A First-in-Human Phase I Study of BXQ-350, a First-in-Class Sphingolipid Metabolism Regulator, in Patients with Advanced/Recurrent Solid Tumors or High-Grade Gliomas

Olivier Rixe[1], John L. Villano[2], Robert Wesolowski[3], Anne M. Noonan[3], Vinay K. Puduvalli[4], Trisha M. Wise-Draper[5], Richard Curry III[6], Emrullah Yilmaz[7], Charlie Cruze[8], Besim Ogretmen[9], Gilles Tapolsky[8], and Ray Takigiku[8]

ABSTRACT

Purpose: BXQ-350, a nanovesicle formulation of saposin C, is an allosteric sphingolipid metabolism regulator that increases proapoptotic ceramide and decreases oncogenic sphingosine-1-phosphate levels. We conducted a first-in-human phase I study of BXQ-350.

Patients and Methods: Adults (≥18 years old) with advanced/recurrent, treatment-refractory solid tumors or high-grade gliomas received BXQ-350 intravenously in five dose cohorts (0.7–2.4 mg/kg) in a 3+3 dose escalation and expansion design. The primary endpoints during dose escalation were dose-limiting toxicities and maximum tolerated dose; the primary objective in expansion parts was assessment of antitumor activity (RECIST v1.1/Response Assessment in Neuro-Oncology criteria).

Results: Eighty-six patients were enrolled. Dose-limiting toxicities were not observed during dose escalation ($n = 18$), and a maximum tolerated dose was not identified. An additional 68 patients received the 2.4 mg/kg dose. Nine patients (10%) discontinued due to adverse events. The most common treatment-related adverse events were nausea (24%) and fatigue (23%). Eight patients had a progression-free survival of ≥6 months. Two of these achieved a partial response, and six had stable disease, among whom three had a reduction in ≥1 target lesion. Of those with progression-free survival of ≥6 months, seven remained on study for >12 months, five for >24 months, and after 7 years, two remained on study without disease progression.

Conclusions: BXQ-350 was well-tolerated as monotherapy at doses up to 2.4 mg/kg. It provided some lasting clinical benefit in patients with recurrent solid malignancies across several tumor types, consistent with a decreased systemic sphingosine-1-phosphate/ceramide metabolic rheostat. BXQ-350 warrants further clinical investigation alone and combined with standard of care for advanced solid tumors.

Introduction

Dysregulated metabolism has been recognized as one of the hallmarks of cancer (1). Glucose and lipid metabolisms are intertwined, and lipid metabolites have been identified as possessing fundamental signaling properties in cell death, cell survival, and immune responses (2). A particular area of interest has been the impact of lipids, and especially sphingolipids, on cancer initiation, progression, and development of therapeutic resistance (2–4).

Sphingolipids have Janusian properties in regard to cancer. Broadly, ceramides [particularly medium-chain (C14–18, including C18:1) ceramides] are proapoptotic, and higher ceramide levels have been associated with a favorable prognosis; conversely, sphingosine-1-phosphate (S1P) is implicated in pathways involved with cancer progression, and higher S1P levels are associated with a worse prognosis (2). This concept is known as the S1P/ceramide rheostat (5, 6). Enzymes controlling sphingolipid metabolism may, therefore, play an essential role in cancer progression, with sphingosine kinases 1 and 2, acid ceramide synthases 1 to 6, glucosylceramidase, and others being preclinically or clinically investigated as novel therapeutic targets (7–11). Several early-phase clinical studies investigating sphingolipid modulators have been undertaken; however, mixed efficacy results have been observed (9–16), potentially due to ineffective inhibition of S1P or lack of ceramide level increase.

BXQ-350 is a novel nanovesicle formulation of recombinant human saposin C (SapC) and the synthetic phospholipid, sodium dioleoylphosphatidylserine. Dioleoylphosphatidylserine is a synthetic molecular species that resembles the cellular lipid bilayer component, phosphatidylserine. SapC is a lysosomal allosteric activator of multiple enzymes involved in sphingolipid metabolism, including glucosylceramidase, which increases ceramide levels by transforming glucosylceramides to ceramides (17–20). Preclinical and preliminary data suggest that by modulating the activity of these different enzymes, BXQ-350 increases the concentration of ceramides, with the concentration of C18:1 ceramide being increased consistently across most cancer cell lines, and concomitantly decreases the concentration of S1P (21, 22). By doing so, BXQ-350 may induce an antitumor effect, rebalancing the S1P/ceramide rheostat toward homeostasis (23). Furthermore, the nanovesicular formulation of BXQ-350 may enable the drug to cross the blood–brain barrier (24, 25), notably enabling targeting of primary brain malignancies and solid tumors that metastasize to the brain. BXQ-350 is currently

[1]University of New Mexico, Albuquerque, New Mexico. [2]University of Kentucky, Lexington, Kentucky. [3]The Ohio State University Comprehensive Cancer Center, Columbus, Ohio. [4]MD Anderson Cancer Center, Houston, Texas. [5]University of Cincinnati Cancer Center, Cincinnati, Ohio. [6]CTI, Covington, Kentucky. [7]Cleveland Clinic, Cleveland, Ohio. [8]Bexion Pharmaceuticals, Covington, Kentucky. [9]Medical University of South Carolina, Hollings Cancer Center, Charleston, South Carolina.

Clinical trial registration: NCT02859857

O. Rixe and G. Tapolsky contributed equally to this article.

Corresponding Author: Gilles Tapolsky, Pharmacology and Translational Sciences, Bexion Pharmaceuticals, 632 Russell Street, Covington, KY 41011. E-mail: gtapolsky@bexionpharma.com

Clin Cancer Res 2024;XX:XX–XX

doi: 10.1158/1078-0432.CCR-24-1721

Translational Relevance

Sphingolipid metabolism plays a critical role in cancer outcomes: higher levels of proapoptotic ceramides are associated with tumor suppression and a favorable prognosis. In contrast, elevated sphingosine-1-phosphate levels are associated with tumor promotion and a worse prognosis. BXQ-350 is a novel biologic and a nanovesicle formulation of recombinant human saposin C, an allosteric activator of enzymes involved in sphingolipid metabolism, including glucosylceramidase, and the synthetic phospholipid, sodium dioleoylphosphatidylserine. No dose-limiting toxicities were observed in this first-in-human monotherapy trial in advanced/recurrent cancers, and BXQ-350 had a favorable safety profile. Some patients experienced lasting disease control from BXQ-350; notably, two are still in the study without disease progression after 7 years. Pharmacodynamic analyses show that among patients with a reduction in tumor burden, BXQ-350 increases systemic ceramide levels while decreasing sphingosine-1-phosphate. Further clinical investigation of BXQ-350 as a sphingolipid metabolism regulator in patients with cancer is warranted.

under clinical investigation in metastatic colorectal cancer in combination with standard of care (NCT05322590; ref. 26) and for the treatment of pediatric patients with diffuse intrinsic pontine glioma or diffuse midline glioma in combination with radiation (NCT04771897; ref. 27).

