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ORIGINAL RESEARCH

Phase I Study of Anti-GM2 Ganglioside Monoclonal Antibody BIW-8962 as Monotherapy in Patients with Previously Treated Multiple Myeloma

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ABSTRACT

Introduction: BIW-8962 is a monoclonal antibody to GM2 ganglioside that shows preclinical activity towards multiple myeloma (MM) cell lines and in animal models bearing MM xenografts. The objective of this study was to determine the safety, tolerability, maximum tolerated dose (MTD), pharmacokinetics, potential immunogenicity, and preliminary clinical efficacy of BIW-8962 in patients with heavily pretreated MM.

Methods: Patients ($n = 23$) received escalating doses of BIW-8962 (0.03–3 mg/kg) intravenously every 2 weeks in phase Ia. The highest anticipated dose (10 mg/kg) was not tested and the study was discontinued without proceeding to phases Ib and II.

Results: The MTD of BIW-8962 was not established and BIW-8962 was relatively well tolerated. No pattern of consistent toxicity could be inferred from treatment-related AEs grade ≥3 and only two dose-limiting toxicities were recorded (atrial thrombosis + cardiomyopathy and chest pain, respectively). In the efficacy evaluable population ($n = 22$), no patient had a response (complete or partial) and 16 (72.7%) had a best response of stable disease, which was generally not durable.

Conclusion: BIW-8962 did not show evidence of clinical activity. The study was therefore stopped and further development of BIW-8962 in MM was halted.

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INTRODUCTION

Multiple myeloma (MM) is a B cell malignancy characterized by abnormal monoclonal expansion of plasma cells, usually accompanied by a monoclonal protein (myeloma-protein; M-protein) found in the blood and/or urine. Clinical features include renal failure, anemia, recurrent infections, skeletal destruction, and hypercalcemia [1]. MM accounts for around 18% of hematological malignancies, resulting in an estimated 30,330 new cancer cases and 12,650 deaths in the USA for 2016 [2]. Advances in the treatment of newly-diagnosed MM over recent decades, including high-dose chemotherapy with autologous stem cell transplantation, immunomodulatory drugs (e.g., thalidomide, lenalidomide), and proteasome inhibitors (e.g., bortezomib) have improved clinical outcome [3, 4]. This is reflected in the significant improvement in 5-year relative survival rate in the USA from 25% in 1975–1977 to 49% in 2005–2011 [2]. Virtually all MM patients ultimately relapse or become refractory after first- or second-line therapy [5] and these patients represent a clinical challenge because of their poor clinical outcome [6]. Alternative immunomodulatory drugs (pomalidomide) [7] and proteasome inhibitors (carfilzomib, ixazomib) [8, 9], new agents such as the histone deacetylase inhibitor panobinostat [10], and novel monoclonal antibodies (mAbs) directed at different targets such as CD38 (daratumumab) [11] and SLAMF7 (elotuzumab) [12] have been approved more recently for treatment of relapsed/refractory MM. Novel agents that act through different mechanisms are still needed and are under investigation [13].

Gangliosides are ubiquitous cell membrane components composed of a carbohydrate chain with sialic acid at the cell surface and a hydrophobic ceramide in the lipid bilayers [14]. Some of these gangliosides play a role in cell–cell recognition [15] and cell–matrix attachment [16] that regulate cell growth and differentiation [17, 18]. Quantitative and qualitative changes are known to occur in the expression of gangliosides through the oncogenic transformation of cells [15], so attention has been directed to gangliosides as therapeutic targets [19, 20]. The recognition of potential immunologic differences between cancer cells and normal cells led to an immunotherapy trial in an attempt to immunize metastatic melanoma patients against the GM2 ganglioside [21]. GM2 ganglioside is expressed in a range of other tumor cell types, e.g. neuroblastoma, leukemia, and it was noted that the majority of myeloma cell lines (70%) and myeloma cells in patient marrow specimens (64%) expressed GM2 ganglioside on the cell surface [22].

