

## Research Article

## Journal of Cancer Biology and Therapeutics

**BIW-8962, an Anti-GM2 Ganglioside Monoclonal Antibody, in Previously Treated Advanced/Recurrent Lung Cancer: a Phase I/II Study**Park K<sup>1\*</sup>, Lee JS<sup>2</sup>, Kim SW<sup>3</sup>, Lee DH<sup>3</sup>, Kang JH<sup>4</sup>, Cho BC<sup>5</sup>, Kim JH<sup>5</sup>, Shiraishi N<sup>6</sup> and Strout V<sup>7</sup><sup>1</sup>Division of Hematology-Oncology, Department of Medicine, Samsung Medical Center, Seoul, Republic of Korea<sup>2</sup>Department of Hematology-Oncology, Seoul National University Bundang Hospital, Seongnam, Republic of Korea<sup>3</sup>Department of Oncology, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Republic of Korea<sup>4</sup>Division of Medical Oncology, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Republic of Korea<sup>5</sup>Division of Medical Oncology, Department of Internal Medicine, CHA Bundang Medical Center, CHA University, Seongnam, Republic of Korea<sup>6</sup>Kyowa Hakko Kirin Co., Ltd., Tokyo, Japan<sup>7</sup>Kyowa Kirin Pharmaceutical Development, Inc., Princeton, NJ, USA

**\*Correspondence:** Keunchil Park, Division of Hematology-Oncology, Department of Medicine, Samsung Medical Center, 81, Irwon-ro, Gangnam-gu, Seoul 135-710, Republic of Korea, Tel: +82 2 3410 3450; Fax: +82 2 3410 1754; E-mail: kpark@skku.edu

Received: October 17, 2018; Accepted: November 15, 2018; Published: November 20, 2018

**Abstract**

BIW-8962 is a monoclonal antibody to GM2 ganglioside that shows pre-clinical activity towards lung cancer cell lines and in an animal model bearing small-cell lung cancer (SCLC) xenografts. In phase I, patients ( $N=16$ ) with advanced, recurrent lung cancer received BIW-8962 (1-10 mg/kg) intravenously every 3 weeks. There were no dose-limiting toxicities and the maximum tolerated dose was not established. The highest dose (10 mg/kg) was administered to patients with advanced, recurrent SCLC ( $N=21$ ) in phase II. The phase II study was prematurely terminated due to lack of efficacy. Objective response rate was 5% (95% CI: 0.1-24.9%) in the efficacy evaluable population ( $N=20$ ). One patient showed a durable partial response and there were a few with stable disease, which was generally not durable. No pattern of consistent toxicity was observed across the phases: there were no treatment-related adverse events (AEs) grade  $\geq 3$ , serious AEs, AEs leading to discontinuation of BIW-8962, or deaths. Exploratory analysis of circulating tumor cells and other potentially predictive orpharmacodynamic markers did not reveal any results consistent with an effect from BIW-8962. Given the complete lack of response in the study population, further development of BIW-8962 has been discontinued. The reason for the lack of clinical activity with BIW-8962 is unknown. It is hoped that the negative findings of this study will contribute to other investigations of GM2 as a therapeutic target, possibly by combination therapy, and of alternative tumor markers in patients with SCLC.

**Keywords:** BIW-8962, Anti-GM2 ganglioside monoclonal antibody, Lung cancer, Mesothelioma

**Abbreviations:** 5-HT3: 5-hydroxytryptamine 3; ADCC: Antibody-dependent Cell-mediated Cytotoxicity; AE: Adverse Event; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; AUC: Area Under the serum concentration-time Curve; AUC<sub>∞</sub>: Area Under the serum concentration-time Curve from time zero extrapolated to infinity; AUC<sub>21d</sub>: Area Under the serum concentration-time Curve over the dose interval; BMI: Body Mass Index; CBR: Clinical Benefit

Rate; CDC: Complement-dependent Cytotoxicity; CI: Confidence Interval; CL: Total Systemic Clearance;  $C_{max}$ : Maximum Serum Concentration;  $C_{min}$ : Minimum (trough) Serum Concentration; CTC: Circulating Tumor Cell; DLT: Dose-limiting Toxicity; ECG: Electrocardiogram; ECOG: Eastern Cooperative Oncology Group; FUT8: Fucosyltransferase 8; GD2: Disialic ganglioside 2; GM2: Monosialic Ganglioside 2; HIV: Human Immunodeficiency Virus; IHC: Immunohistochemistry; IP-10: Interferon Gamma-inducible Protein 10; IV: Intravenously; mAb: monoclonal Antibody; MedRA: Medical Dictionary for Regulatory Affairs; Mip-1alpha: Macrophage Inflammatory Protein 1 alpha; mRNA: messenger Ribonucleic Acid;  $MRT_{\infty}$ : Mean Residence Time extrapolated to infinity; MTD: Maximum Tolerated Dose; NCI-CTCAE: National Cancer Institute Common Terminology Criteria for Adverse Events; NSCLC: Non-small Cell Lung Cancer; ORR: Objective Response Rate; OS: Overall Survival; PFS: Progression-free Survival; PR: Partial Response; RECIST: Response Evaluation Criteria in Solid Tumors; RP2D: Recommended Phase II Dose; SAE: Serious Adverse Event; SCID: Severe Combined Immunodeficiency; SCLC: Small Cell Lung Cancer; SD: Standard Deviation;  $SpO_2$ : Peripheral Capillary Oxygen Saturation;  $t_{1/2}$ : Elimination Half-life;  $T_{max}$ : Time to Maximum serum concentration; V: Volume;  $V_{ss}$ : Volume of distribution at Stead State