Here, we report results from the first-in-human phase I study of BXQ-350 in patients with advanced solid tumors and high-grade gliomas (HGG).

Patients and Methods

Study design

This open-label, phase I, first-in-human, first-in-class study (NCT02859857) aimed to characterize the safety, tolerability, pharmacokinetics (PK), and preliminary antitumor activity of single-agent BXQ-350 in patients with advanced solid tumors and HGG. Pharmacodynamics and immunogenicity [antidrug antibodies (ADA)] were also assessed.

The study was organized into three parts: dose escalation (part 1) and expansion (parts 2 and 3). The primary objective in part 1 was the characterization of the safety profile, maximum tolerated dose (MTD), and dose-limiting toxicities (DLT; details of which are defined in Supplementary Methods S1). In part 1, patients received an intravenous infusion of BXQ-350 in dose cohorts of 0.7, 1.1, 1.4, 1.8, and 2.4 mg/kg, administered in 28-day cycles; treatment administration is detailed in Supplementary Methods S1. Dose escalation proceeded by following single-patient dose cohorts until a grade ≥2 treatment-related adverse event (TRAE) occurred. At that point, two additional patients were added to the dose cohort, and a standard 3+3 design was used, with subsequent dose level cohorts including at least three patients.

The MTD was assessed during the first 28-day cycle of study therapy and was defined as the dose at which two of six patients experienced a DLT. The recommended phase II dose was the cohort dose immediately below the MTD in which a maximum of one of six patients would have experienced a DLT, or in the absence of an MTD, 2.4 mg/kg.

The primary objective in parts 2 and 3 was preliminary antitumor activity in patients with unselected advanced solid tumors including HGG (part 2) or ependymoma, gastrointestinal tumors, or solid tumors other than HGG (part 3). Secondary objectives were to characterize the safety profile of BXQ-350, evaluate PK, and measure preliminary activity in terms of progression-free survival (PFS) at 6 months, time to response, and duration of response. The pharmacodynamic effects of BXQ-350 were an exploratory objective.

Patients

Eligible patients were ≥18 years old with advanced solid tumors or HGG and an Eastern Cooperative Oncology Group Performance Status of 0 to 2. Key inclusion criteria were confirmatory positive histologic or cytologic evidence of disease, disease progression having exhausted all approved therapies, and measurable or nonmeasurable disease. Eligible patients must have had acceptable organ function and coagulation parameters that met established clinical test specifications. Patients with HGG required confirmatory evidence of tumor recurrence or progression with MRI scans or positive histologic data, measurable or nonmeasurable disease per Response Assessment in Neuro-Oncology (RANO) criteria, and prior treatment with radiotherapy and temozolomide.

Key exclusion criteria were prior anticancer therapies within 2 weeks before dose assignment (for patients with solid tumors) and anticancer therapies within 2 to 12 weeks (based on therapy type) before dose assignment (for patients with HGG).

The complete list of inclusion and exclusion criteria is provided in Supplementary Methods S1.

The study protocol (and any amendments), the informed consent form, and patient recruitment materials were approved by the institutional review boards (IRB) of the Western IRB, University of Cincinnati IRB, and University of Kentucky IRB. The trial was conducted according to the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice. All patients gave written informed consent before enrollment.

Assessments

Safety and tolerability

Dose escalation and study cessation determinations during the 28-day DLT evaluation period (cycle 1) were based on initial screening and periodically scheduled clinical laboratory analyses, including hematology, blood chemistry, coagulation test results, vital sign measurements, electrocardiograms, and neurologic assessments. Adverse events (AE) were recorded [termed using the Medical Dictionary for Regulatory Activities (v19.1)] throughout the study, and severity was graded using Common Terminology Criteria for Adverse Events v4.03. Investigators determined the relationship to treatment for TRAEs. Serious AEs (SAE) were defined according to US FDA guidelines (28).

PK

Plasma samples were analyzed for SapC using a validated electrochemiluminescence method based on a Mesoscale Discovery Platform Instrument. The assay performed well over a concentration range of 1.25 to 15,400 ng/mL with the targeted validated concentration linear concentration range of 5 to 1,920 ng/mL. The lower limit of quantitation was 5 ng/mL.

Blood samples for PK analysis were collected from patients in parts 1 and 2. For part 1, samples were collected predose on days 1 to 5, 8, and 22. In addition to predose samples, serial sampling was

performed on days 1, 4, and 22. Sample times were 0.25, 0.5, 1, 2, 3, 4, 6, and 8 hours after infusion. Days 2 and 5 predose samples were the 24-hour postdose samples for days 1 and 4, respectively. For part 2, day 4, and day 22, serial sampling was limited to predose and end of infusion. PK parameters calculated using noncompartmental methods included the area under the concentration versus time curve (AUC), maximum plasma concentration (C_{max}), elimination half-life ($t_{½}$), and clearance (CL). The noncompartmental analysis module of PKanalix version 2020R1 (Antony, France: Lixoft SAS, 2020) was used to perform PK analyses.

ADAs

Blood samples were collected from all patients for ADAs on days 1, 8, 22, 113, the end of the study visit, and at every odd-numbered cycle beyond 6 months. Detection was based on a MESO QuickPlex SQ120 system with a 2.5 ng/mL sensitivity. Study samples were screened for the presence of anti-SapC antibodies. In a subsequent assessment, putative positive samples were confirmed for both the presence and absence of SapC. Confirmed positive samples were further evaluated and characterized to determine the antibody titer. Samples with an unexpected or sporadic dilution pattern of results were marked as inconclusive.

Antitumor activity

Tumor evaluations were performed at baseline, at 4 weeks, and at 8 weeks after the first dose of BXQ-350, then every 8 weeks (two cycles) for the duration of treatment. Investigators evaluated response using RECIST v1.1 for solid tumors or RANO criteria for HGG.