BIW-8962 is a recombinant, humanized, non-fucosylated immunoglobulin G1 mAb directed against the GM2 ganglioside. BIW-8962 was produced in Chinese Hamster Ovary (CHO) cells that lack the FUT8 gene, rendering the mAb devoid of fucose in the carbohydrate structure. Non-fucosylated mAbs have been shown to have up to 100-fold higher antibody-dependent cell-mediated cytotoxicity

(ADCC) against tumor cells compared to conventional fucosylated antibodies [23]. Preclinical studies employed a precursor mAb, KM8969, with the same complementarity-determining regions as BIW-8962. The binding activity of KM8969 was assessed with an enzyme-linked immunoassay using various immobilized gangliosides as previously reported [24]. KM8969 reacted strongly with *N*-acetyl-GM2 and *N*-glycolyl-GM2 but weakly with GD2. In vitro preclinical studies (data on file, Kyowa Kirin Pharmaceutical Development, Inc.) showed that KM8969 bound to many MM cell lines in flow cytometric analysis and exhibited potent ADCC and complement-mediated cytotoxicity towards MM cell lines. In vivo, KM8969 effected dose-dependent antitumor activity that plateaued at 3–10 mg/kg in the KMS-11 human MM xenograft severe combined immunodeficiency mouse model after intravenous (iv) administration twice weekly for 3 weeks.

The aim of the current first-in-human phase I study was to determine the safety, tolerability, maximum tolerated dose (MTD), pharmacokinetics, potential immunogenicity, and preliminary clinical efficacy of BIW-8962 administered by iv infusion as monotherapy in patients with previously treated multiple myeloma.

METHODS

Study Design

The primary phase I objective was to establish the safety profile and recommended phase II dose as determined by either the MTD or the active biologic dose (ABD) of BIW-8962 in patients with previously treated MM. Secondary objectives were to determine the pharmacokinetic profile of BIW-8962, to evaluate preliminary evidence of antitumor activity, and to screen for potential antibodies against BIW-8962.

As this was the first-in-class human study of BIW-8962, the starting dose level was based on a 12-week toxicology study in cynomolgus monkeys (data on file, Kyowa Kirin Development, Inc.), which showed the no observed adverse effect level was 0.1 mg/kg administered weekly. The selected human starting dose of BIW-8962 0.03 mg/kg iv once every 2 weeks provided a safety factor of six with the dosing regimen difference factored in, which is sufficiently high for relapsed MM patients.

The study had a multi-center, open-label design consisting of three sequential parts: dose escalation to determine the MTD or ABD (phase Ia) followed by dosing regimen determination using adjustment with a loading dose (phase Ib) followed by an efficacy assessment (phase II). Phases Ib and II were not conducted due to lack of preliminary efficacy in phase Ia and, as such, are not described herein. Phase Ia employed a standard $3 + 3$ dose-escalation design. Increasing doses of BIW-8962 (0.03, 0.1, 0.3, 1, 3, and 10 mg/kg) were administered every 2 weeks. 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.

BIW-8962 was administered by iv infusion in 0.9% saline (25–250 ml depending on dose level) over 60 min, except the 0.03 mg/kg dose which was delivered over 15–20 min. All infusions were delivered with an infusion pump through a 0.22-μm protein-sparing/low-protein-binding in-line filter. Routine premedication for the prophylaxis of infusion reactions was not allowed. Patients were allowed to continue treatment until disease

progression, unacceptable toxicity, grade 3/4 infusion reactions, or any event that required >2 dose reductions.

Dose-limiting toxicity (DLT) was defined as any grade ≥3 hematologic or non-hematologic toxicity that was considered by the investigator to be probably or possibly related to BIW-8962. Patients who experienced grade 3 nausea, vomiting, or diarrhea were not considered as DLT if they could be managed and reduced to grade ≤1 within 24 h and, in subsequent courses, when subjects are given appropriate prophylaxis, they do not recur at grade >2.

