

Phase I Clinical Trial of Marizomib (NPI-0052) in Patients with Advanced Malignancies Including Multiple Myeloma: Study NPI-0052-102 Final Results

Simon J. Harrison¹, Paul Mainwaring², Timothy Price³, Michael J. Millward^{4,5}, Peeter Padrik⁶, Craig R. Underhill⁷, Paul K. Cannell⁸, Steven D. Reich⁹, Mohit Trikha⁹, and Andrew Spencer¹⁰

Abstract

Purpose: Marizomib (NPI-0052) is an irreversible proteasome inhibitor, derived from a marine actinomycete, with activity and specificity that is distinct from other proteasome inhibitors.

Experimental Design: Phase I study (NPI-0052-102) evaluated the MTD, pharmacokinetics, and pharmacodynamics of marizomib intravenously on two dosing schedules.

Results: Forty-two patients with advanced malignancies received Schedule A (0.1–0.9 mg/m² over 1–10 minutes on days 1, 8, 15 in 4-week cycles); 44 patients with relapsed and/or refractory multiple myeloma (RRMM) and other hematologic malignancies received Schedule B (0.075–0.6 mg/m² over 1 minute to 2 hours on days 1, 4, 8, 11, in 3-week cycles). The Schedule A recommended phase II dose was 0.7 mg/m² over 10 minutes; Schedule B was 0.5 mg/m² over 2 hours. The most common (>25% of patients) related adverse events were

fatigue, nausea, diarrhea, and infusion site pain (Schedule A); and fatigue (Schedule B). Overall response rate of 11% was seen in 27 efficacy-evaluable RRMM Schedule B patients (1 very good partial response, 3 partial responses, 4 minimal responses, and 12 stable disease). One Schedule A patient with transformed marginal zone lymphoma had complete response. Marizomib has a short half-life (<30 minutes), with high volume of distribution (~15–416 L) and clearance (~0.9–22 L/minutes).

Conclusions: Marizomib does not exhibit the severe peripheral neuropathy or hematologic toxicity observed with other proteasome inhibitors. Marizomib was generally well tolerated with low-dose dexamethasone, demonstrated activity in heavily pretreated RRMM patients, and warrants further evaluation. *Clin Cancer Res*; 22(18): 4559–66. ©2016 AACR.

Introduction

Marizomib (NPI-0052; salinosporamide A) is a proteasome inhibitor derived from a marine actinomycete that irreversibly inhibits the three major catalytic activities of the 20S core of the ubiquitin-26S proteasome (1, 2). Marizomib rapidly (within seconds) enters cells and forms covalent chemical bonds at all three active enzyme sites in the proteasome, termed β 1 (caspase-like; C-L), β 2 (trypsin-like; T-L), and β 5 (chymotrypsin-like; CT-L). Within minutes, irreversible inhibition both *in vitro* and *in vivo* occurs, with biologic inhibition

reversed through cell replacement and/or proteasome resynthesis (3). Marizomib induces a broader spectrum of proteasome inhibition than the other proteasome inhibitors, bortezomib (4, 5), carfilzomib (6, 7), or ixazomib (8, 9), that are approved for the treatment of multiple myeloma. Marizomib at pharmacologic concentrations does not demonstrate toxicity to bone marrow–derived progenitor cells of any lineage and exhibits marked synergistic antitumor activity when combined with lenalidomide and pomalidomide in *in vitro* and *in vivo* models of myeloma (10, 11). Marizomib does penetrate the blood/brain barrier and inhibits proteasome activity in the central nervous system (CNS) with minimal cytotoxic effects on normal human neural stem/progenitor cells compared with malignant glioma stem-like cells or established glioma cell lines (12).

Marizomib began clinical development with a phase I trial conducted in patients with solid tumors and refractory lymphoma (NPI-0052-100, NCT00396864). Two additional phase I/II trials were conducted. A U.S. trial explored first a weekly dosing schedule and then a twice weekly dosing schedule (NPI-0052-101, NCT00461045). Herein we report the results of the study NPI-0052-102 (NCT00629473) conducted in Australia and Estonia, which explored both the weekly and twice weekly schedules in patients with solid tumors, lymphoma, chronic lymphocytic leukemia (CLL), Waldenstrom's macroglobulinemia, and relapsed and/or refractory (RR) multiple myeloma.