Pharmacodynamic and biomarker analyses

Tissue and blood collection for pharmacodynamic and biomarker analyses were optional for study participants. Peripheral blood samples were taken before BXQ-350 administration on cycle 1 day 1, typically before BXQ-350 administration on cycle 2 day 1 (day 29 after initiation of BXQ-350), and periodically after that. Lipidomic analysis was performed (to analyze sphingomyelin C14–26, sphingosine, S1P, and ceramides C14–26) at the Medical University of South Carolina Lipidomics Shared Resource by liquid chromatography with tandem mass spectrometry methodology as previously described (29–31), and the S1P/C18:1 ratio in pre– and post–BXQ-350 plasma samples was calculated.

Statistical analysis

No formal hypothesis testing or statistical comparison was planned. Safety and tumor response were summarized using descriptive statistics. A *post hoc* two-tailed Mann–Whitney unpaired (U) test was conducted to compare post-S1P/C18:1 values for subsets of patients with/without clinical benefit. SAS software (v2.0) was used for data analysis.

Safety evaluations were conducted on all patients who received ≥1 infusion of BXQ-350 (safety analysis set). Efficacy was evaluated in patients with measurable disease who underwent baseline assessment and at least one efficacy assessment (efficacy analysis set). PK analyses were conducted on patients in parts 1 and 2 who had received ≥1 infusion during the first 28-day cycle (PK analysis set).

Data availability

The data generated in this study are not publicly available as there may be information that could compromise patient privacy or consent, but are available upon reasonable request from the corresponding author.

Results

Patients

Between September 1, 2016 and July 7, 2020, 18 patients were enrolled in part 1 (dose escalation), 37 in part 2, and 31 in part 3.

In part 1, all patients at the second and third dose levels (1.1 mg/kg and 1.4 mg/kg; $n = 3$ for each) discontinued the study due to disease progression, as did most patients in the fourth (1.8 mg/kg; $n = 2/3$; 67%) and fifth dose levels (2.4 mg/kg; $n = 5/8$; 63%). In parts 2 and 3, eight patients completed six cycles; 3 (8%) and 5 (16%) in parts 2 and 3, respectively. The most common reason for discontinuation was disease progression, with 28 (76%) in parts 2 and 22 (71%) in part 3. A summary of patient disposition in parts 1, 2, and 3 is shown in Supplementary Table S1.

Table 1 shows baseline characteristics. In part 1, patients were 24 to 69 years old and mostly (61%) male. Nine patients had a non-HGG solid tumor; of the nine who had HGG, the majority (8; 89%) had neural HGGs. Most patients (83%) had received multiple lines of prior chemotherapy; the remaining patients had prior surgery, radiation, or both. Representativeness of study participants is shown in Supplementary Table S2.

Safety and tolerability

No DLTs occurred among the 18 patients in part 1; an MTD was not identified. In part 1, 12/18 patients (67%) experienced TRAEs, and three patients experienced SAEs; no SAE was considered related to treatment (**Table 2**).

All patients in parts 2 ($n = 37$) and 3 ($n = 31$) received at least one dose of BXQ-350 and were included in safety analyses. Twenty-six patients experienced TRAEs, the majority of which were grade 1 or 2. The most frequently reported TRAEs were nausea and fatigue, which was grade ≥3 in one patient (**Table 2**). Across parts 2 and 3, SAEs occurred in 23 patients; one was considered related to BXQ-350 (infusion reaction; the patient recovered without sequelae); other SAEs were considered related to disease progression by the investigators. There were no treatment-related deaths.

PK

The PK analysis set ($n = 55$) comprised 18 patients from part 1 and 37 from part 2.

The mean plasma concentrations of SapC following intravenous infusion of BXQ-350 on day 1 are shown in **Fig. 1**. For patients on day 1 of part 2 who were administered the highest dose (2.4 mg/kg), C_{max} was 25,522 ng/mL, $AUC_{0-\infty}$ was 45,127 hours·ng/mL, CL was 60.2 mL/hours/kg, V_{ss} was 165.5 mL/kg, and $t_{1/2}$ was 4.02 hours. Following BXQ-350 infusion, SapC plasma levels decreased rapidly, displaying a polyexponential decay. Plasma SapC values on days 4 and 22 had similar PK profiles to those observed following dosing on day 1 (Supplementary Fig. S1A and S1B). The PK parameters for day 1 are shown in Supplementary Table S3.

Allometric scaling of animal toxicokinetic studies predicted a human CL of 63 mL/kg/hours and AUCs of 11,100 and 38,100 ng·hour/mL for the 0.7 and 2.4 ng/kg BXQ-350 doses, respectively; these were in good agreement with the observed clinical values and consistent between the 2.4 mg/kg groups in parts 1 and 2.

ADAs

Very low levels of endogenous SapC were present (5–18 ng/mL) in the plasma of 22 patients before the first dose of BXQ-350; four of these patients also had detectable ADA levels (1:10).

Table 1. Baseline demographics and clinical characteristics.

	Part 1 (*n* = 18)	Part 2 (*n* = 37)	Part 3 (*n* = 31)
Dose, mg/kg	0.7–2.4	2.4	2.4
Median (range) age, years	59 (24–69)	55 (27–80)	59 (26–81)
Sex			
Female	7 (39)	13 (35)	16 (52)
Male	11 (61)	24 (65)	15 (48)
Race			
Black	1 (6)	1 (3)	0
White	17 (94)	36 (97)	29 (94)
Asian	0	0	2 (6)
Ethnicity			
Hispanic or Latino	1 (6)	1 (3)	2 (6)
Not Hispanic or Latino	17 (94)	36 (97)	29 (94)
Median (range) body weight, kg	86 (49–149)	79 (47–155)	83 (48–141)
ECOG PS			
0	6 (33)	13 (35)	14 (45)
1	12 (67)	19 (51)	16 (52)
2	0	5 (14)	1 (3)
Solid tumor site (non-HGG)	*n* = 9	*n* = 19	*n* = 31
Neural	0	1 (5)	2 (6)
Lung	1 (11)	3 (16)	2 (6)
Head/neck	0	2 (11)	1 (3)
Colorectal	3 (33)	3 (16)	10 (32)
Pancreas	1 (11)	4 (21)	7 (23)
Skin	0	1 (5)	0
Other	4 (44)[a]	5 (26)	9 (29)
HGG site	*n* = 9	*n* = 18	*n* = 0
Head/neck	1 (11)	2 (11)	–
Neural	8 (89)	13 (72)	–
Brain	0	3 (17)	–
Prior anticancer therapy			
Chemotherapy	15 (83)	34 (92)	31 (100)
Other[b]	4 (22)	15 (41)	4 (13)

Data are *n* (%) unless otherwise specified. Percentages may not sum to 100 due to rounding.
Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status.
[a]Appendix (*n* = 1); esophagus (*n* = 1); ovaries (*n* = 1); prostate (*n* = 1).
[b]Excluding radiation, surgery, or procedures.