Patients

Eligible patients included adults (≥18 years) with ECOG performance status ≤2 and adequate hematological and organ function who presented measurable, symptomatic MM documented by IMWG criteria [1] who had failed ≥2 prior MM therapies. Full inclusion/exclusion criteria are detailed as supplementary material (available online).

Safety and Clinical Assessment

Demographic and medical/cancer histories were recorded at screening. Bone marrow aspiration and biopsy were performed during screening. Physical examination and laboratory value assessments were undertaken at screening, on day 1 of each course (every 2 weeks), at the end of treatment, and at follow up. Vital signs were recorded at all visits. ECG was undertaken at screening, post-infusion on day 1 of each course, and at end of treatment. Serum and urinary M protein and free light chain analyses were performed at screening, on day 1 of course 1 and on day 1 of every other course, and at study termination. Fluorescence-activated cell sorting analysis was performed on T, B and NK cells at screening and prior to each treatment course. Immunogenicity samples were taken at screening, immediately before doses 3 and 4, and 8 weeks after the last dose using an enzyme-linked immunosorbent assay for detection of human antibodies to BIW-8962. All patients were followed after the last dose until confirmation of progression or start of alternative treatment.

Adverse events (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) v 3.0. 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. Serious AEs (SAEs) were reported in an expedited manner.

Response Assessment

Best overall response was determined in the efficacy evaluable population, which included those patients with baseline and at least one on-study assessment for response. Confirmation of response required two consecutive assessments. Response was assessed by IMWG criteria every 4 weeks. Response assessment included quantitation of M-protein (serum and urine), urinary Bence-Jones protein for patients who do not have complete monoclonal immunoglobulins, or, in the absence of M-protein detectable by either electrophoresis or immunofixation, free light chain (FLC)

analysis. As clinically appropriate, response assessment may also include bone marrow analysis and imaging of plasmacytoma. Samples for M-protein (serum and urine) and FLC analyses (serum and urine) were analyzed at a central laboratory (ICON Laboratories, Farmingdale, NY).

Pharmacokinetics

Blood samples were taken pre-dose, and at 0 (end of infusion) 1, 2, 4, 6, 24, 72, 96, 168, 216, and 336 h following the first dose of BIW-8962. After the second dose of BIW-8962, blood samples were taken pre-dose, and at 0, 24, 96, 216, and 336 h. Plasma samples were analyzed at a central laboratory (Tandem, Inc., West Trenton, NJ) using a validated sandwich electrochemiluminescence assay. The quantification range was 80–5120 ng/ml. Pharmacokinetic parameters including area under the plasma concentration–time curve from time zero to the time of the last measurable concentration (AUC_{last}) and to infinity (AUC_{∞}), maximum plasma concentration (C_{max}), time to C_{max} (T_{max}), total systemic clearance (CL), volume of distribution in the terminal phase (Vz), and elimination half-life ($t_{1/2}$) were calculated using non-compartmental methods with WinNonLin version 5.0 software (Pharsight A Certara Company, Mountain View, CA).

Statistics

Safety, efficacy, and pharmacokinetics were summarized by descriptive statistics.

Compliance with Ethical Guidelines

The study was conducted in accordance with the Declaration of Helsinki and International Conference for Harmonization of Good Clinical Practice Guidelines. The protocol and its subsequent amendments were approved by the Institutional Review Board at each of the four study centers (H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL; Barbara Ann Karmanos Cancer Institute, Detroit, MI; Cleveland Clinic Foundation, Cleveland, OH; Duke University Medical Center, Durham, NC). The study was registered in ClinicalTrials.gov (NCT00775502). All patients provided written informed consent prior to study registration.

RESULTS

Patient Characteristics

The study is complete and was conducted between 13 February 2009 and 30 November 2010. The baseline clinical and demographic characteristics of the patients are summarized in Table 1. All patients had received at least 3 prior systemic therapies and 83% had received >4 prior systemic therapies for treatment of myeloma. All patients had received prior bortezomib and corticosteroids, and all but one had received prior lenalidomide. The patients had also received many other standard and investigational therapies, as well as stem cell transplantation.

