhea, and electrolyte imbalances lasting greater than 48 hours despite optimal medical intervention.

Treatment administration

BXQ-350 was administered intravenously, over 45-minute (± 15 minutes) infusions. The administration was as follows for the first six cycles:

Cycle 1 Week 1	Cycle 1 Week 2	Cycle 1 Week 3 and 4	Cycle 2 and thereafter
Days 1-5 (5 consecutive days)	3×/week (Every other day)	Once every 7 (±3) days	Once every 28 (±3) days

After 6 cycles, patients who demonstrated evidence of ongoing benefit and without disease progression, based on radiological and clinical criteria, could continue treatment every 28 days.

According to the investigator’s judgment, all patients could receive pre-medication with diphenhydramine 25–50 mg (or equivalent).

Key inclusion criteria

- Age ≥ 18 years with an Eastern Cooperative Oncology Group performance status (ECOG PS) 0–2.
- Histologically or cytologically confirmed diagnosis of an advanced cancerous solid tumor for which there was no further standard therapy or when standard therapy was contraindicated.
- Patients with high-grade gliomas (HGG) must have shown unequivocal evidence for recurrence or progression by magnetic resonance imaging (MRI) scan or had histologically proven tumor recurrence.
- Patients with HGG had to have previously received radiation therapy and temozolomide.
- Patients with HGG and receiving glucocorticoid therapy had to be on stable or decreasing equivalent daily dose of glucocorticoids for 2 weeks (14 days) prior to dose assignment.
- Measurable or non-measurable disease per RECIST v1.1 for solid tumors and RANO criteria for HGG.
- Acceptable liver function, which was defined as:
    - Total serum bilirubin ≤1.5 × upper limit of normal for the study site (ULN) (in patients with known Gilbert Syndrome, total bilirubin ≤3 × ULN, with direct bilirubin ≤1.5 × ULN)
    - Aspartate aminotransferase (AST), serum glutamic oxaloacetic transaminase (SGOT), alanine aminotransferase (ALT), serum glutamic-pyruvic transaminase (SGPT) ≤3 × ULN (if liver metastases were present, then ≤5 × ULN was allowed)
    - Serum albumin ≥3 g/dL.
- Acceptable renal function, defined as serum creatinine ≤1.5 × ULN, or calculated creatinine clearance ≥ 45 mL/min for patients with creatinine levels above 1.5 mg/dL.
- Acceptable bone marrow function, which was defined as:
    - Absolute neutrophil count (ANC) ≥1,500 cells/mm^3
    - Platelet count ≥100,000 cells/mm^3
    - Hemoglobin > 9.0 g/dL.

- Acceptable coagulation parameters:
    - International normalized ratio (INR) ≤2 × ULN.
    - Activated partial thromboplastin time (aPTT) within normal limits.

Key exclusion criteria

- Had a concurrent malignancy or have had another malignancy within 1 year before screening (except for adequately treated basal or squamous cell carcinoma, melanoma in situ, early-stage prostate cancer [T1a-cN0M0], ductal carcinoma in situ of the breast or cervical carcinoma in situ).
- Patients with solid tumors: had received anticancer therapies, including radiation therapy, cytotoxic agents, targeted agents, or endocrine therapy within 2 weeks before dose assignment.
- Patients with HGG: anticancer therapies including:
    - Radiation therapy to the current disease site within 12 weeks of dose assignment.
    - Targeted agent therapy within 2 weeks of dose assignment.
    - Nitrosoureas within 6 weeks of dose assignment.
    - Procarbazine within 3 weeks of dose assignment.
    - Other cytotoxic agents within 4 weeks of dose assignment.
- Had not recovered from the toxicity of prior therapy at the time of dose assignment (excluding alopecia, neuropathy, and lymphopenia).
- Had received prior treatment with any investigational drug within 28 days before dose assignment.
- Had major surgery within 28 days before dose assignment or had not recovered from significant side effects of the surgery if more than 4 weeks had elapsed since surgery.
- Had a history of cardiac dysfunction including any of the following:
    - Myocardial infarction within 6 months before initiation of screening.
    - History of documented congestive heart failure within 6 months before screening.
    - Active cardiomyopathy.
    - ECG with QTc >450 ms in male patients or >470 ms in female patients.
- History of human immunodeficiency virus (HIV) seropositivity.
- Pregnant or nursing (lactating).