## Introduction

Lung cancer is estimated as the most commonly diagnosed and the leading cause of cancer death globally [1]. Small-cell Lung Cancer (SCLC) comprises ~15% of all lung cancers and is the most aggressive form of the disease [2]: survival is inferior compared to Non-small-cell Lung Cancer (NSCLC) [3]. It is characterized by rapid growth and a tendency to metastasize early in the disease course. About one-third of patients with SCLC present with limited-stage disease, which is usually responsive to first-line therapy (usually with cisplatin and etoposide) although survival remains poor. The disease typically develops drug resistance during first-line therapy in almost all cases, resulting in recurrences that respond poorly to additional treatment [4]. Patients with limited-stage SCLC treated with etoposide, concurrent thoracic radiotherapy, and subsequent prophylactic cranial radiotherapy may expect a representative median Overall Survival (OS) of 14.5 months, and 2- and 5-year OS rates of 25% and 10%, respectively [5]. In extensive-stage SCLC, etoposide chemotherapy can palliate symptoms and prolong OS [4]: median OS for patients with extensive-stage disease has remained fairly constant at 9-10 months regardless of whether etoposide is dose intensified or whether irinotecan is substituted for etoposide [6,7]. Objective Response Rates (ORRs) to second-line chemotherapies tend to range between 12% and 25% and median OS from the start of second-line therapy is only 4-6 months [8-10]. There is consequently an unmet need for novel, more effective agents in the treatment of SCLC.

Cell membrane gangliosides comprise a carbohydrate chain with sialic acid at the cell surface and a hydrophobic ceramide in the lipid bilayer [11]; some are involved in cell-cell recognition [12] and cell-matrix attachment [13] that regulate cell growth and differentiation [14,15]. Gangliosides have been proposed as therapeutic targets for cancer therapy [16,17] as their quantitative

and qualitative expression can increase during cellular oncogenic transformation [12]. GM2 ganglioside is a tumor-associated antigen that is over expressed in a high proportion of several malignancies, e.g., SCLC, NSCLC, mesothelioma, melanoma, neuroblastoma, multiple myeloma [18-21].

BIW-8962 is a recombinant, humanized, non-fucosylated immunoglobulin G1 monoclonal antibody (mAb) directed against GM2 ganglioside. BIW-8962 is produced in Chinese Hamster Ovary cells that lack the *FUT8* gene, rendering the mAb devoid of fucose. Non-fucosylated mAbs have up to 100-fold higher antibody-dependent cell-mediated cytotoxicity (ADCC) against tumor cells compared to conventional fucosylated mAbs [22]. Assessment of the binding activity of KM8969 (a precursor mAb of BIW-8962 with the same complementarity-determining regions) in an enzyme-linked immunoassay using a series of immobilized gangliosides showed strong reaction with *N*-acetyl-GM2 and *N*-glycolyl-GM2 but weak reaction with GD2 [23]. BIW-8962 (and/or KM8969) demonstrated ADCC and Complement-dependent Cytotoxicity (CDC) against GM2-positive human SCLC [23,24] or mesothelioma [21] cell lines using human peripheral blood mononuclear cells as the effector cells or human serum as the complement source, respectively, in *in vitro* assays. *In vivo* activity of BIW-8962 has been demonstrated in murine SCID models using GM2-positive SCLC or mesothelioma cells [21,24]. The *in vivo* antitumor activity of BIW-8962 (3 mg/kg intravenously [IV] once weekly for 3 weeks) has been demonstrated in a nude rat xenotransplant model using a human SCLC cell line, NCI-N417, with no severe toxicity [data on file, Kyowa Kirin Pharmaceutical Development, Inc.]. BIW-8962 has been investigated in a phase I clinical study in patients with heavily pre-treated multiple myeloma: the maximum tolerated dose (MTD) was not reached at up to 3 mg/kg IV every 2 weeks and no clinical activity was demonstrated at this dose using

monotherapy [25].

The aim of the current phase I/II study was to determine the safety, tolerability, MTD, pharmacokinetics, potential immunogenicity, and preliminary clinical efficacy of BIW-8962 administered as monotherapy in patients with previously treated advanced/recurrent lung cancer.