¹The Peter MacCallum Cancer Centre, East Melbourne and Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Australia. ²Mater Adult Hospital South Brisbane, Australia (currently Icon Cancer Care, South Brisbane, Australia). ³Queen Elizabeth Hospital, Adelaide, Australia. ⁴Sir Charles Gairdner Hospital, Nedlands, Western Australia. ⁵University of Western Australia, Perth, Australia. ⁶Tartu University Hospital, Tartu, Estonia. ⁷Border Medical Oncology, Wodonga, Australia. ⁸Royal Perth Hospital, Perth Australia, Australia. ⁹Triphase Accelerator, San Diego, California. ¹⁰The Alfred Hospital, Melbourne, Australia.

Corresponding Author: Simon J. Harrison, Division of Cancer Medicine, Level 5, Peter MacCallum Cancer Centre, Locked Bag 1, A'Beckett Street, Melbourne, Victoria 8006, Australia. Phone: 613-9656-1076; Fax: 613-9656-1408; E-mail: Simon.Harrison@petermac.org

doi: 10.1158/1078-0432.CCR-15-2616

©2016 American Association for Cancer Research.

Translational Relevance

Marizomib (NPI-0052) is a novel, third-generation proteasome inhibitor that irreversibly binds to all 3 subunits [β 1 (caspase-like; C-L), β 2 (trypsin-like; T-L), and β 5 (chymotrypsin-like; CT-L)] of the 20S proteasome. The compound was studied in patients with metastatic solid tumors and advanced hematologic malignancies. The compound has activity in relapsed and refractory multiple myeloma with a good safety profile and a noncross-reactive toxicity profile compared with the current standard-of-care agents such as thalidomide, lenalidomide, bortezomib, carfilzomib, and ixazomib. The findings in this clinical trial and preclinical studies form the rationale for further exploring marizomib (NPI-0052) in a recommended phase II dose study in combination with pomalidomide and dexamethasone in patients with refractory multiple myeloma, which remains an area of unmet clinical need.

Materials and Methods

Study design

This was a multicenter, open-label, phase I trial of marizomib administered by two dosing schedules (Schedule A in patients advanced malignancies and Schedule B in patients with multiple myeloma or other hematologic malignancies). During the dose-escalation stage, patients were enrolled using a classic 3 + 3 study

design for each of the two schedules to determine the MTD and the recommended phase II dose (RP2D). Once the MTD was determined for each schedule, additional subjects were treated at the RP2D to further define the safety and efficacy profiles of marizomib. In addition to determining the MTD and the RP2D, primary objectives of the study included evaluating the blood pharmacokinetics of marizomib when administered on the two schedules. Figure 1 provides the distribution of patients for the two dosing schedules.

Secondary objectives were to evaluate the safety and toxicity profile of repeated dosing of marizomib; to assess the biologic activity and pharmacodynamics of marizomib using laboratory correlative studies (including proteasome inhibition); to recommend a dose for future study of marizomib; and to describe and assess any preliminary evidence of antitumor activity of marizomib using standard response assessment criteria in patients with solid tumors, CLL, lymphoma, or multiple myeloma.

The study was conducted according to the Declaration of Helsinki and was approved by the independent ethics committee/Institutional Review Board at each of the participating centers. It was registered at clinicaltrials.gov as NCT00629473. Here we report the results from the safety, efficacy, and pharmacokinetic analysis. The pharmacodynamic data will be reported separately.

Inclusion and exclusion criteria

Patients were ≥ 18 years of age, had Karnofsky performance status $\geq 70\%$, had recovered from toxicity of prior treatments, and