- Symptomatic brain metastases or leptomeningeal disease.
- Active (acute or chronic) or uncontrolled severe infections.
- Active poor wound healing (delayed healing, wound infection, or fistula).
- Poorly controlled hypertension, defined as blood pressure >160/90 on at least 2 repeated determinations on separate days within 2 weeks (14 days) before screening.
- Evidence of active clinically significant bleeding (e.g., gastrointestinal bleed, hemoptysis, or gross hematuria).

Supplementary results

Supplementary Table S1. Patient disposition in Parts 1, 2, and 3.

Cohort	Part 1 (n=18)					Part 2 (n=37)	Part 3 (n=31)
	0.7 mg/kg (n=1)	**1.1 mg/kg (n=3)**	**1.4 mg/kg (n=3)**	**1.8 mg/kg (n=3)**	**2.4 mg/kg (n=8)**		
Completed 6 cycles	1 (100)	0	0	1 (33)	1 (13)	3 (8)	5 (16)
Discontinued before 6 cycles	0	3 (100)	3 (100)	2 (67)	7 (88)	34 (92)	26 (84)
Disease progression	0	3 (100)	3 (100)	2 (67)	5 (63)	28 (76)	22 (71)
Withdrew consent	0	0	0	0	1 (13)	3 (8)	1 (3)
Death	0	0	0	0	0	2 (5)	1 (3)
AE	0	0	0	0	0	1 (3)	2 (6)
Lost to follow-up	0	0	0	0	1 (13)	0	0

AE, adverse event.

Supplementary Table S2. Representativeness of study participants

Cancer type	Advanced/recurrent solid tumors
Considerations related to:	
Sex	In 2022 in the United States (US), 983,160 and 934,870 new cases of cancer were estimated to occur in male and female patients, respectively. Of these new cases, more than 90% were estimated to be solid tumors in both male and female patients.[1,2]
Age	The median age of diagnosis of cancer at any site in the US is 67 years. Cancer is most frequently diagnosed between the ages of 65 to 74 years. The median age at death is 73 years.[2]
Race/ethnicity	In the US, the incidence rates for cancer are highest among White people and lowest among Asian people or Pacific Islanders. However, Black patients have the highest mortality rates.[1,2]
Geography	In patients of all ages, cancer is the second leading cause of death in the United States. Cancer is the leading cause of death for both male and female patients aged 60 to 79 years.[1]
Overall representativeness of this study	The median (range) age in this study was 59 (24–69), 55 (27–80), and 59 (26–81) years in Parts 1, 2, and 3, respectively, slightly lower than the median age of patients diagnosed with cancer in the US overall. In Parts 1, 2, and 3 of this study, patients were 61%, 65%, and 48% male, respectively, broadly consistent with higher rates of diagnosis in male patients than female patients in the US. White patients comprised ~95% of the patients in this study, followed by Asian and Black patients (~2% each). This study was conducted at 4 sites in the US, potentially limiting the representativeness of racial distributions compared with populations in other geographic areas. As a pan-tumor phase 1 study, a broad range of solid tumors was included.

TableS2 references:

1. Siegel R, et al. *CA Cancer J Clin* 2022;72:7–33; doi: 10.3322/caac.21708;

2. SEER*Explorer: An interactive website for SEER cancer statistics [Internet]. Surveillance Research Program, National Cancer Institute; 2024 Apr 17. [cited 2024 May 2]. Available from: https://seer.cancer.gov/statistics-network/explorer/. Data source(s): SEER Incidence Data, November 2023 Submission (1975-2021), SEER 22 registries.