## Patients and Methods

### Study design

The primary objectives of the phase I study were to determine the safety, tolerability, and MTD/recommended phase II dose (RP2D) of BIW-8962 in patients with advanced/recurrent lung tumors. Secondary objectives in phase I was to evaluate preliminary efficacy, determine the pharmacokinetic profile of BIW-8962, and screen for potential development of antibodies against BIW-8962. The primary objective of the phase II study was to determine the preliminary efficacy of BIW-8962 in patients with advanced/recurrent SCLC. Secondary objectives in phase II were to further evaluate the safety and tolerability of BIW-8962, and to screen for potential development of antibodies against BIW-8962.

The starting dose and schedule for administration of BIW-8962 in phase I was 1 mg/kg IV every 3 weeks. The starting dose level and its escalation were based on the safety and pharmacokinetic data from the first-in-human study of BIW-8962 in patients with relapsed/recurrent multiple myeloma, which examined doses up to 3 mg/kg IV every 2 weeks without reaching the MTD [25].

Phase I was conducted at a single study center (Samsung Medical Center) employing a standard 3+3 dose-escalation design with increasing doses of BIW-8962 (1, 3, 6, and 10 mg/kg) to determine the MTD and RP2D. Patients not receiving at least two full doses of BIW-8962 in a dose cohort were replaced, except for those who experienced BIW-8962-related toxicity. Dose-limiting toxicity (DLT) was defined as any of the following treatment-related toxicities: grade 3 or 4 infusion reaction; grade 4 anemia or thrombocytopenia; grade 4 neutropenia  $\geq 5$  days despite treatment with granulocyte colony stimulating factor; grade 3 or 4 neutropenia if accompanied by fever  $\geq 38.5^{\circ}\text{C}$  for  $\geq 4$  hours; grade  $\geq 3$  non-hematologic toxicity (except nausea/vomiting or diarrhea that can be reduced to grade  $\leq 2$  within 24 hours with medical management, or grade 3 laboratory adverse event (AE) that is asymptomatic or rapidly reversible); and any other toxicity considered a clinically significant

hazard to the patient.

Phase II was conducted at all five study centers using the RP2D for BIW-8962 in a maximum of 40 efficacy evaluable patients. Phase II included a Bayesian stopping rule for futility (lack of efficacy), with assessment of futility initiated after objective response of the first 15 patients was confirmed.

BIW-8962 was administered after dilution in 250 mL normal saline by IV infusion through a 0.22  $\mu\text{m}$  protein-sparing/low-protein binding in-line filter over  $\geq 2$  hours. Prophylactic pre-medication for potential infusion-related reactions was administered 30-60 min before BIW-8962 administration according to the standard practice of each study center. Patients were allowed to continue treatment until disease progression, unacceptable toxicity, grade 3/4 infusion reactions, or withdrawal of consent.

### Patients

Adult ( $\geq 19$  years) patients of either gender with Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1 and a histopathological or cytological diagnosis of measurable or non-measurable, unresectable, locally advanced primary recurrent, metastatic SCLC, NSCLC, or pleural mesothelioma unresponsive to standard therapy or for which there was no standard therapy available were eligible for inclusion in phase I. This was restricted to patients with SCLC exclusively in phase II. Other inclusion criteria included: life expectancy  $> 3$  months; adequate hematologic, hepatic, renal, and cardiac function; and preserved lung function ( $\text{SpO}_2 \geq 92\%$  on room air only in phase I and  $\geq 94\%$  by pulse oximeter with or without supplemental oxygen in phase II). Standard clinical trial advice was given to men and women to preclude and avoid conception.

Exclusion criteria included: use of chemotherapy, oral biologic agents, hormonal therapy, radiotherapy (unless limited to non-visceral structures), immunotherapy, or investigational agents  $< 3$  weeks prior to the first dose of BIW-8962; use of mAb of any type or for any form of disease  $< 4$  weeks prior to the first dose; major surgery  $< 4$  weeks prior to the first dose; known symptomatic brain metastases; leptomeningeal disease; uncontrolled intercurrent illnesses; known HIV disease; psychiatric illness, disability, or social situation likely to compromise safety, ability to provide consent, or limit compliance with study requirements; known hypersensitivity to mAbs or other therapeutic proteins, or any component of the BIW-8962 formulation, and the reaction could not

be subsequently controlled or prevented with standard treatment (e.g., antihistamines, 5-HT<sub>3</sub> antagonists, corticosteroids); history of other malignancy except for non-melanomatous skin cancer, cervical carcinoma *in situ*, or other primary solid tumor treated with curative intent and no known active disease present and no treatment administered for > 3 years; neurological paraneoplastic syndrome; and women who were pregnant.