Thirty-eight patients developed low ADA titers over several dosing time points, and 12 of these patients' titers reverted to baseline levels or disappeared with continued dosing. At least 13 patients had quantifiable ADA titers on a scheduled PK sample day. There was no discernible effect of ADAs on the PK parameters of SapC. Although a higher proportion of patients with no detectable ADAs had PFS >6, >12, and >24 months, no clear association between ADAs and clinical benefit could be made (Supplementary Table S4).

Antitumor activity

In total, 73 patients were evaluable for tumor response, including all patients whose tumor burden was reassessed at the end of cycle 2 and patients with HGG enrolled in part 1 at the end of cycle 1 (day 29).

Overall, 3/16 (19%), 5/29 (17%), and 5/28 (18%) patients had a clinical benefit [stable disease (SD) or better for ≥2 postbaseline visits] as assessed by RECIST v1.1 or RANO criteria in parts 1, 2, and 3, respectively.

Of all response-evaluable patients, 13 (18%) remained in the study with SD or partial response until cycle 6 tumor measurements (**Fig. 2A**). Of these 13 patients, 3 (two with ependymoma and one with pancreatic cancer) had a transitory clinical benefit as the size of one of their target lesions regressed over two assessment cycles or more (−34% and −42% reduction in tumor size for the patients with ependymoma and complete response of the primary lesion for the patient with pancreatic cancer); however, as other target lesions progressed, these patients came off the study by cycle 6 with progressive disease.

Eight patients had a PFS of greater than 6 months (**Fig. 2B**). These patients had between 2 and 5 prior lines of chemotherapy (with a median of three prior chemotherapeutic treatments). In addition, these patients had prior radiation, surgery or both. Two of these patients (one with metastatic mucinous adenocarcinoma of the appendix and one with glioblastoma multiforme) achieved a partial response at any point. They remained in the study for approximately 2 and 3 years, respectively. In addition, two other patients (one with glioblastoma multiforme and one with rectal adenocarcinoma) are alive 7 years after initiating treatment with no radiologic progression on periodic evaluation and no clinical evidence of disease progression; brief narratives for these patients are described in Supplementary Table S5.

Pharmacodynamics

Sphingolipid analysis was performed on pre– and post–BXQ-350 dosing plasma samples of a subset of patients enrolled in this study;

Table 2. Safety summary and AEs in parts 1, 2, or 3.

Patients with an AE, *n* (%)	Part 1 (*n* = 18)		Part 2 (*n* = 37)		Part 3 (*n* = 31)	
Any-cause TEAEs	18 (100)		37 (100)		31 (100)	
Grade ≥3	7 (39)		20 (54)		17 (55)	
Related to treatment	12 (67)		26 (70)		25 (81)	
Grade ≥3	0		4 (11)		3 (10)	
SAEs (any cause)	3 (17)		12 (32)		11 (35)	
Related to treatment	0		0		1 (3)	
TEAEs leading to discontinuation	0		5 (14)		4 (13)	
TEAEs leading to death	0		1 (3)		1 (3)	
Most common TRAEs (≥2 patients in a study part)						
	Any grade	**Grade ≥3**	**Any grade**	**Grade ≥3**	**Any grade**	**Grade ≥3**
Nausea	5 (28)	0	8 (22)	1 (3)	8 (26)	0
Fatigue	5 (28)	0	7 (19)	0	8 (26)	0
Infusion-related reaction	2 (11)	0	2 (5)	0	6 (19)	0
Vomiting	0	0	4 (11)	1 (3)	4 (13)	0
Decreased appetite	1 (6)	0	2 (5)	0	4 (13)	0
Electrocardiogram QT prolonged	4 (22)	0	2 (5)	0	0	0
Anemia	0	0	5 (14)	1 (3)	1 (3)	0
Hypokalemia	0	0	3 (8)	0	2 (6)	0
Dizziness	1 (6)	0	2 (5)	0	2 (6)	0
Pyrexia	0	0	4 (11)	0	0	0
Diarrhea	0	0	2 (5)	0	2 (6)	0
ALT increased	0	0	2 (5)	0	2 (6)	0
Lymphocyte count decreased	0	0	2 (5)	1 (3)	2 (6)	1 (3)
AST increased	1 (6)	0	2 (5)	0	1 (3)	0
Blood ALP increased	0	0	1 (3)	0	3 (10)	0
Blood fibrinogen increased	2 (11)	0	2 (5)	0	0	0
Hypophosphatemia	1 (6)	0	0	0	3 (10)	2 (6)
Abdominal pain	0	0	1 (3)	0	2 (6)	0
Headache	0	0	2 (5)	0	1 (3)	0
Hyperkalemia	0	0	2 (5)	0	1 (3)	0
Insomnia	0	0	1 (3)	0	2 (6)	0
Flushing	1 (6)	0	2 (5)	0	0	0
Stomatitis	0	0	0	0	2 (6)	0
aPTT prolonged	0	0	0	0	2 (6)	0
Arthralgia	0	0	0	0	2 (6)	0
Dyspnea	0	0	2 (5)	0	0	0
Hypotension	0	0	0	0	2 (6)	0

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; aPTT, activated partial thromboplastin time; AST, aspartate aminotransferase; TEAE, treatment-emergent AE.

samples for 21 patients were available and analyzed. Samples included those of 13 patients who had a clinical benefit and eight who did not.

The mean percent change in the S1P/C18:1 ratio among all 21 patients was +13.0% following treatment. The mean pre– versus post–BXQ-350 change in S1P/C18:1 among patients who had a clinical benefit ($n = 13$) was approximately −11%, whereas the mean change among those who did not have a clinical benefit ($n = 8$) was +36%; this difference was not statistically significant (Supplementary Fig. S2A). However, a statistically significant difference was observed when comparing post-S1P/C18:1 ratios between the "clinical benefit" and "no clinical benefit" subsets (median 11.95 vs. 24.20; $P = 0.045$; Supplementary Fig. S2B). A similar observation was made when comparing the percent change in the pre- and post-S1P/C18:1 ratios among the four patients with ependymoma enrolled in this study: a notable decrease in the S1P/C18:1 ratios was observed for the three patients who had a clinical benefit. In contrast, the ratio was unchanged for the patient who experienced disease progression by the first imaging reassessment (Supplementary Fig. S2C).