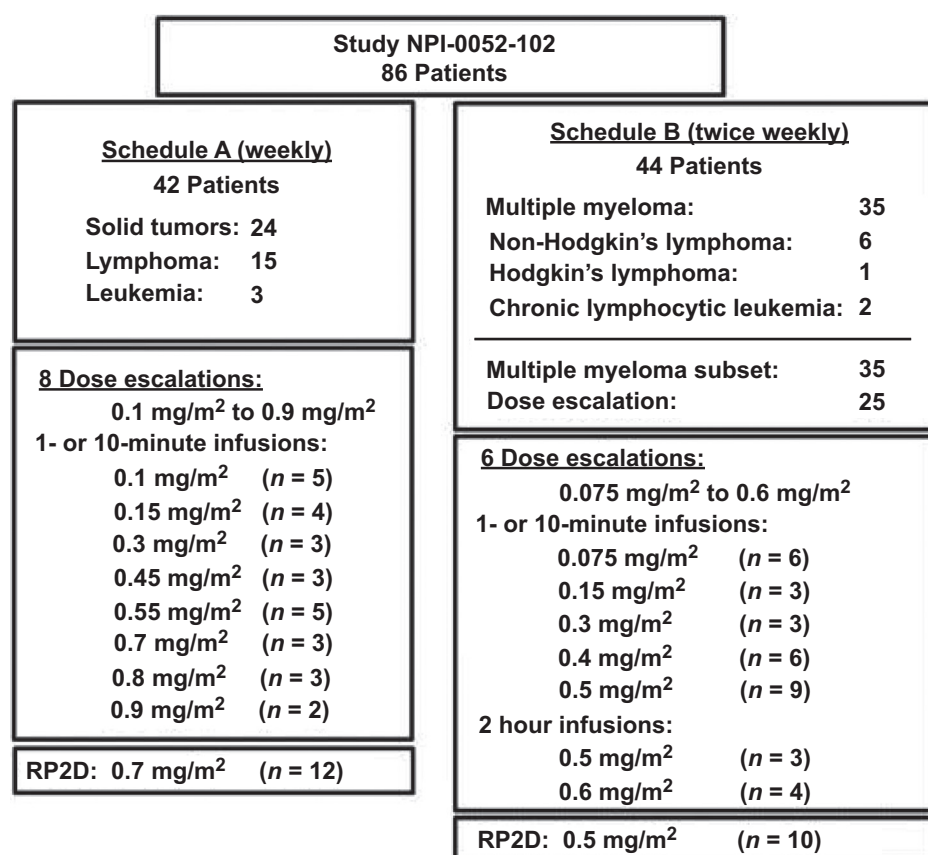


Figure 1.
Distribution of patients.

had adequate organ function. All patients provided written informed consent.

For the dose-escalation stages, Schedule A patients had to have histologically confirmed, advanced malignancy (solid tumors, lymphoma, or leukemia) and Schedule B patients had to have CLL, lymphoma, or multiple myeloma. For both Schedules, patients had to have disease for which a standard, approved therapy was not available and that was measurable.

For the Schedule A RP2D cohort, patients with solid tumors were excluded.

For the Schedule B RP2D cohort, patients were required to have RRMM with measurable disease as defined as one of the following:

- Serum M-protein ≥ 0.5 g/dL;
- Urine M-protein ≥ 200 mg/24 hours; or
- Involved serum-free light chain (FLC) level ≥ 10 mg/dL, provided serum FLC ratio was abnormal.

Relapsed and Refractory was defined as:

- Must have received at least two prior treatment regimens.
- Must have received prior treatment with at least two cycles of lenalidomide and at least two cycles of bortezomib (either in separate regimens or within the same regimen).
- Must have received a cytotoxic chemotherapy agent (e.g., alkylating agent).
- Relapsed disease: Must have progressive disease after having achieved at least stable disease for at least one cycle of treatment during at least one prior regimen.
- Refractory disease: Must have documented evidence of progressive disease during or within 60 days (measured from the end of the last cycle) of completing the most recently received antimyeloma drug regimen before study entry.

Exclusion criteria included administration of chemotherapy, radiotherapy, biologic, immunotherapy, or investigational agent within 28 days before receiving first study drug administration (6 weeks for nitrosourea or mitomycin C; 12 weeks for radioimmunotherapy or bone marrow transplant); surgery, other than diagnostic surgery, within 4 weeks before first study drug administration; patients with ongoing coagulopathies and/or taking anticoagulants; significant cardiac disease; pregnant or breast-feeding women; patients with proteinuria \geq grade 2; any pre-existing kidney disease (acute or chronic) that would impose excessive risk to the patient; bacterial or fungal infection requiring systemic therapy; or patients who were known to be HIV positive or have active Hepatitis A, B, or C infection.