## Clinical and safety assessments

Demographics and medical/cancer histories were recorded together with pregnancy testing if applicable, echocardiography or multigated acquisition scan, and contrast magnetic resonance brain imaging at screening. Physical examination including vital signs, laboratory tests (blood chemistry, hematology, coagulation parameters, and urinalysis), 12-lead Electrocardiogram (ECG), and determination of antibodies to BIW-8962 using a bridging electrochemiluminescence assay were undertaken at screening and regular intervals during treatment. All patients were followed after the last dose until confirmation of disease progression or start of alternative treatment.

Response was evaluated by imaging and physical examination every 6 weeks. Best response (ORR and Clinical Benefit Rate [CBR]) was determined using Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 criteria [26] in the efficacy evaluable population, which included those patients with baseline and at least one on-study assessment for response. Confirmation of response was required  $\geq 4$  weeks after initial response. OS, progression-free survival (PFS), and response duration were estimated using the Kaplan-Meier method. OS and PFS were defined as the time from the date of the first dose of study drug until the first date on which death due to any cause or progressive disease was documented; patients were censored at the date of the last evaluable tumor assessment for patients who were lost to follow-up or who were alive at the time of analysis.

AEs were recorded following observations by the investigator during clinic visits or in response to non-leading questions, spontaneous reporting by the patient, or on the basis of clinical or laboratory tests. They were graded by National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v4.0. And classified by the investigator with respect to relationship to treatment with BIW-8962 (definitely, probably, possibly, unlikely or unrelated). Treatment-related AEs included those considered definitely, probably, or possibly related

to BIW-8962. The safety analysis population included all patients who received at least one dose of BIW-8962. SAEs were reported in an expedited manner.

## Pharmacokinetics

Blood samples were taken pre-dose, and at 0 (end of infusion), 2, 6-8, 24, 48, 96, 192, 288, and 360 hours following the first and third dose of BIW-8962 as well as pre-dose following the second and fourth dose in phase I. Serum samples were analyzed at a central laboratory using a validated sandwich electrochemiluminescence assay. Pharmacokinetic parameters including area under the serum concentration-time curve from time zero extrapolated to infinity ( $AUC_{\infty}$ ) and over the dose interval ( $AUC_{21d}$ ), maximum serum concentration ( $C_{max}$ ), time to  $C_{max}$  ( $T_{max}$ ), total systemic clearance (CL), volume of distribution at steady state ( $V_{ss}$ ), elimination half-life ( $t_{1/2}$ ), and mean residence time extrapolated to infinity ( $MRT_{\infty}$ ) were calculated using non-compartmental methods with WinNonlin software (Pharsight-A Certara Company, Mountain View, CA).

## Exploratory assessments

When pre-treatment tumor biopsy samples were available, GM2 positivity was determined by immunohistochemistry (IHC) staining using BIW-8962 as the detection agent. Exploratory objectives in phase I were to assay circulating tumor cells (CTCs) in blood using the Cell search™ immunomagnetic enrichment system and to detect CTC GM2 positivity by fluorescence imaging before and after exposure to BIW-8962, and, in phase II, to assay exploratory variables including GM2 synthase mRNA, GM2 activator protein, chemokines (e.g., IP-10, Mip-1alpha), and complement consumption in blood before and after exposure to BIW-8962 as potential pharmacodynamic and/or predictive biomarkers.

## Statistics

The sample size for phase II was based on the one-sided upper-tailed test for a single one sample proportion at a significance level of 0.025 and 20% beta (80% power). The threshold value of ORR under the null hypothesis and target ORR and the value under the alternative hypothesis were set to 5% and 20%, respectively. This test was equivalent to constructing a 95% confidence interval (CI) on the ORR and rejected the null hypothesis if the lower limit of the CI exceeded 5%. Under these assumptions, a sample size of 40 efficacy evaluable subjects provided  $\geq 80\%$  probability to demonstrate that the lower limit of the exact 95% CI for ORR exceeded the threshold ORR of 5%.

Safety, efficacy, and pharmacokinetics were summarized by descriptive statistics. Response data were reported with the exact 95% CI.

## Results

### Patient characteristics

The study is complete and was conducted between 10 June 2013 and 23 June 2016. The baseline clinical and demographic characteristics of the patients enrolled in phases I and II are summarized in table 1. Patients in phase I had SCLC ( $n=8$ ) or NSCLC ( $n=8$ ) and those in phase II exclusively had SCLC ( $n=21$ ). All 16 patients in phase I were included in safety and efficacy populations. The safety and efficacy populations in phase II included 21 and 20 patients, respectively: one patient was excluded from efficacy evaluation because of a lack of post-baseline tumor assessment. Patient disposition and drug exposure are summarized in table 2. In both phase I and II, all patients received the total planned dose. Infusion of BIW-8962 was temporarily interrupted in one patient (6.3%) due to grade 2 infusion-related reaction in the cohort receiving 6 mg/kg in phase I and in two patients (9.5%) due to grade 2 infusion-related reaction and grade 2 hypoglycemia, respectively. It was only possible to obtain pre-study biopsy samples for two patients with SCLC (1 each in phase I and phase II), both of which showed cell surface GM2 over expression of moderate intensity (50-75% staining) on IHC testing.