Supplementary Table S3. BXQ-350 PK parameters in Part 2, Day 1 (2.4 mg/kg)

PK parameter, mean (SD)	**2.4 mg/kg (n=37)**
$C_{max,}$ ng/mL	25522 (25051)
$AUC_{0-\infty}$, h·ng/mL	45127 (19664)
CL, mL/h/kg	60.2 (23.2)
$V_{ss,}$ mL/kg	165.5 (50.6)
$t_{1/2,}$ h	4.02 (0.75)

$AUC_{0-\infty}$, area under the concentration-time curve; C_{max}, maximum plasma concentration; CL, clearance; SD, standard deviation; $t_{1/2}$, terminal elimination half-life; V_{ss}, volume of distribution at steady state.

Supplementary Table S4. ADAs among patients with PFS >6, >12, and >24 months

	>6 months (n=8)	**>12 months (n=7)**	**>24 months (n=4)**	**Still on study (n=2)**
No detectable ADAs	5 (63)	6 (86)	3 (75)	1 (50)[a]
Detectable ADAs	3 (38)	1 (14)	1 (25)	1 (50)

Data are n (%).

[a]For the first two years.

ADA, anti-drug antibody; PFS, progression-free survival.

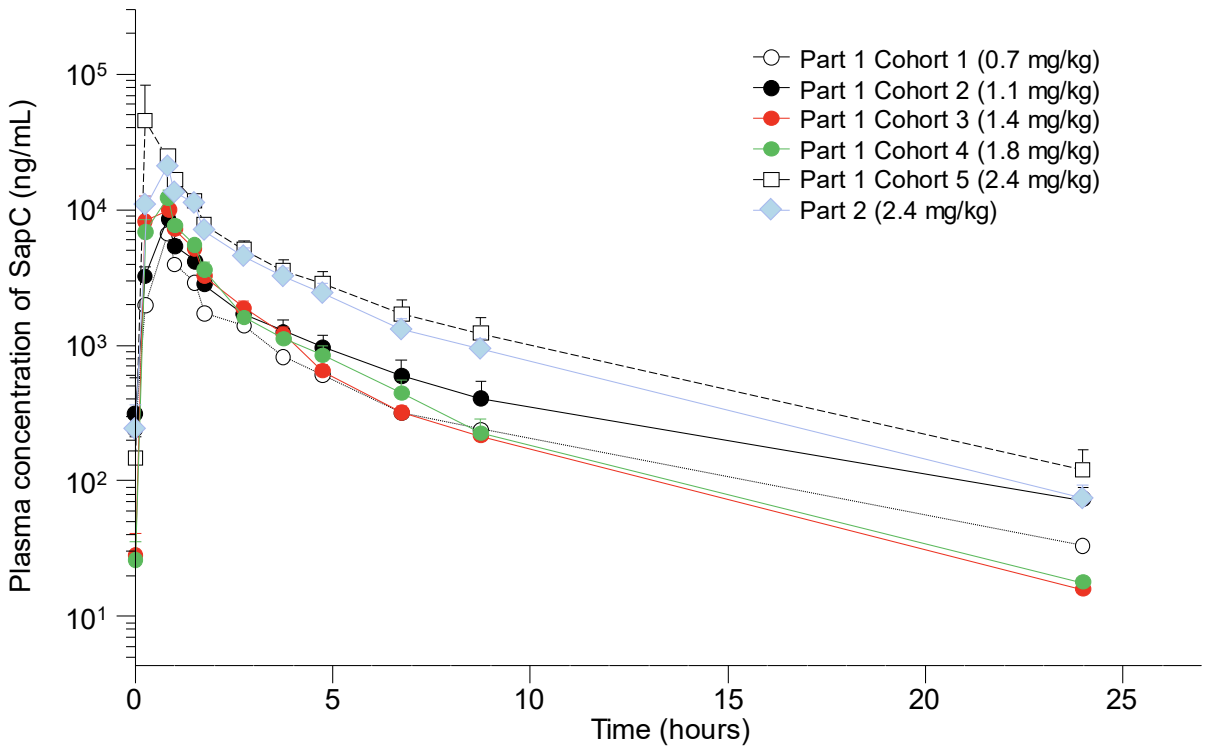
Supplementary Table S5. Narratives for patients with long-term clinical benefit continuing to receive BXQ-350.