Patient disposition and drug exposure are summarized in Table 2. The safety and efficacy populations included 23 and 22 patients, respectively. The reasons for discontinuation from the study were progressive disease ($n = 22$, 95.7%) and AEs ($n = 1$, 4.3%).

Dose-Limiting Toxicity

Two patients developed DLTs: one at 0.03 mg/kg and one at 1 mg/kg. The number of evaluable

Table 1 Baseline clinical and demographic characteristics

Characteristic	Total ($n = 23$)
Median age, years (min–max)	66 (49–79)
Gender, *n* (%)	
Male	14 (60.9)
Female	9 (39.1)
Race, *n* (%)	
White	17 (73.9)
African American	6 (26.1)
ECOG performance status, *n* (%)	
0	2 (8.7)
1	20 (87.0)
2	1 (4.3)
No. of prior systemic therapies, *n* (%)	
0–2	0
3	1 (4.3)
4	3 (13.0)
>4	19 (82.6)
Median (min–max)	6 (3–28)
MM type, *n* (%)	
IgG	12 (52.2)
IgA	3 (13.0)
IgM	1 (4.3)
Light chain kappa	1 (4.3)
Light chain lambda	5 (21.7)
Not recorded	1 (4.3)

Percentages may not equal 100% exactly due to rounding
ECOG Eastern Cooperative Oncology Group, *MM* multiple myeloma

patients was therefore increased to six in each of these cohorts. Seven patients eventually entered the 0.03 mg/kg cohort as one patient received only one dose and discontinued study medication due to disease progression, which necessitated replacement. No additional patients developed DLT. The DLT in the 0.03 mg/kg cohort was grade 3 atrial thrombosis and cardiomyopathy possibly related to the study drug. The DLT in the 1 mg/kg cohort was grade 3 chest pain probably related to the study drug. The latter patient died during the study but this was not considered drug-related (see next section). Both DLTs led to discontinuation of study drug.

Neither the MTD nor the ABD was reached. No patients were recruited to the highest planned dose level of 10 mg/kg prior to discontinuation of the trial.

Safety

AEs are summarized in Table 3. Treatment-related AEs occurred in 8 (34.8%) patients, did not appear related to dose, and, by preferred term, were reported in individual patients except for alopecia ($n = 2$). There were no treatment-related life-threatening AEs or deaths. Treatment-related grade 3 AEs were reported in two patients (atrial thrombosis + cardiomyopathy in one patient and chest pain in the other): these were the DLTs reported above. The patient who experienced grade 3 chest pain probably related to BIW-8962 occurred after receiving a partial dose of study drug (1 mg/kg cohort) on day 1 in the context of a grade 2 infusion reaction: the patient died on day 6 due to cardiopulmonary arrest that was considered unrelated to the study drug. The patient who experienced grade 3 atrial thrombosis and cardiomyopathy had a medical history of congestive heart failure with pre-existing cardiomyopathy. Both these DLTs were classed as SAEs. One other patient experienced a treatment-related SAE: this involved a patient who received 3 mg/kg and experienced grade 3 fatigue plus a grade 2 infusion reaction, which