### Dose-limiting toxicity

None of the patients in phase I developed DLT. The MTD was not reached. The RP2D was therefore the highest tested dose of 10 mg/kg every 3 weeks in the phase I trial.

### Safety

AEs are summarized in table 3. Of particular note, there were no treatment-related AEs grade  $\geq 3$ , serious AEs (SAEs), AEs leading to discontinuation of BIW-8962, or deaths in either phase. In phase I, overall treatment-related AEs (grade 1 or 2) did not appear related to dose across the cohorts and, by preferred term, were reported in individual patients except for infusion-related reaction ( $n=2$ ). In phase II, overall treatment-related AEs (grade 1 or 2) occurred in seven of 21 (33.3%) of patients and, by preferred term, were reported in individual patients except for fatigue ( $n=3$ ), and decreased appetite, increased Alanine Aminotransferase (ALT), and increased Aspartate Aminotransferase (AST) (each  $n=2$ ). The only

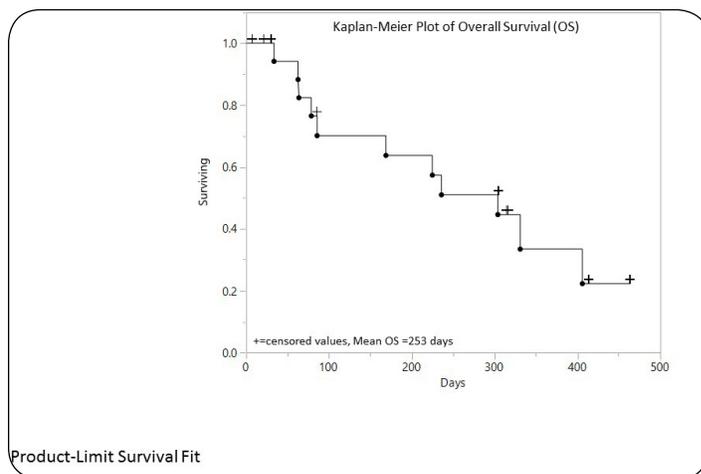
SAEs occurred in one patient receiving 10 mg/kg in phase I (atelectasis) and three patients receiving 10 mg/kg in phase II (2 with fatigue and 1 with hypercalcemia), all of which were considered unrelated to BIW-8962.

No unexpected trends or safety concerns were identified from laboratory parameter, vital sign, or ECG assessments. Anti-BIW-8962 antibodies were not detected in serum of any patient before or following treatment.

### Anti-tumor activity

Across all cohorts in phase I, ORR was 0%. Four patients experienced stable disease at 3 mg/kg ( $n=1$ ) and 10 mg/kg ( $n=3$ ). CBR was 25.0% (95% CI: 7.3-52.4%).

Among the 20 efficacy-evaluable patients in phase II, ORR was 5% (95% CI: 0.1-24.9%) and CBR was 15% (95% CI: 3.2-37.9%). Only one patient achieved a response (a partial response [PR]) with PFS of 463 days and response duration of 382 days. Median OS was 304.0 days (95% CI: 70.0-406 days) (Figure 1) and median PFS was 43.0 days (95% CI: 38.0-43.0 days) in the efficacy evaluable set.



**Figure 1:** Kaplan-Meier plot of overall survival.

### Pharmacokinetics

A summary of BIW-8962 pharmacokinetic parameters following the administration of the first dose of BIW-8962 is shown in table 4. Exposure to BIW-8962 based on  $C_{max}$  and area under the serum concentration-time curve (AUC) values increased in a dose proportional manner over the 1-10 mg/kg dose range following single-dose IV infusion.  $Meant_{1/2}$ ,  $CL$ , and  $V_{ss}$  were 235.3-310.0 hours, 0.1597-0.3897  $\mu\text{g}\cdot\text{hr}/\text{mL}$ , and 68.90-100.37 mL/kg, respectively across the dose cohorts. Serum concentration versus time curves following single and repeated doses of BIW-