Patient	Primary tumor type	Study part and dose	Narrative
1	Recurrent GBM	Part 1 (0.7 mg/kg; subsequently increased to 2.4 mg/kg)	This male patient received 30 doses of BXQ-350 during his enrolment in this study. Between Days 394 and 562, MRI scans showed progressive contrast enhancement despite no clinical neurologic decline, at which time he was withdrawn from the study due to suspected disease progression. However, a subsequent subtotal resection revealed necrotic tissue and not disease progression; therefore, the patient was deemed to have had clinical benefit from BXQ-350 (and not disease progression). Given this finding, the patient was enrolled in the expanded-access continued-treatment study.
2	Metastatic rectal adenocarcinoma	Part 1 (1.8 mg/kg; subsequently increased to 2.4 mg/kg)	This female patient received 51 doses of BXQ-350 during enrolment on this study (RECIST sum of diameters of 1.5 cm at screening) until Day 1185, when she had a lesion sum of diameters of 1.3 cm. At that time, she was enrolled in the expanded-access continued-treatment study.

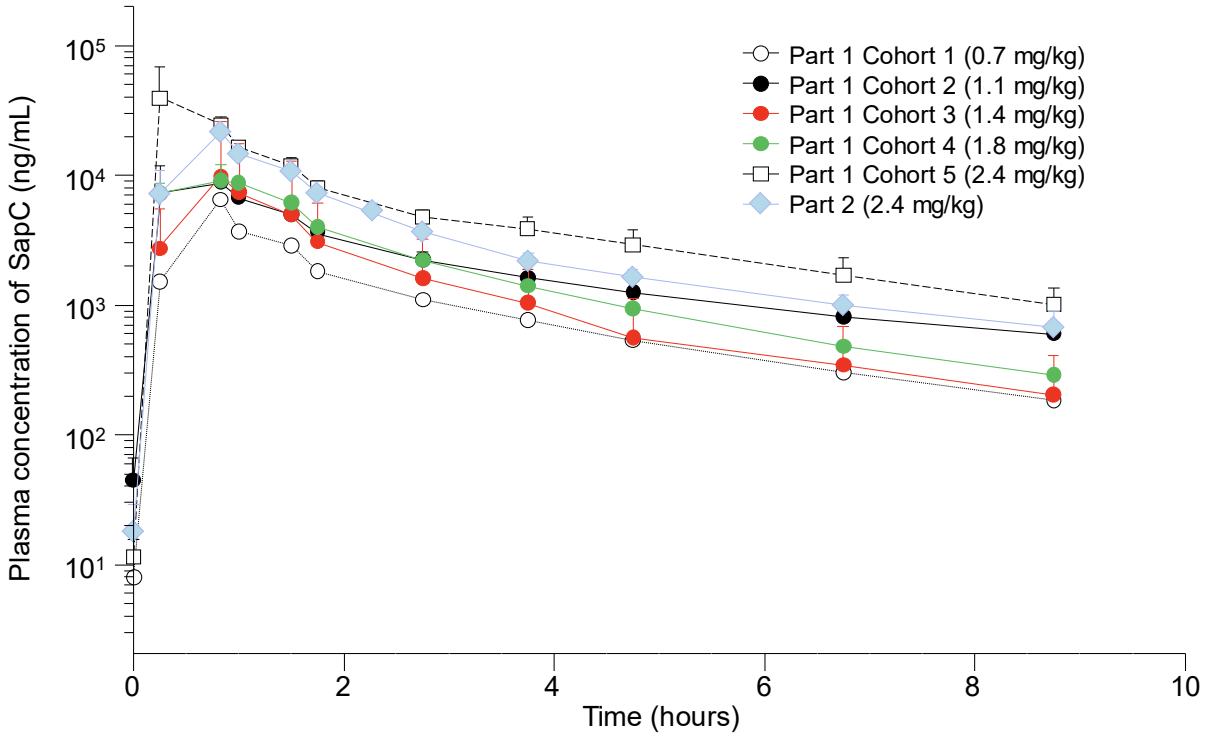
GBM, glioblastoma multiforme; MRI, magnetic resonance imaging.