Treatment schedules

For Schedule A, marizomib was administered on days 1, 8, and 15 every 4 weeks by intravenous injection over 1 or 10 minutes (depending upon dose), starting at a dose of 0.1 mg/m^2 . Dose increments of up to 100% were allowed.

For Schedule B, marizomib was administered on days 1, 4, 8, and 11 every 3 weeks by intravenous infusion over 1 to 120 minutes with dose increments of $\leq 50\%$. Patients in the 0.075 and 0.15 mg/m^2 cohorts were treated with 1-minute infusions, in the 0.3 mg/m^2 cohort, patients were treated with 1- to 10-minute infusions. The 0.4 and the initial 0.5 mg/m^2 cohorts were treated with 10-minute infusions to account for the additional volume of drug at the higher doses. In an attempt to ameliorate the overall toxicity of marizomib, infusions were lengthened to 2 hours starting at the 0.5 mg/m^2 dose level and for the 0.6 mg/m^2 and

RP2D cohorts. All but the first patient with multiple myeloma received dexamethasone 20 mg orally or intravenously on the day of marizomib infusion and either the day before or day after marizomib infusion. Those with other malignancies did not receive dexamethasone.

Before marizomib infusion, patients received 350 mL of normal saline administered at approximately 350 mL/hour , which continued after injection to complete a 1-L infusion.

Efficacy assessments

For patients with solid tumors or lymphoma disease, response was to be assessed after two cycles of marizomib treatment (at approximately 8 weeks in patients having no delay in the administration schedule) and every two to three cycles thereafter. For patients with hematologic malignancies, response was to be assessed at the end of every cycle. Standard response assessment criteria were to be used [e.g., Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST); ref. (13) for solid tumors; European Group for Blood and Marrow Transplantation (EBMT; ref. 14), and International Uniform Response Criteria (IURC; ref. 15) for multiple myeloma; Cheson and colleagues 1999 (16) for lymphoma; Cheson and colleagues 1996 (17) for CLL].

Pharmacokinetic assessments

Pharmacokinetic parameters were assessed in up to 3 patients per cohort during the dose-escalation stages and in up to 6 patients from the RP2D cohorts. Blood samples for pharmacokinetic assessment were to be collected before and after the first and last injections in cycle 1 (cycle 1/days 1 and 15; Schedule A), or before and after the first and fourth injections (cycle 1/days 1 and 11; Schedule B) for determination of marizomib blood concentrations. Compartmental-independent pharmacokinetic analyses were performed. The following pharmacokinetic parameters were calculated using standard noncompartmental analysis: maximum observed blood drug concentration (C_{max}), time of maximum blood concentration (T_{max}), elimination half-life ($t_{1/2}$), area under the blood concentration-time curve (AUC_{inf}), clearance (CL), and volume of distribution (V_d). Blood concentrations and computed pharmacokinetic parameters for marizomib were listed and summarized by cohort [mean, geometric mean, SD, coefficient of variation (CV), minimum, maximum, and number of observations].

Statistical analysis and analytical methods

Sample size. The sample size of approximately 85 patients was chosen for this investigation based on practical considerations regarding recruitment and conventional ways of determining dose-limiting toxicities (DLT). Approximately 85 patients were to be treated to determine the toxicity profile, DLTs, and MTD for marizomib. It was estimated that 3 to 6 evaluable patients per dose level would provide sufficient data to assess the toxicity and pharmacokinetic profile for marizomib, followed by cohorts of 10 to 12 patients in targeted diagnoses to confirm the effects of the drug in these populations before initiating phase II studies.