Table 2 Patient disposition, drug exposure, and TTP

	BIW-8962 cohort					
	Cohort 1 0.03 mg/kg	**Cohort 2 0.1 mg/kg**	**Cohort 3 0.3 mg/kg**	**Cohort 4 1 mg/kg**	**Cohort 5 3 mg/kg**	**Total**
Patient disposition, *n* (%)						
Safety population	7	3	3	6	4	23 (100.0)
Efficacy population	7	3	3	5	4	22 (95.7)
Reason for withdrawal						
Disease progression	7	3	3	5	4	22 (95.7)
Adverse event	0	0	0	1	0	1 (4.3)
Drug exposure						
Total doses administered, mean ± SD	4.0 ± 2.04	2.33 ± 0.58	7.67 ± 7.37	3.8 ± 2.23	3.25 ± 0.96	4.04 ± 3.11
Actual dose, mg	2.90 ± 0.62	7.22 ± 1.27	23.53 ± 0.12	76.12 ± 22.24	319.34 ± 108.17	71.85 ± 110.28
Dose intensity, %[a]	100	100	100	93.73 ± 22.35	100	98.37 ± 11.57
Median doses before disease progression (min–max)	3 (1–7)	2 (2–3)	5 (2–16)	4 (1–7)	3.5 (2–4)	3 (1–16)
Median TTP, weeks (min–max)	6.3 (2.1–14.4)	3.4 (3.1–7.1)	9.1 (3.7–34.1)	9.7 (4.1–14.1)	7.05 (3.1–9.1)	6.7 (2.1–34.1)

SD standard deviation, *TTP* time to disease progression

[a] Calculated as actual dose/planned dose × 100%

Table 3 Treatment-emergent adverse events

	No. of patients (%)					
	BIW-8962 cohort					Total ($n = 23$)
	Cohort 1 0.03 mg/kg ($n = 7$)	Cohort 2 0.1 mg/kg ($n = 3$)	Cohort 3 0.3 mg/kg ($n = 3$)	Cohort 4 1 mg/kg ($n = 6$)	Cohort 5 3 mg/kg ($n = 4$)	
AE	7	3	3	6	4	23 (100)
Treatment-related AE[a]	4	1	0	1	2	8 (34.8)
AE grade ≥3	5	3	1	4	3	16 (69.6)
Treatment-related AE grade ≥3[a]	1	0	0	1	0	2 (10.2)
Serious AE	2	2	0	2	1	7 (30.4)
Treatment-related serious AE[a]	1	0	0	1	1	3 (13.5)
AE leading to discontinuation of BIW-8962	1	1	0	2	0	4 (17.4)
Death[b]	0	0	0	1	0	1 (4.3)
Treatment-related AE[a] by preferred term[c]						
Anorexia	2	0	0	0	0	2 (8.7)
Alopecia	1	0	0	0	0	1 (4.3)
Anemia	1	0	0	0	0	1 (4.3)
Atrial thrombosis	1	0	0	0	0	1 (4.3)
Cardiomyopathy	1	0	0	0	0	1 (4.3)
Chest pain	0	0	0	1	0	1 (4.3)
Fatigue	0	0	0	0	1	1 (4.3)
Headache	0	0	0	0	1	1 (4.3)
Hyperkalemia	1	0	0	0	0	1 (4.3)
Hyperuricemia	1	0	0	0	0	1 (4.3)
Infusion-related reaction	0	0	0	0	1	1 (4.3)
Musculoskeletal discomfort	0	0	0	0	1	1 (4.3)

Table 3 continued

	No. of patients (%)					
	BIW-8962 cohort					Total ($n = 23$)
	Cohort 1 0.03 mg/kg ($n = 7$)	Cohort 2 0.1 mg/kg ($n = 3$)	Cohort 3 0.3 mg/kg ($n = 3$)	Cohort 4 1 mg/kg ($n = 6$)	Cohort 5 3 mg/kg ($n = 4$)	
Neutropenia	0	0	0	0	1	1 (4.3)
Non-cardiac chest pain	0	0	0	0	1	1 (4.3)
Pain in extremity	0	0	0	0	1	1 (4.3)
Thrombocytopenia	0	1	0	0	0	1 (4.3)
Treatment-related AE[a] grade ≥3 by preferred term[c]						
Atrial thrombosis	1	0	0	0	0	1 (4.3)
Cardiomyopathy	1	0	0	0	0	1 (4.3)
Chest pain	0	0	0	1	1	1 (4.3)

AE adverse events

[a] Considered by the investigator as possibly, probably, or definitely related to treatment

[b] Death was unrelated to study medication

[c] Coded by MedDRA version 11.0

were considered probably and definitely related to study drug, respectively. All other SAEs were not considered related to the study drug and were generally typical of the underlying disease process, e.g. hypercalcemia, plasmacytoma, infection, fracture.