Supplementary Figure S1. Mean concentration-time profiles of SapC on (A) day 4 and (B) day 22

A



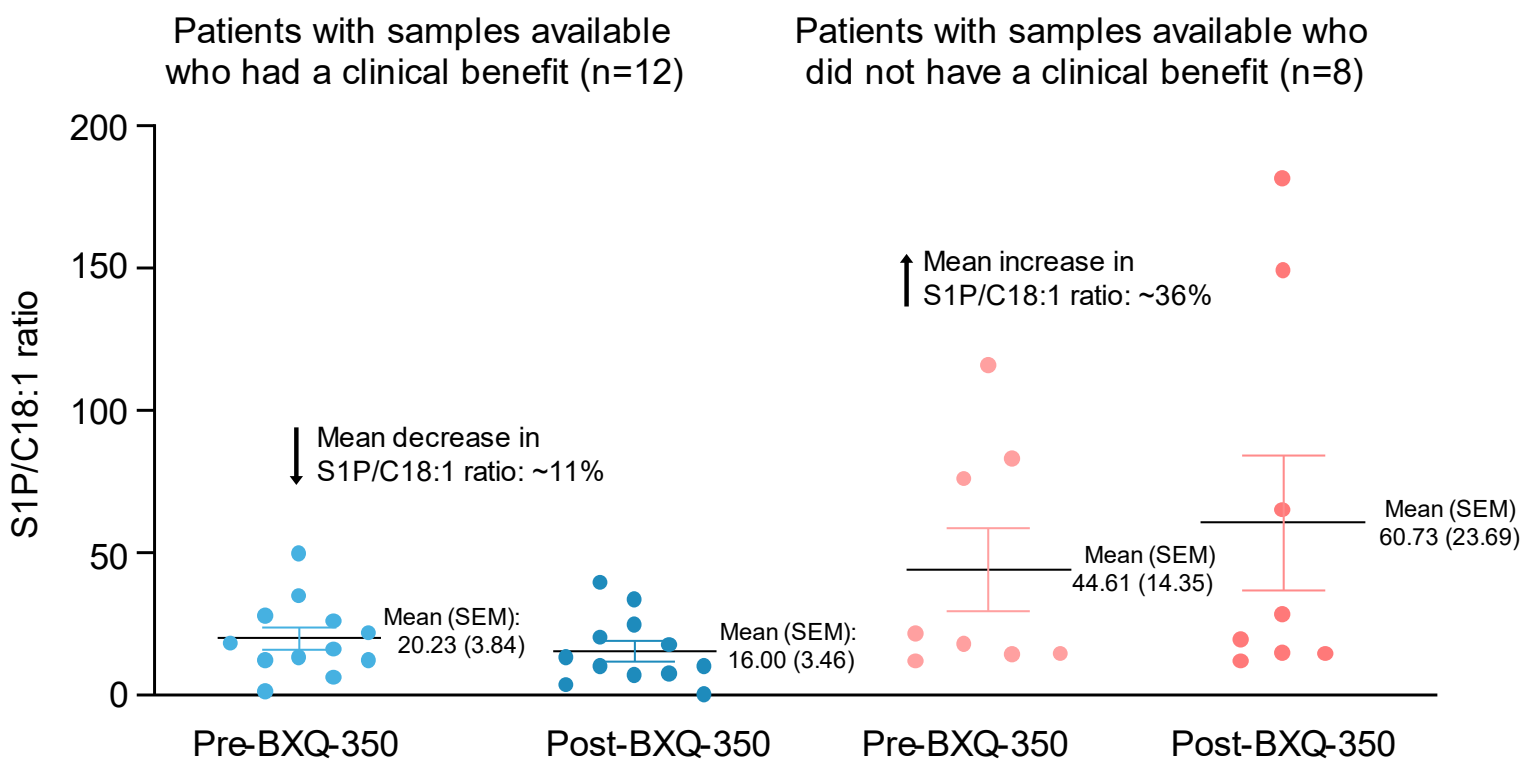
B



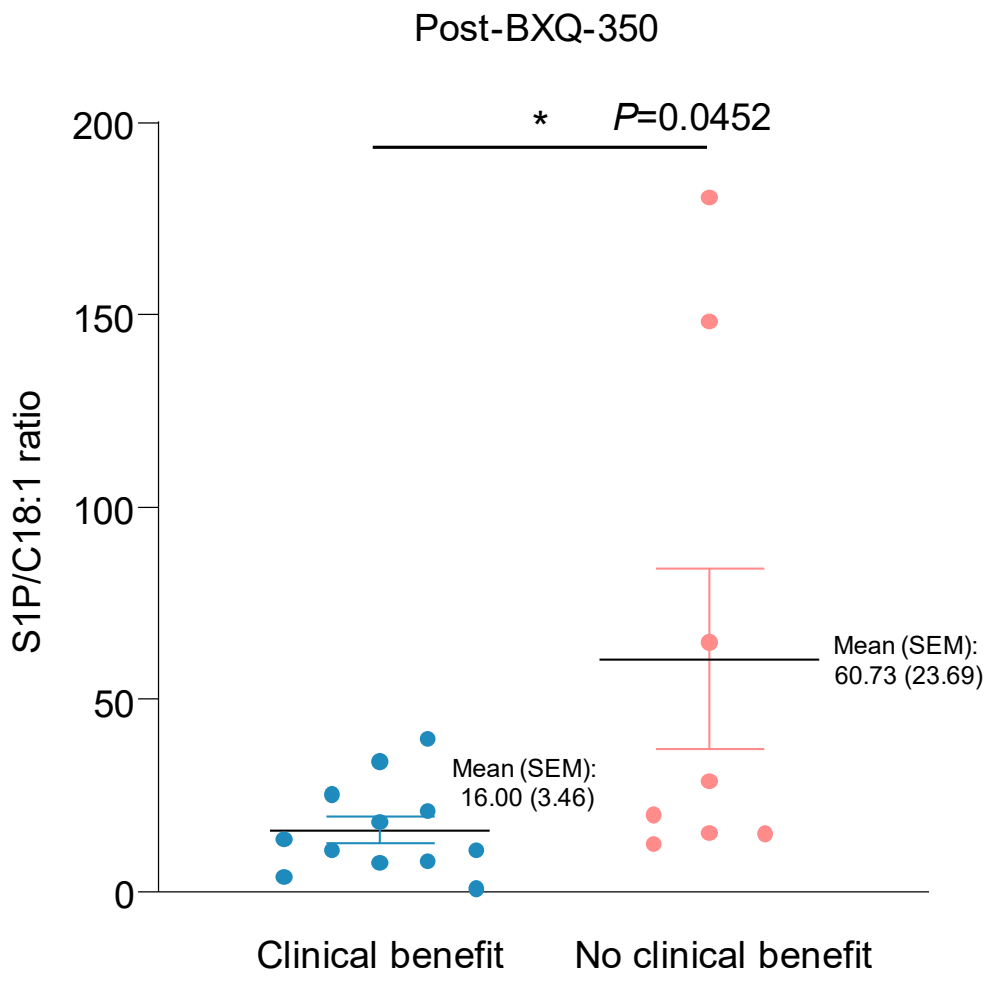
SapC, saposin C.

Supplementary Figure S2. Mean percent changes in S1P/C18:1 ratios following BXQ-350 treatment among patients for which samples were available: (**A**) Comparison of the pre- versus post-BXQ-350 S1P/C18:1 changes for the subset of patients experiencing a clinical benefit (CB; subset of 12/13 patients) and for the subset of patients who did not experience a CB (8 patients); (**B**) Comparison of the S1P/C18:1 ratios following BXQ-350 for the CB subset versus non-CB subset; (**C**) Comparison of the Pre vs. Post S1P/C18:1 changes for the four patients with ependymoma.