**Table 1:** Baseline clinical and demographic characteristics of the patients.

Characteristic	Phase I	Phase II
	(N=16)	(N=21)
<b>Median age, years (range)</b>	59.5 (45-78)	65.5 (51-77)
<b>Gender, n (%)</b>		
Male	4 (25.0)	4 (19.0)
Female	12 (75.0)	17 (81.0)
<b>Median BMI, kg/m<sup>2</sup> (range)</b>	21.91 (18.4-25.9)	25.47 (19.1-30.3)
<b>Smoking status</b>		
Never smoked	4 (25.0)	2 (9.5)
Past smoker	12 (75.0)	17 (81.0)
Current smoker	0	2 (9.5)
<b>ECOG performance status, n (%)</b>		
0	1 (6.3)	7 (33.3)
1	15 (93.8)	14 (66.7)
<b>Cancer type, n (%)</b>		
SCLC	8 (50.0)	21 (100.0)
NSCLC	8 (50.0)	0
<b>Cancer status, n (%)</b>		
Advanced	15 (93.8)	18 (85.7)
Recurrent	1 (6.3)	3 (14.3)
<b>Previous therapy, n (%)</b>		
Chemotherapy	16 (100.0)	21 (100.0)
Radiotherapy	7 (43.7)	15 (71.1)
Surgery	8 (50.0)	9 (42.9)
<b>No. of previous chemotherapies, n (%)</b>		
1	1 (6.2)	4 (19.0)
2	3 (18.7)	5 (23.8)
3	4 (25.0)	5 (23.8)
4	2 (12.5)	3 (14.3)
≥5	6 (37.5)	4 (19.0)

BMI body mass index, ECOG Eastern Cooperative Oncology Group, NSCLC, non-small cell lung cancer, SCLC, small cell lung cancer

Note: Percentages may not equal 100% exactly due to rounding.

8962 1-10 mg/kg are shown in figure 2. Given the limited numbers of patients evaluable following repeated cycles of BIW-8962 ( $n=1$  for 3 mg/kg and  $n=1-3$  for 10 mg/kg), any results should be interpreted cautiously. In Cycle 3,  $t_{1/2}$  ranged from 211 to 352 hours for the 3 and 10 mg/kg doses. Accumulation for BIW-8962 based on  $C_{max}$ , trough serum concentration ( $C_{min}$ ), and  $AUC_{21d}$  in Cycle 3 was modest for the 3 and 10 mg/kg doses ranging from 1.27 to 1.80. Visual inspection of BIW-8962  $C_{min}$  values following repeated administration of BIW-8962 indicated that steady state was reached at the end of Cycle 3.

### Exploratory analyses

Determination of CTCs in phase I revealed measurable CTC counts (median 5 [range, 2-19] per 7.5 mL of whole blood) at baseline prior to BIW-8962 in six of 16 patients. After dosing with BIW-8962, CTCs were detected in 9 patients (median 3 [range, 1-40] per 7.5 mL of whole blood). Only one patient had a CTC sample that was GM2 positive and that was in a post-dose sample. None of the other pharmacodynamic biomarker assessments revealed predictive changes in relation to BIW-8962 administration or data collected were insufficient for meaningful analysis.

**Table 2:** Patient disposition and drug exposure.

	BIW-8962 cohort/phase				
	Phase I				Phase II
	Cohort 1 1 mg/kg (n=3)	Cohort 2 3 mg/kg (n=4)	Cohort 3 6 mg/kg (n=3)	Cohort 4 0 mg/kg (n=6)	(N=21)
<b>Patient disposition, n (%)</b>					
<b>Safety population</b>	3	4	3	6	21 (100.0)
<b>Efficacy population</b>	3	4	3	6	20 (95.7)
<b>Reason for withdrawal</b>					
Disease progression	3	4	3	6	19 (90.4)
Adverse events	0	0	0	0	0
Withdrawal of consent	0	0	0	0	1 (4.8)
Other	0	0	0	0	1 (4.8)
<b>Drug exposure, mean (SD)</b>					
<b>No. of cycles completed</b>	2.0 (0.00)	2.0 (0.82)	1.7 (0.58)	3.7(2.25)	3.1 (4.41)
<b>Actual dose, mg</b>	107.3 (19.38)	429.0 (235.9)	589.7 (216.6)	2120.5 (1153.5)	2005.5 (2774.6)

Note: Percentages may not equal 100% exactly due to rounding.

**Table 3:** Treatment-emergent adverse events.

	No. of patients (%)				
	Phase I				Phase II
	Cohort 1 1 mg/kg (n=3)	Cohort 2 3 mg/kg (n=4)	Cohort 3 6 mg/kg (n=3)	Cohort 4 10 mg/kg (n=6)	(N=21)
<b>AE</b>	3	2	3	5	18 (85.7)
<b>Treatment-related AE<sup>a</sup></b>	2	0	3	1	7 (33.3)
<b>AE grade ≥3</b>	0	0	1	1	3 (14.3)
<b>Treatment-related AE grade ≥3<sup>a</sup></b>	0	0	0	0	0
<b>Serious AE</b>	0	0	0	1	3 (14.3)
<b>Treatment-related serious AE<sup>a</sup></b>	0	0	0	0	0
<b>AE leading to discontinuation of BIW-8962</b>	0	1	0	0	0
<b>Treatment-related AE leading to discontinuation of BIW-8962<sup>a</sup></b>	0	0	0	0	0
<b>Treatment-related AE<sup>a</sup> by preferred term<sup>b</sup></b>					
Fatigue	1	0	1	1	3 (14.3)
Infusion-related reaction	0	0	2	0	1 (4.8)
Decreased appetite	0	0	1	0	2 (9.5)
ALT increased	0	0	0	0	2 (9.5)
AST increased	0	0	0	0	2 (9.5)
Asthenia	0	0	0	1	1 (4.8)
Rash	0	0	0	1	1 (4.8)
Vomiting	0	0	0	0	1 (4.8)
Platelet count decreased	0	0	0	0	1 (4.8)
Nausea	1	0	0	1	0
Pruritus	0	0	0	1	0