No unexpected trends or safety concerns were identified from laboratory parameter, vital sign, or ECG assessments. Anti-BIW-8962 antibodies were not detected in plasma for any patients except one who developed a weakly positive response.

Anti-Tumor Activity

No patient had a complete or partial response, with no patient showing a ≥50% reduction in serum M protein and a ≥90% reduction in 24-h urinary M protein or to <200 mg/24 h. Sixteen of 22 evaluable patients (72.7%) had stable disease (SD). The longest duration of SD was ~9 months in a patient who received BIW-8962 0.3 mg/kg (Fig. 1). This patient was diagnosed in 2003 and received four cycles of vincristine, doxorubicin, and dexamethasone, and then underwent high-dose chemotherapy and stem cell transplantation in November 2003. Remission lasted for 2 years until March 2006. She then received six cycles of bortezomib with a response and was subsequently placed on thalidomide maintenance therapy, which she received intermittently until February 2008 when she again experienced progressive disease. She then received four cycles of bortezomib, lenalidomide, and dexamethasone for 4 cycles and was then subsequently maintained on lenalidomide. In December 2009, she experienced progressive disease and entered the current trial of BIW-8962. Three additional patients had SD for ~3 months. Median time to disease progression was 6.7 (range 2.1–34.1) weeks (Table 2).