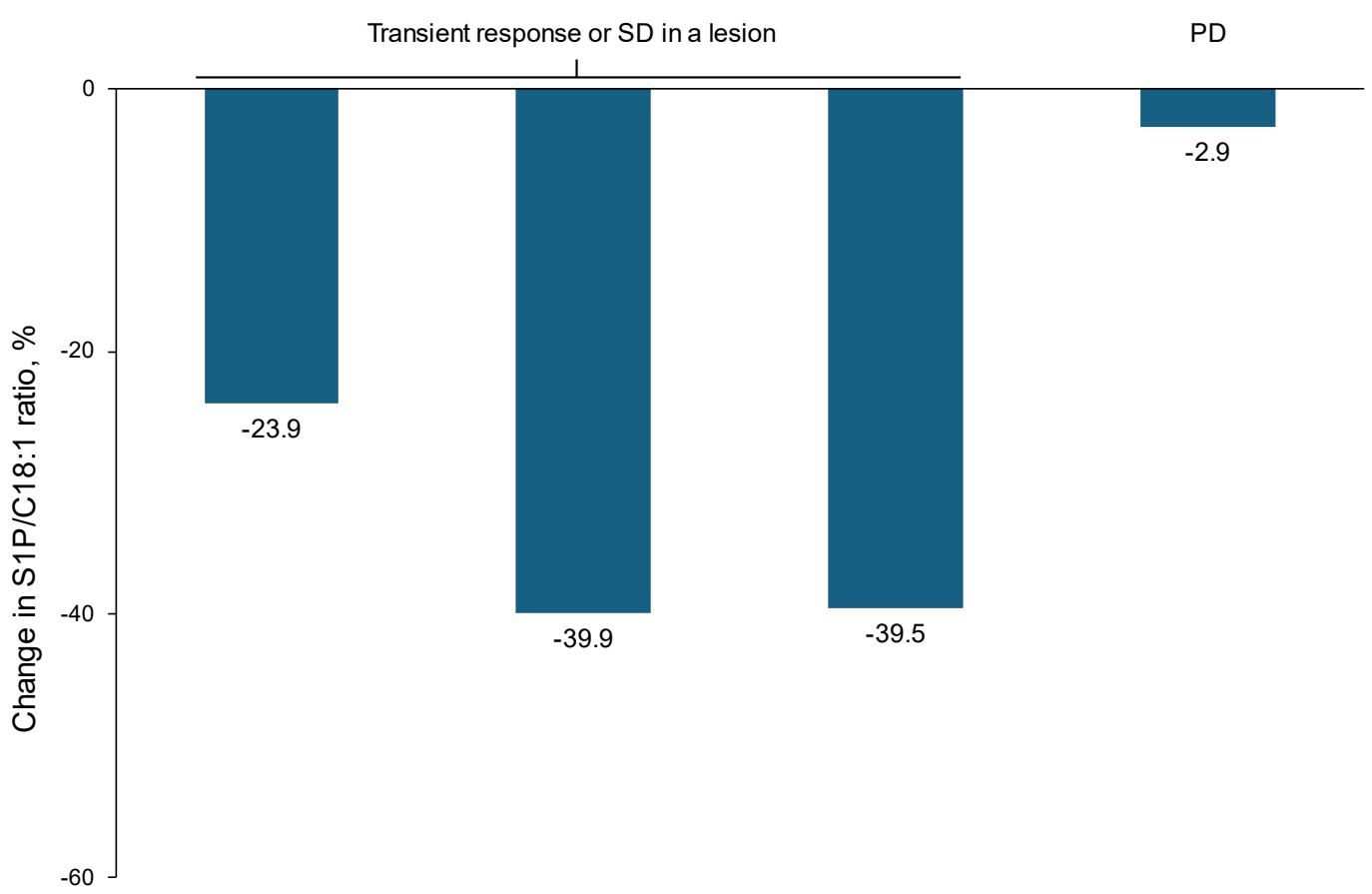
A



B



C



C18:1, C18:1 ceramide; CB, clinical benefit; PD, progressive disease; S1P, sphingosine-1-phosphate; SD, stable disease; SEM, standard error of the mean.