Discussion

This first-in-human study aimed to determine the safety profile of BXQ-350, its PK parameters, and to investigate preliminary signs of clinical activity in adult patients with advanced/recurrent, treatment refractory solid tumors or HGG.

BXQ-350 was well-tolerated across the range of doses studied. No DLT was observed, and an MTD was not reached. The treatment-emergent AEs related to BXQ-350 were mild to moderate in severity and mainly limited to nausea, vomiting, and fatigue. Of the 10 patients who experienced a treatment-related infusion reaction, one

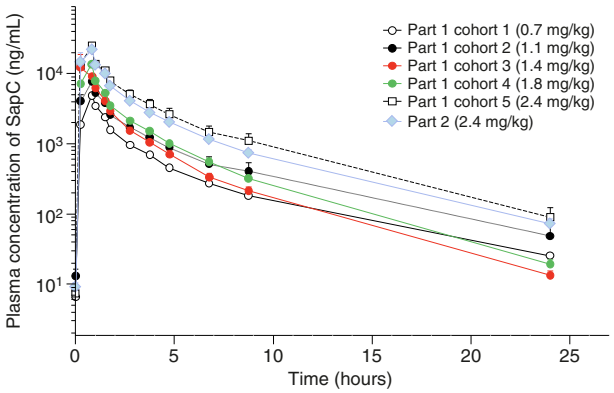

Figure 1.

Concentration–time profile of SapC. Mean plasma concentration of SapC following BXQ-350 infusion over the course of study day 1 in the PK analysis set.

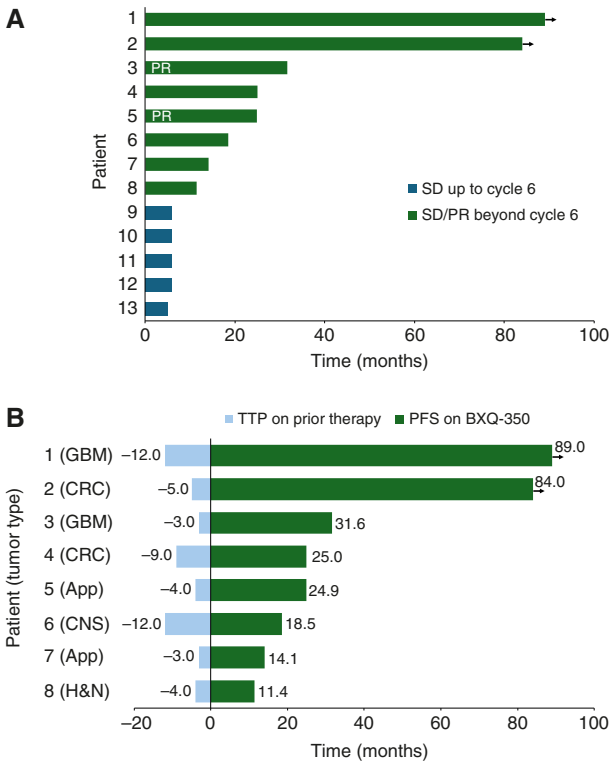

Figure 2.

Clinical benefit in patients with PFS of ≥6 cycles. **A,** Swimmer plot of the duration of clinical benefit among patients with PFS of at least six cycles. **B,** TTP of individual patients (left; blue) compared with their PFS (right; green). Arrows indicate ongoing time on study/PFS. Patient numbers correspond between figure panels. App, adenocarcinoma of the appendix; CNS, central nervous system; CRC, colorectal cancer; GBM, glioblastoma multiforme; H&N, head and neck cancer; PR, partial response; TTP, time to progression.

experienced a grade 4 infusion reaction that led to their discontinuation of the study; the patient recovered without sequelae. Approximately half of the patients had anti-SapC antibodies detected in the samples assessed for ADA; patients did not seem to develop an allergic reaction upon repeated administrations of BXQ-350.

Multiple patients had prolonged PFS of ≥6 months, despite advanced and aggressive disease at the time of enrollment (with these eight patients having up to five prior lines of chemotherapy). The PFS of the eight patients with PFS ≥6 months was notably longer than the time-to-progression on their prior line of therapy, suggesting these patients benefited from BXQ-350 treatment. Furthermore, three patients who did not reach the PFS ≥6-month milestone had noticeable regression in at least one of their target lesions over several imaging assessments. Taken together, this duration of disease control extended beyond what may be expected in these patients with recurrent tumors who had exhausted all approved therapies.

The fact that two patients are alive without disease progression 7 years after initiating BXQ- 350 treatment is remarkable. The patient with recurrent glioblastoma multiforme received 30 doses of BXQ-350 and showed no clinical neurologic decline despite suspected progression on MRI. A subsequent subtotal resection revealed necrotic tumor tissue; given that finding, this patient restarted treatment with BXQ-350. The other patient, who had recurrent rectal adenocarcinoma, clinically improved after initiating therapy and, from regular imaging assessments, has experienced SD since enrollment with neither radiologic nor clinical evidence of disease progression.

The patients in this study had a range of tumor types. Among the eight patients who had a PFS of ≥6 months and the additional three who had a regression in at least one target lesion following BXQ-350 administration, five had primary brain/central nervous system tumors (glioblastoma multiforme, $n = 2$; ependymoma, $n = 2$; choroid plexus papilloma, $n = 1$), four others had gastrointestinal diseases ($n = 2$ with colorectal cancer and $n = 2$ with mucinous adenocarcinoma of the appendix), and one had head and neck cancer (salivary gland cancer). The clinical benefits observed in these patients suggest that BXQ-350 distributes throughout the body, effectively crosses the blood–brain barrier, and accumulates at clinically relevant concentrations in multiple organs and tissues.

PK exposure seemed linear with the administered dose in the investigated range. The plasma half-life predicted by scaling the animal data was 0.8 to 4.8 hours, which was in agreement with the actual half-life, which had a geometric mean of 4.0 hours. Despite the short half-life, SapC was detected in the plasma above baseline on several occasions in samples taken before administration on days 8 and 22.