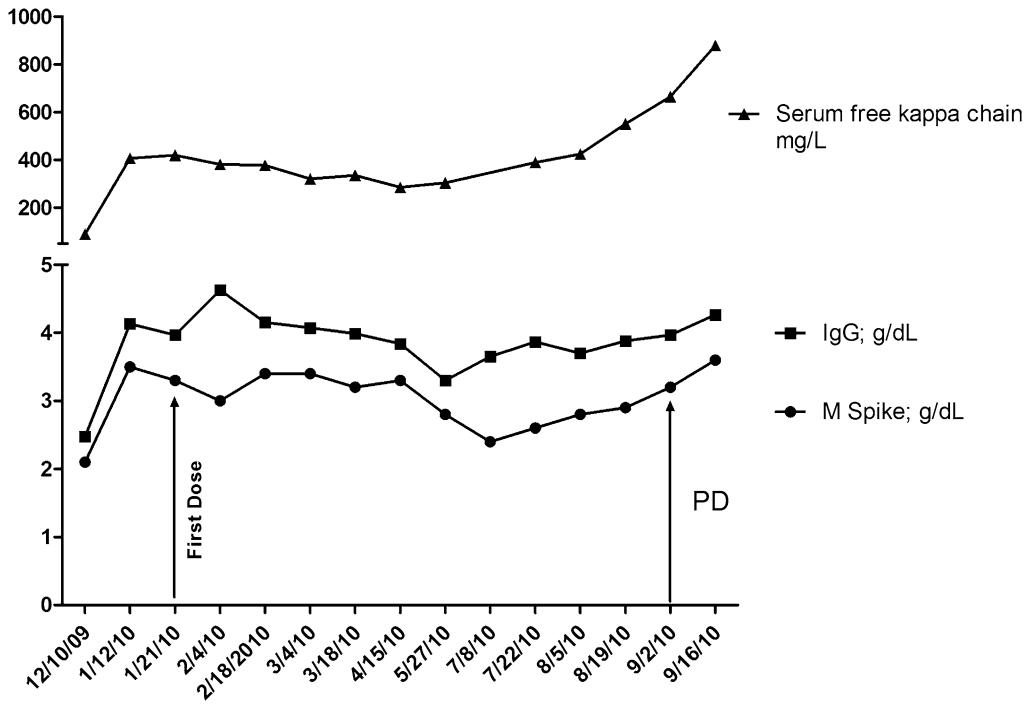

Fig. 1 Response in the patient with sustained (~9 months) stable disease

Pharmacokinetics

A summary of BIW-8962 pharmacokinetic parameters following the administration of the first and second dose of BIW-8962 every 2 weeks is shown in Table 4. C_{max} increased in a dose proportional manner over the 0.03–3 mg/kg dose range. Systemic exposure based on AUC_{last} and AUC_{∞} values increased in a dose proportional up to 1 mg/kg but was greater than the dose increase at 3 mg/kg. The ratio of AUC_{last} comparing the second and first dose of BIW-8962 was ~1.3 at 0.03–0.3 mg/kg doses and ~1.6 at 1 and 3 mg/kg doses. Mean $t_{1/2}$ ranged from 80.2 to 266 h across the dose cohorts and appeared longer after the second dose, particularly at the higher doses of 1 and 3 mg/kg. Mean Vz ranged from 53.0 to 94.4 ml/kg and did not appear dose related. Mean CL ranged from 0.229 to 0.544 μg h/ml and may have been slower at the highest dose of 3 mg/kg.

DISCUSSION

Neither the MTD nor the ABD for BIW-8962 were determined in this first-in-class human study of this monoclonal antibody to the ganglioside GM2 in patients with heavily pretreated MM. The study was stopped prematurely during dose escalation in phase Ia and the highest dose of BIW-8962 10 mg/kg was not tested. The study was stopped because of insufficient evidence of clinical activity and the study did not progress to phase Ib or phase II as initially planned.

BIW-8962 administered iv up to 3 mg/kg twice weekly did not show any efficacy. None of the 22 patients evaluable for efficacy showed a response (complete or partial). Sixteen of 22 evaluable patients (72.7%) had SD. The longest duration of SD was ~9 months and three additional patients had SD for ~3 months, so there was little evidence of patients achieving durable SD.

The MTD was not reached. At the doses tested, BIW-8962 was relatively well tolerated. No pattern of consistent toxicity could be noted from treatment-related AEs grade ≥3 and only two DLTs were recorded during dose escalation.

Pharmacokinetic analysis showed that BIW-8962 distribution appears primarily confined to serum following iv administration. It is eliminated slowly with a mean half-life ranging from 80 to 266 h across the dose cohorts. Systemic exposure increased in a dose-related manner up to 1 mg/kg, although, at 3 mg/kg, the increase in systemic exposure was greater than the dose increase. Trough levels of BIW-8962 were within the range which would have been expected to cause cytotoxicity if in vitro data against MM cell lines and in the preclinical animal MM model that showed activity for BIW-8962 were extrapolated to patients.

The reason for the lack of clinical activity is unknown. It may be that preclinical activity in vitro and in vivo for BIW-8962 does not translate in patients. Since the conclusion of study and decision to discontinue the development of BIW-8962, we have become aware of studies with elotuzumab in MM patients. Single-agent treatment with elotuzumab showed no objective clinical responses in a phase I study in heavily pretreated MM patients [25]. Given that lenalidomide and bortezomib enhanced the activity of elotuzumab in preclinical models, further clinical studies were conducted of elotuzumab in combination lenalidomide [26, 27] and bortezomib [28, 29] that demonstrated additive or synergistic activities

Table 4 Pharmacokinetics of BIW-8962 after the first and second dose following iv administration every 2 weeks in patients with multiple myeloma during dose escalation in phase Ia