Safety and DLT definitions. All subjects receiving at least 1 dose of marizomib were included in the safety analysis. All adverse events (AE) were graded according to the CTCAE (version 3.0)

Table 1. Patient demographics and baseline characteristics

Characteristics	Schedule A (n = 42)	Schedule B (n = 44)	Multiple myeloma subset (Schedule B), n = 35
Median age (range)	63.5 y (23–80)	62.5 y (38–79)	63.0 y (38–79)
Male	22 (52.4%)	26 (59.1%)	19 (54.3%)
Race			
Asian	2 (4.8%)	1 (2.3%)	1 (2.9%)
White	33 (78.6%)	40 (90.9%)	32 (91.4%)
Other	7 (16.7%)	3 (6.8%)	2 (5.7%)
Median time since initial diagnosis (mo)	43.2 (11.1–177.5)	68.2 (14.8–161.1)	71.4 (21.2–161.1)
Median Karnofsky performance status (KPS) score	90.0 (70–100)	90.0 (70–100)	90.0 (70–100)
Median number of prior oncology regimens (range)	4.5 (1–15)	6.0 (2–13)	7.0 (2–13)

grading system for severity and marizomib-related causality defined. Each subject was counted only once within each MedDRA (Medical Dictionary for Regulatory Activities) preferred term.

The MTD was defined as the dose level below the one that caused at least two DLTs in up to 6 patients. The RP2D was to be the MTD dose unless other safety considerations required lowering the dose.

A DLT was defined as the occurrence of drug-related toxicities observed during the first treatment cycle including grade 4 neutropenia, anemia, or thrombocytopenia, despite transfusion or growth factor support of duration >5 days or febrile neutropenia or the requirement for G-CSF support or platelet transfusion; grade 3 nausea, diarrhea, or vomiting despite maximal supportive care and prophylaxis; clinically significant grade 3 or greater nonhematologic toxicity (not including alopecia, anorexia, or fatigue); and treatment delay of ≥ 2 weeks due to prolonged recovery from a drug-related toxicity.

Efficacy analysis

Tumor response data were reported by descriptive statistics for the patients according to tumor type, and were reported separately for those patients with multiple myeloma treated on Schedule B at the RP2D. Response was determined by a computer program and verified by clinical staff.

Results

Patient characteristics and disposition

Eighty-six patients were enrolled between July 2007 and December 2012 at seven sites in Australia and one site in Estonia. Forty-two patients were treated on Schedule A over eight dose-escalation cohorts (5 patients at 0.1 mg/m², 4 at 0.15 mg/m², 5 at 0.3 mg/m², 3 at 0.45 mg/m², 5 at 0.55 mg/m², 3 at 0.7 mg/m², 2 at 0.9 mg/m², and 3 at 0.8 mg/m²), and an additional 12 patients were then treated at the RP2D of 0.7 mg/m². A further 44 patients were treated on Schedule B over six dose-escalation cohorts (6 patients at 0.075 mg/m², 3 at 0.15 mg/m², 3 at 0.3 mg/m², 6 at 0.4 mg/m², 9 at 0.5 mg/m² using a short infusion, 3 at 0.5 mg/m² using a 2-hour infusion, and 4 at 0.6 mg/m² using a 2-hour infusion) and an additional 10 patients were treated at the RP2D of 0.5 mg/m² infused over 2 hours. Thirty-five patients treated on Schedule B had RRMM, including 25 during the escalation phase and 10 at the RP2D. Distribution of patients is shown in Figure 1.

For Schedule A, the most common diagnoses in the 24 patients with solid tumors were melanoma (25.0%), colorectal cancer (16.7%), stomach cancer (12.5%), and prostate cancer (12.5%).

The most common diagnosis in the 15 patients with lymphoma was B-cell non-Hodgkin's lymphoma (80%). All 3 patients with leukemia had CLL.

For Schedule B, the most common diagnosis for the 44 patients was multiple myeloma (79.5%). The other patients had non-Hodgkin's lymphoma (13.6%), Hodgkin's lymphoma (2.2%), and CLL (4.5%).

Table 1 provides the demographics and baseline characteristics for patients treated on Schedule A, Schedule B, and the subset of multiple myeloma patients treated on Schedule B. Table 2 provides baseline disease characteristics of the subset of multiple myeloma patients treated on Schedule B.