AE adverse event, ALT alanine aminotransferase, AST aspartate aminotransferase

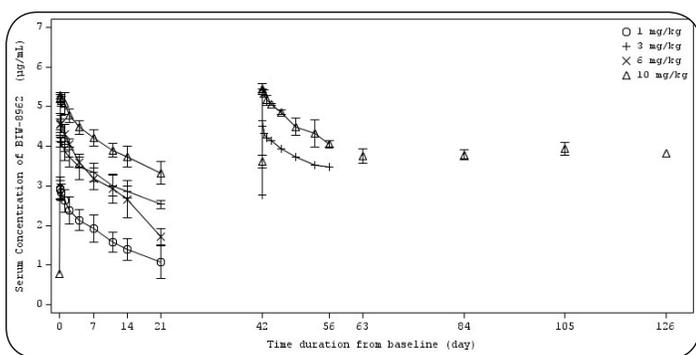
<sup>a</sup>Considered by the investigator as possibly, probably, or definitely related to treatment.

<sup>b</sup>Coded by Medical Dictionary for Regulatory Affairs (MedDRA) v19.0.

**Table 4:** Pharmacokinetics of BIW-8962 following first-dose intravenous administration patients during phase 1.

	<b>Cohort 1 1 mg/kg (n=3)</b>	<b>Cohort 2 3 mg/kg (n=4)</b>	<b>Cohort 3 6 mg/kg (n=3)</b>	<b>Cohort 4 10 mg/kg (n=6)</b>
<b>Mean <math>C_{max}</math>, <math>\mu\text{g/mL}</math> (SD)</b>	19.53 (4.535)	69.43 (7.290)	100.47 (27.086)	203.50 (27.581)
<b>Median <math>T_{max}</math>, h (range)</b>	1.70 (1.6-3.6)	3.35 (1.2-3.5)	3.30 (2.0-4.6)	4.55 (4.3-22.9)
<b>Mean <math>AUC_{\infty}</math>, <math>\mu\text{g}\cdot\text{h/mL}</math> (SD)</b>	3,246.0 (872.00)	13,241.7 (2,567.45) <sup>a</sup>	13,516.0 (3,787.64)	33,389.5 (5,825.76)
<b>Mean <math>AUC_{21d}</math>, <math>\mu\text{g}\cdot\text{h/mL}</math> (SD)</b>	4,785.3 (2,099.61)	18,904.7 (2,073.28) <sup>a</sup>	17,631.7 (8,741.49)	47,330.7 (15,391.48)
<b>Median <math>MRT_{\infty}</math>, h (range)</b>	338.0 (303-612)	347.0 (331-621) <sup>a</sup>	208.0 (200-493)	350.0 (291-693)
<b>Mean <math>t_{1/2}</math>, h (SD)</b>	310.0 (140.52)	324.3 (143.03) <sup>a</sup>	235.3 (127.27)	300.0 (117.49)
<b>Mean <math>CL</math>, mL/h/kg (SD)</b>	0.2357 (0.09404)	0.1597 (0.01779) <sup>a</sup>	0.3897 (0.15120)	0.2268 (0.05996)
<b>Mean <math>V_{ss}</math>, mL/kg (SD)</b>	89.70 (19.416)	68.90 (25.129) <sup>a</sup>	100.37 (7.355)	83.18 (13.733)

$AUC_{\infty}$  area under the serum concentration-time curve from time zero extrapolated to infinity,  $AUC_{21d}$  area under the serum concentration-time curve over the dose interval,  $C_{max}$  maximum serum concentration,  $CL$  total systemic clearance,  $MRT_{\infty}$  mean residence time extrapolated to infinity,  $T_{max}$  time to  $C_{max}$ ,  $V_{ss}$  volume of distribution at steady state, <sup>a</sup>n=3.



**Figure 2:** Mean (SD) BIW-8962 serum concentration-time profiles following intravenous infusion of 1, 3, 6, and 10 mg/kg in Cycles 1, 3, and 4-7 in phase I.

Note: n=3, 4, 3, and 6 for 1, 3, 6, and 10 mg/kg, respectively, in Cycle 1; n=1 and 3 for 3 and 10 mg/kg, respectively, in Cycle 3; n=2 for 10 mg/kg in Cycles 4, 5, and 6; and n=1 for 10 mg/kg in Cycle 7.

## Discussion

This study was prematurely terminated due to lack of efficacy. ORR was 5% (95% CI: 0.1-24.9%) in phase II, which did not meet the efficacy hypothesis of the study, i.e., the lower limit of the exact 95% CI for ORR was 0.1%. Clinical development of BIW-8962 has been discontinued.