BIW-8962 dose	Day	No. of patients	Mean ± SD						
			T_{max} (h)	C_{max} (ng/ml)	AUC_{last} (µg h/ml)	AUC_{∞} (µg h/ml)	CL (ml h/kg)	Vz (ml/kg)	$t_{1/2}$ (h)
0.03 mg/kg	1	7	0.674 ± 0.471	0.646 ± 0.196	63.5 ± 30.7	118 ± 48.7[a]	0.299 ± 0.139[a]	69.6 ± 17.1[a]	184 ± 80.6[a]
	15	6	–	0.634 ± 0.406	80.2 ± 56.4	178 ± 138[b]	–	–	203 ± 109[b]
0.01 mg/kg	1	3	1.28 ± 0.631	1.37 ± 0.3	166 ± 38.9	292 ± 178	0.42 ± 0.19	94.4 ± 9.77	185 ± 96.4
	15	3	–	1.72 ± 0.616	243 ± 147	408 ± 340	–	–	187 ± 140
0.3 mg/kg	1	3	1.31 ± 0.603	5.11 ± 1.81	537 ± 351	908 ± 661[c]	0.45 ± 0.327[c]	76.8 ± 56.9[c]	118 ± 2.17[c]
	15	3	–	5.19 ± 2.46	669 ± 397	1100 ± 1040[c]	–	–	116 ± 62.6[c]
1 mg/kg	1	5	2.43 ± 1.64	19.3 ± 5.08	1940 ± 554	2250 ± 1170[d]	0.544 ± 0.3[d]	53.0 ± 7.61[d]	80.2 ± 34.6[d]
	15	5	–	21.6 ± 7.14	3070 ± 988	4820 ± 2140	–	–	161 ± 66.7
3 mg/kg	1	4	7.96 ± 11.4	85.9 ± 13.0	8900 ± 2640	12,500 ± 3800[d]	0.255 ± 0.0745[d]	60.8 ± 15.7[d]	174 ± 65.1[d]
	15	4	–	78.3 ± 8.27	14,300 ± 10,200	27,800 ± 12,800[d]	–	–	266 ± 129[d]

AUC_{last} area under the serum concentration–time curve from time zero to the time of the last measurable concentration, $AUC_{0-\infty}$ area under the serum concentration–time curve from time zero to infinity, C_{max} maximum serum concentration, *CL* total systemic clearance, *IV* intravenous,*SD* standard deviation, T_{max} time to C_{max}, $t_{1/2}$ elimination half-life, *Vz* volume of distribution in the terminal phase

[a] $n = 6$

[b] $n = 5$

[c] $n = 2$

[d] $n = 3$

in MM patients. We have not investigated the potential synergism of BIW-8962 with other agents such as lenalidomide, bortezomib, or carfilzomib and cannot therefore rule out this possibility. Similarly, while nivolumab (an anti-PD-1 mAb) was not associated with single-agent activity in MM [30], pembrolizumab (another anti-PD-1 mAb) in combination with lenalidomide has demonstrated responses in lenalidomide-refractory MM patients [31]. As opposed to elotuzumab, mAbs targeting CD38 (daratumamab, isatuximab, and MOR03087) have shown evidence of single-agent activity in patients with relapsed/refractory MM [32]. This was the basis for the approval of daratumumab in the USA.

A limitation of our study, which may have masked potential clinical activity of BIW-8962, was that we were unable to determine the GM2 status of patients by flow cytometric analysis of bone biopsy samples due to either limited stability of GM2 or because the external laboratory that performed the analysis did not first enrich the samples for CD138+ by use of a preparatory column.

CONCLUSION

Further development of BIW-8962 in MM was discontinued given the complete lack of clinical efficacy.

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Compliance with Ethical Guidelines. The study was conducted in accordance with the Declaration of Helsinki and International Conference for Harmonization of Good Clinical Practice Guidelines. The protocol and its subsequent amendments were approved by the Institutional Review Board at each of the four study centers (H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL; Barbara Ann Karmanos Cancer Institute, Detroit, MI; Cleveland Clinic Foundation, Cleveland, OH; Duke University Medical Center, Durham, NC). The study was registered in ClinicalTrials.gov (NCT00775502). All patients provided written informed consent prior to study registration.

Data Availability. The datasets during and/or analyzed during the current study are available from corresponding author on reasonable request.

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