Immunogenicity and infusion reactions are common issues associated with products administered parenterally. Interestingly, although 38 patients had detectable ADA titers at any point in the present study, 12 of these patients' titers reverted to baseline levels or disappeared with continued dosing. In ongoing trials of BXQ-350 (26, 27, 32), premedication has been implemented, and infusion reactions have not been an issue in adult and pediatric patients with cancer. Nonetheless, infusion reactions and immunogenicity will continue to be monitored, along with feedback from investigators and patients. ADAs had no clear effect on PK, and whereas most patients who experienced a clinical benefit in did not have detectable ADA levels, the small sample size precludes any association to be drawn between ADAs and PFS; notably, one of the two patients with prolonged PFS still on study had detectable ADAs during the first 2 years, and the other did not.

Although exploratory, this study's analysis of S1P and C18:1 ceramide levels may suggest that patients who derived clinical benefit from BXQ-350 treatment had greater decreases in S1P/C18:1 ratio than patients with disease progression. These results are from a small number of patients with recurrent disease and a range of tumor types and should be interpreted with caution. However, although these results will need to be investigated further, they are consistent with preclinical results that suggest that BXQ-350 increases the concentration of specific ceramides and concurrently lowers the concentration of S1P across cancer cell lines (22). As observed preclinically, changes in C18:1 and S1P, and their ratio, were observed across many of these patients with a diverse range of advanced/recurrent tumors. It may have been expected that other medium-chain ceramides (C14, C16, and C18) would have changed in a similar way; further research is needed to determine why, among medium-chain ceramides, C18:1 levels were particularly increased by BXQ-350. S1P is a critical sphingolipid whose signaling properties support cell proliferation and upregulate multiple oncogenic pathways (2, 33, 34) and is a known immuno-modulator that favors a protumoral microenvironment (35–37). Preclinical studies have shown proapoptotic and anticancer properties of ceramides across cancer types (2, 23, 38). Changes in systemic concentrations of C18:1 ceramide and S1P induced by BXQ-350 would be consistent with the efficacy profile observed in multiple tumor types, for which preclinical and clinical published data suggest that sphingolipids may be significant contributors to disease progression (4, 39–42). Given these characterized pro- and antitumor roles of S1P and C18:1 ceramide, respectively, one would expect to observe an increase in S1P levels with disease progression. With S1P plasma levels higher than C18:1 levels, this may account for the increase in S1P/C18:1 ratio observed in the combined overall cohort of 21 patients and in the subset who did not have a clinical benefit.

Limitations of this phase I study include interpretation of the preliminary antitumor activity, given that this safety dose escalation study was open to all solid recurrent tumor types (patients with 20 cancer types were enrolled). Although these results from patients with diverse and advanced tumors are interesting, particularly given that a proportion of patients derived clinical benefit for an extended time beyond what may be expected, further studies are needed to ascertain the activity of BXQ-350 and more clearly characterize patients who have a response to BXQ-350. Similarly, pharmacodynamic results were only available from a small number of patients and firm conclusions with regard to the potential association of S1P/C18:1 ratio modulation and clinical benefit cannot be drawn. Further research is needed with regard to biomarkers of response to BXQ-350, including detailed sphingolipid-based biomarker analysis in more patients, a tumor-selected population, and potentially to delineate antitumor effects of other sphingolipid species. Reports in the literature suggest that C14, C16, and C18 (including C18:1) are key proapoptotic ceramides in various tumor types. For example, preclinical data suggest that C18 ceramide, including C18:1 ceramide, may induce lethal mitophagy in head and neck cancer and acute myeloid leukemia cells and tumors (43, 44). Decreased levels of C18 ceramide were also associated with increased metastatic disease and poor survival in patients with head and neck cancer (45). In contrast, increased serum levels of C18 ceramide were associated with improved responses to chemotherapy in patients with advanced head and neck cancer (46). C18:1 may also be a key ceramide for anticancer activity in glioblastoma (25, 47). Lipidomic and sphingolipid analyses in ongoing studies will enable further investigation of changes in sphingolipid profiles and their relationship to clinical benefits.

In conclusion, the results of this first-in-human safety and dose escalation study in patients with advanced/recurrent solid tumors or HGG showed that BXQ-350 in doses of up to 2.4 mg/kg is well-tolerated in this patient population. TRAEs observed in all study parts were typical of this patient population. The favorable safety profile of BXQ-350, combined with the signs of single-agent antitumor activity across multiple tumor types, warrants further clinical investigation of BXQ-350 alone or in combination with other anticancer agents. A phase Ib/II study is ongoing in newly diagnosed patients with metastatic colorectal cancer investigating BXQ-350 with the standard of care (ASIST study; NCT05322590).

Authors' Disclosures

O. Rixe reports grants from Bexion Pharmaceuticals during the conduct of the study, as well as other support from Bexion Pharmaceuticals outside the submitted work. R. Wesolowski reports other support from Bexion Pharmaceuticals during the conduct of the study. A.M. Noonan reports personal fees from AstraZeneca, Taiho Oncology, Elevar Therapeutics, DAVA Oncology, and OncLive outside the submitted work, as well as a patent for PCT/US2022/011731 pending. V.K. Puduvalli reports other support from Bexion Pharmaceuticals during the conduct of the study, as well as personal fees from Bayer, Boehringer Ingelheim, Servier, Telix Pharma, Tango Pharmaceuticals, Insightec, Novocure, Orbus Therapeutics, and Med-IQ and nonfinancial support from Karyopharm outside the submitted work. R. Curry III reports other support from Bexion Pharmaceuticals outside the submitted work. E. Yilmaz reports personal fees from Astellas Pharma and Johnson & Johnson outside the submitted work. C. Cruze reports personal fees from Bexion Pharmaceuticals during the conduct of the study, as well as personal fees from Bexion Pharmaceuticals outside the submitted work; in addition, C. Cruze reports a patent for Bexion Pharmaceuticals pending. G. Tapolsky reports a patent for U.S. App. No. 18/260677 pending. R. Takigiku reports other support from Bexion Pharmaceuticals, Inc outside the submitted work, as well as a patent for US 10682411 issued, a patent for US 11590227 issued, a patent for WO2021111842A1 pending and issued, and a patent for WO2020228150A1 pending and issued. No disclosures were reported by the other authors.