In phase I, the MTD of BIW-8962 was not reached within the dose range selected for study, so the RP2D was set at 10 mg/kg every 3 weeks, which was the highest tested dose. No DLTs were detected during phase I and no consistent pattern or severity of treatment-induced toxicity was noted in phase II. It might therefore be considered that the upper limit of the dose range studied

in phase I might have been insufficient and a maximally effective dose was not reached for phase II study. However,  $C_{min}$  values of BIW-8962 were within the range which would have been expected to cause cytotoxicity if *in vitro* data against SCLC cell lines and in the pre-clinical animal SCLC model that showed activity for BIW-8962 (see Introduction) were extrapolated to patients. The reason for the lack of *in vivo* activity is unknown. It may be that pre-clinical activity *in vitro* and *in vivo* for BIW-8962 does not translate in patients. Similar results concerning lack of efficacy for BIW-8962 have been found during the first-in-human phase I study in patients with relapsed/refractory multiple myeloma [25]. The potential synergism of BIW-8962 with other agents has not been investigated pre-clinically in animal lung tumor models and remains a possible option for investigation. None of the predictive biomarkers that were investigated showed pharmacodynamic-like changes following treatment with BIW-8962 that may have been indicative of a therapeutic effect, which may also be reflective of a lack of efficacy by BIW-8962. A limitation of the current study was that GM2 positivity was only able to be determined in two patients from biopsy samples, both of whom had SCLC GM2-positive tumors: one of whom had SD and the other was not evaluable for efficacy. The unlikely recruitment of predominance of patients with GM2-negative lung tumors would bias against detection of a therapeutic effect by BIW-8962.

BIW-8962 exposure increased in a generally dose-proportional manner across the dose range of 1-10 mg/kg following a single IV infusion. In general, the pharmacokinetic properties of BIW-8962 were similar

following single and multiple administrations with mean  $t_{1/2}$  at approximately 235-324 hours. Accumulation of BIW-8962 was less than 2-fold and it appeared that stable BIW-8962 serum levels were reached as early as the end of Cycle 3 following multiple dose administration. The pharmacokinetic properties of BIW-8962 were similar to those previously reported following single-dose IV infusion in patients with relapsed/refractory multiple myeloma [25].

Only one patient demonstrated a response (PR) across phases I and II. Few patients demonstrated stable disease in phase I ( $n=4$ , including 3 at 10 mg/kg) or phase II ( $n=3$ ), with little evidence of durable stable disease. The one patient with PR in phase II achieved response throughout the study and subsequently received BIW-8962 as part of a compassionate use program, attaining PFS of 463 days and duration of response of 382 days. This patient was a 46-year-old woman who had never smoked and was diagnosed with stage IV SCLC (T4N0M1b); she had received radiation therapy to the right lung and whole brain with no surgery; and she had received four lines of prior chemotherapy prior to BIW-8962 administration. The GM2 over expression status of her tumor was not determined as pre-study biopsy samples were not available.

In conclusion, further development of BIW-8962 has been discontinued given the complete lack of response in the study population. It is hoped that the negative findings of this study will contribute to other investigations of GM2 as a therapeutic target, possibly by combination therapy, and of alternative tumor markers in patients with SCLC.

## Acknowledgements

The authors thank Jihyun Koo and Sookyeong Park (Kyowa Hakko Kirin Korea Co., Ltd., Seoul, Korea) for management of the trial, and Peter Todd (Tajut Ltd., Kaiapoi, New Zealand) for writing and editorial support in the preparation of this manuscript, which was funded by Kyowa Kirin Pharmaceutical Development, Inc. (Princeton, NJ, USA).

## Funding

This study was funded by Kyowa Kirin Pharmaceutical Development, Inc. (Princeton, NJ, USA). No funding or sponsorship was received for the publication of this article.

## Conflicts of interest

NS and VS are employees of Kyowa Hakko Kirin Co., Ltd. (Tokyo, Japan) and Kyowa Kirin Pharmaceutical Development, Inc. (Princeton, NJ, USA), respectively. The other authors have no competing interests.

## Ethical approval

The protocol and its subsequent amendments were approved by the Institutional Review Board at each of the five study centers (Division of Hematology-Oncology, Department of Medicine, Samsung Medical Center; Severance Biomedical Science Institute, Yonsei University College of Medicine; Lung Cancer Center, Asan Medical Center; Division of Medical Oncology, Seoul St. Mary's Hospital, The Catholic University of Korea; Division of Hematology and Medical Oncology, Department of Internal Medicine, Seoul National University Bundang Hospital, Seoul, Republic of Korea). The study was conducted in accordance with the Declaration of Helsinki and International Conference for Harmonisation of Good Clinical Practice Guidelines.

## Informed consent

All patients provided written informed consent prior to study registration.

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