Authors' Contributions

O. Rixe: Conceptualization, investigation, methodology, project administration, writing–review and editing. **J.L. Villano:** Investigation, project administration, writing–review and editing. **R. Wesolowski:** Supervision, data curation, investigation, project administration, writing–review and editing. **A.M. Noonan:** Data curation, investigation, writing–review and editing. **V.K. Puduvalli:** Investigation, writing–review and editing. **T.M. Wise-Draper:** Investigation, project administration, writing–review and editing. **R. Curry:** Formal analysis, investigation, writing–review and editing. **E. Yilmaz:** Investigation, writing–review and editing. **C. Cruze:** Conceptualization, data curation, formal analysis, methodology, writing–review and editing. **B. Ogretmen:** Formal analysis, writing–review and editing. **G. Tapolsky:** Data curation, formal analysis, writing–original draft, writing–review and editing. **R. Takigiku:** Funding acquisition, methodology, writing–original draft, writing–review and editing.

Acknowledgments

We thank the patients and their families for making this trial possible and the investigators and clinical study teams who participated. We thank Drs Mike Gazda and Tariq Arshad for their editorial review and comments. Adam Gill, MRes (Rude Health Consulting Ltd.) provided medical writing and editorial support, which was funded by Bexion Pharmaceuticals, Inc. Research was supported in part by the Medical University of South Carolina's Lipidomics Shared Resource through funding of laboratory space for the Analytical Unit located in 505C Children's Research Institute: Hollings Cancer Center (P30 CA138313), the Lipidomics Shared Resource in the South Carolina Lipidomics and Pathobiology COBRE; Medical University of South Carolina Department of Biochemistry (P20 RR017677), and the National Center for Research Resources and Office of the Director of the NIH (C06 RR018823). Research was also supported by the NIH/NIC grant 5R44CA136017-06 to Bexion Pharmaceuticals, Inc.

Note

Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Received June 4, 2024; revised July 25, 2024; accepted September 9, 2024; published first September 12, 2024.

References

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646–74.
2. Ogretmen B. Sphingolipid metabolism in cancer signalling and therapy. Nat Rev Cancer 2018;18:33–50.
3. Madigan JP, Robey RW, Poprawski JE, Huang H, Clarke CJ, Gottesman MM, et al. A role for ceramide glycosylation in resistance to oxaliplatin in colorectal cancer. Exp Cell Res 2020;388:111860.
4. Grbčić P, Sedić M. Sphingosine 1-phosphate signaling and metabolism in chemoprevention and chemoresistance in colon cancer. Molecules 2020;25:2436.
5. Hannun YA. The sphingomyelin cycle and the second messenger function of ceramide. J Biol Chem 1994;269:3125–8.
6. Hajj C, Becker-Flegler KA, Haimovitz-Friedman A. Novel mechanisms of action of classical chemotherapeutic agents on sphingolipid pathways. Biol Chem 2015;396:669–79.
7. Stepanovska B, Huwiler A. Targeting the S1P receptor signaling pathways as a promising approach for treatment of autoimmune and inflammatory diseases. Pharmacol Res 2020;154:104170.
8. Zheng X, Li W, Ren L, Liu J, Pang X, Chen X, et al. The sphingosine kinase- 1/ sphingosine-1-phosphate axis in cancer: potential target for anticancer therapy. Pharmacol Ther 2019;195:85–99.
9. White C, Alshaker H, Cooper C, Winkler M, Pchejetski D. The emerging role of FTY720 (Fingolimod) in cancer treatment. Oncotarget 2016;7:23106–27.
10. Nagahashi M, Miyoshi Y. Targeting sphingosine-1-phosphate signaling in breast cancer. Int J Mol Sci 2024;25:3354.
11. Kunkel GT, Maceyka M, Milstien S, Spiegel S. Targeting the sphingosine-1-phosphate axis in cancer, inflammation and beyond. Nat Rev Drug Discov 2013;12:688–702.
12. Sabbadini RA. Sphingosine-1-phosphate antibodies as potential agents in the treatment of cancer and age-related macular degeneration. Br J Pharmacol 2011;162:1225–38.
13. Dickson MA, Carvajal RD, Merrill AH Jr, Gonen M, Cane LM, Schwartz GK. A phase I clinical trial of safingol in combination with cisplatin in advanced solid tumors. Clin Cancer Res 2011;17:2484–92.
14. Pal SK, Drabkin HA, Reeves JA, Hainsworth JD, Hazel SE, Paggiarino DA, et al. A phase 2 study of the sphingosine-1-phosphate antibody sonepcizumab in patients with metastatic renal cell carcinoma. Cancer 2017;123:576–82.
15. Gordon MS, Just R, Rosen LS, Dorr A. A phase I study of sonepcizumab (S), a humanized monoclonal antibody to sphingosine-1-phosphate (S1P), in patients with advanced solid tumors. J Clin Oncol 2010;28(Suppl 15):2560.
16. Britten CD, Garrett-Mayer E, Chin SH, Shirai K, Ogretmen B, Bentz TA, et al. A phase I study of ABC294640, a first-in-class sphingosine kinase-2 inhibitor, in patients with advanced solid tumors. Clin Cancer Res 2017;23:4642–50.
17. Vaccaro AM, Tatti M, Ciaffoni F, Salvioli R, Maras B, Barca A. Function of saposin C in the reconstitution of glucosylceramidase by phosphatidylserine liposomes. FEBS Lett 1993;336:159–62.
18. Sandhoff K. Sphingolipid activator proteins, chapter 76. New York (NY): McGraw Hill; 1995.
19. Harzer K, Paton BC, Christomanou H, Chatelut M, Levade T, Hiraiwa M, et al. Saposins (sap) A and C activate the degradation of galactosylceramide in living cells. FEBS Lett 1997;417:270–4.
20. Linke T, Wilkening G, Lansmann S, Moczall H, Bartelsen O, Weisgerber J, et al. Stimulation of acid sphingomyelinase activity by lysosomal lipids and sphingolipid activator proteins. Biol Chem 2001;382:283–90.
21. Tapolsky GH, Stephens T, Wilkins N, Furnish R, Cruze C, Takigiku R. Abstract 4437: BXQ-350: a novel biologic with an innovative mechanism of action targeting sphingolipid metabolism that induces cancer cell death and repolarizes the tumor microenvironment. Cancer Res 2023;83(Suppl 7):4437.
22. Tapolsky GH, Cruze C, Furnish R, Gazda M, Stephens T, Stamper L, et al. Abstract 3072: BXQ-350: a novel biologic that allosterically activates glucosylcerebrosidase and demonstrates signs of activity in cancer patients. Cancer Res 2024;84(Suppl 6):307.
23. Hannun YA, Obeid LM. Principles of bioactive lipid signalling: lessons from sphingolipids. Nat Rev Mol Cell Biol 2008;9:139–50.
24. N'Guessan KF, Patel PH, Qi X. SapC-DOPS - a phosphatidylserine-targeted nanovesicle for selective cancer therapy. Cell Commun Signal 2020;18:6.
25. Wojton J, Chu Z, Mathsyaraja H, Meisen WH, Denton N, Kwon CH, et al. Systemic delivery of SapC-DOPS has antiangiogenic and antitumor effects against glioblastoma. Mol Ther 2013;21:1517–25.
26. Patel RA, Baron AD, Boulmay BC, Flora DB, Gemmill JAL, Lee FC, et al. BXQ-350: a phase 1b/2 placebo controlled, double blinded study on the efficacy and safety of BXQ- 350 in combination with mFOLFOX7 and bevacizumab in newly diagnosed metastatic colorectal carcinoma (mCRC). J Clin Oncol 2024;42(Suppl 3):TPS224.
27. Dorris K, Fouladi M, Hummel T, Raskin S, Curry R III, Purvis J, et al. TRLS-06. a phase 1, safety and dose escalation study of BXQ-350 in combination with radiation in pediatric patients with diffuse midline glioma or diffuse intrinsic pontine glioma. Neuro Oncol 2023;25(Suppl 1):i80.
28. U.S. Food and Drug Administration (FDA). What is a serious adverse event? 2023. [cited 2024 Jul 1]. Available from: https://www.fda.gov/safety/reporting-serious-problems-fda/what-serious-adverse-event.
29. Bielawski J, Szulc ZM, Hannun YA, Bielawska A. Simultaneous quantitative analysis of bioactive sphingolipids by high-performance liquid chromatography-tandem mass spectrometry. Methods 2006;39:82–91.
30. Bielawski J, Pierce JS, Snider J, Rembiesa B, Szulc ZM, Bielawska A. Comprehensive quantitative analysis of bioactive sphingolipids by high-performance liquid chromatography-tandem mass spectrometry. Methods Mol Biol 2009;579:443–67.
31. Bielawski J, Pierce JS, Snider J, Rembiesa B, Szulc ZM, Bielawska A. Sphingolipid analysis by high performance liquid chromatography-tandem mass spectrometry (HPLC- MS/MS). Adv Exp Med Biol 2010;688:46–59.
32. Bexion Pharmaceuticals, Inc. BXQ-350 pharmacokinetic/ pharmacodynamic study in cancer patients (RETRO). 2023. [updated 2024 Aug 28; cited 2024 Jul 5]. Available from: https://clinicaltrials.gov/study/NCT05291286.
33. Pyne NJ, Tonelli F, Lim KG, Long JS, Edwards J, Pyne S. Sphingosine 1-phosphate signalling in cancer. Biochem Soc Trans 2012;40:94–100.
34. Ponnusamy S, Meyers-Needham M, Senkal CE, Saddoughi SA, Sentelle D, Selvam SP, et al. Sphingolipids and cancer: ceramide and sphingosine-1-phosphate in the regulation of cell death and drug resistance. Future Oncol 2010;6:1603–24.
35. Riboni L, Abdel Hadi L, Navone SE, Guarnaccia L, Campanella R, Marfia G. Sphingosine-1-phosphate in the tumor microenvironment: a signaling hub regulating cancer hallmarks. Cells 2020;9:337.
36. Kim SW, Kim CW, Moon Y-A, Kim HS. Reprogramming of tumor-associated macrophages by metabolites generated from tumor microenvironment. Anim Cell Syst (Seoul) 2024;28:123–36.
37. Gupta P, Kadamberi IP, Mittal S, Tsaih SW, George J, Kumar S, et al. Tumor derived extracellular vesicles drive T cell exhaustion in tumor microenvironment through sphingosine mediated signaling and impacting immunotherapy outcomes in ovarian cancer. Adv Sci (Weinh) 2022;9:e2104452.
38. Gomez-Larrauri A, Das Adhikari U, Aramburu-Nuñez M, Custodia A, Ouro A. Ceramide metabolism enzymes-therapeutic targets against cancer. Medicina (Kaunas) 2021;57:729.
39. Morad SAF, Cabot MC. Ceramide-orchestrated signalling in cancer cells. Nat Rev Cancer 2013;13:51–65.
40. Bao Y, Guo Y, Zhang C, Fan F, Yang W. Sphingosine kinase 1 and sphingosine-1- phosphate signaling in colorectal cancer. Int J Mol Sci 2017;18:2109.
41. Van Brocklyn JR, Jackson CA, Pearl DK, Kotur MS, Snyder PJ, Prior TW. Sphingosine kinase-1 expression correlates with poor survival of patients with glioblastoma multiforme: roles of sphingosine kinase isoforms in growth of glioblastoma cell lines. J Neuropathol Exp Neurol 2005;64:695–705.
42. Alkafaas SS, Elsalahaty MI, Ismail DF, Radwan MA, Elkafas SS, Loutfy SA, et al. The emerging roles of sphingosine 1-phosphate and SphK1 in cancer resistance: a promising therapeutic target. Cancer Cell Int 2024;24:89.
43. Sentelle RD, Senkal CE, Jiang W, Ponnusamy S, Gencer S, Selvam SP, et al. Ceramide targets autophagosomes to mitochondria and induces lethal mitophagy. Nat Chem Biol 2012;8:831–8.
44. Dany M, Gencer S, Nganga R, Thomas RJ, Oleinik N, Baron KD, et al. Targeting FLT3- ITD signaling mediates ceramide-dependent mitophagy and attenuates drug resistance in AML. Blood 2016;128:1944–58.
45. Karahatay S, Thomas K, Koybasi S, Senkal CE, Elojeimy S, Liu X, et al. Clinical relevance of ceramide metabolism in the pathogenesis of human head and neck squamous cell carcinoma (HNSCC): attenuation of C(18)-ceramide in HNSCC tumors correlates with lymphovascular invasion and nodal metastasis. Cancer Lett 2007;256:101–11.
46. Saddoughi SA, Garrett-Mayer E, Chaudhary U, O'Brien PE, Afrin LB, Day TA, et al. Results of a phase II trial of gemcitabine plus doxorubicin in patients with recurrent head and neck cancers: serum C_{18}-ceramide as a novel biomarker for monitoring response. Clin Cancer Res 2011;17:6097–105.
47. Abuhusain HJ, Matin A, Qiao Q, Shen H, Kain N, Day BW, et al. A metabolic shift favoring sphingosine 1-phosphate at the expense of ceramide controls glioblastoma angiogenesis. J Biol Chem 2013;288:37355–64.