For Schedule A, 4.8% of patients did not complete one cycle and 26.2% of patients completed more than three. The median number of cycles completed (range) was 2 (0–15). The reasons for discontinuation were progressive disease (69.0%), patient decision/withdrew consent (16.7%), and adverse events (14.3%). For Schedule B, 11.4% of patients did not complete one cycle and 36.4% of patients completed more than three. The median number of cycles completed (range) was 2 (0–18). The reasons for discontinuation were progressive disease (72.7%), adverse events (13.6%) or patient developed a DLT (4.5%), patient decision/withdrew consent (4.5%), and patient went on to autologous stem cell transplant (4.5%). For patients with multiple myeloma, 8.6% of patients did not complete one cycle and 42.9% of patients completed more than three. The reasons for discontinuation were progressive disease (77.1%), adverse events (8.6%) or patient developed a DLT (2.9%), patient decision/withdrew consent (5.7%), and patient went

Table 2. Baseline disease characteristics of patients with multiple myeloma treated on schedule B

Characteristic	Multiple myeloma subset (Schedule B), n = 35
Time since initial primary diagnosis (mo)	
Mean (SD)	75.5 (37.10)
Median	71.4
Min, Max	21.2, 161.1
Number of relapses	
1	4 (11.4%)
2	12 (34.3%)
3	17 (48.6%)
Multiple myeloma status at screening	
Relapsed	23 (65.7%)
Relapsed/refractory	12 (34.3%)
Primary resistant	0 (0.0%)
Prior treatment with bortezomib	
Prior treatment with bortezomib	28 (80.0%)
Prior bortezomib regimens (median)	1
Refractory to prior bortezomib	5/26 (19.2%)

Table 3. Treatment-emergent adverse events related to study drug occurring in more than 10% of patients or in at least 1 patient as grade 3

Adverse event MedDRA preferred term	Schedule A (n = 42)		Schedule B (n = 44)	
	All grades	Grade 3	All grades	Grade 3
Patients with ≥1 related TEAE, n (%)	40 (95)	11 (26)	39 (89)	11 (25)
Fatigue	23 (55)	0	16 (36)	2 (5)
Application/infusion/injection site pain	23 (55)	3 (7)	8 (18)	0
Nausea	19 (45)	0	9 (21)	1 (2)
Diarrhea	13 (31)	0	8 (18)	1 (2)
Dizziness	10 (24)	2 (5)	2 (5)	0
Headache	9 (21)	0	3 (7)	0
Vomiting	9 (21)	0	3 (7)	1 (2)
Dysgeusia	8 (19)	0	3 (7)	0
Insomnia	8 (19)	2 (5)	5 (11)	0
Anorexia	6 (14)	0	4 (9)	0
Hallucination (not otherwise specified, visual, auditory)	4 (10)	3 (7)	7 (16)	2 (5)
Delirium/confusional state/feeling drunk/disorientation	3 (7)	2 (5)	4 (9)	2 (5)
Gait disturbance	3 (7)	3 (7)	3 (7)	1 (2)
Anemia	3 (7)	1 (2)	3 (7)	0
Coordination abnormal	2 (5)	1 (2)	4 (9)	1 (2)
Asthenia	2 (5)	1 (2)	1 (2)	0
Dyspnea	2 (5)	1 (2)	3 (7)	0
Convulsions	1 (2)	1 (2)	0	0
Loss of consciousness	1 (2)	1 (2)	0	0
Elevated mood	1 (2)	1 (2)	0	0
Pneumonia	1 (2)	1 (2)	1 (2)	0
Cognitive disorder	1 (2)	0	2 (5)	2 (5)
Aphasia	0	0	1 (2)	1 (2)
Thrombocytopenia	0	0	2 (5)	2 (5)
Lymphopenia	0	0	1 (2)	1 (2)

Abbreviations: MedDRA, Medical Dictionary for Regulatory Activities; TEAE, treatment-emergent adverse event.

on to stem cell transplant (5.7%). No deaths were attributable to trial therapy.

Safety

In Schedule A, 95.2% of patients had at least one treatment-emergent adverse event (TEAE; defined as an AE that started after the initial dose of study drug or an existing AE that worsened during the study) related to marizomib, with the majority being mild to moderate and the rest (26.2%) being severe in severity. Table 3 displays TEAEs that were considered related to marizomib in more than 10% of patients or in at least one patient as a grade 3 AE. The most common related TEAEs in Schedule A were fatigue (54.5%), nausea (45.2%), diarrhea (31.0%), vomiting (21.4%), infusion site pain (28.6%), or injection site pain (23.8%), dizziness (23.8%), and headache (21.4%). For Schedule B, 88.6% of patients had at least one related TEAE, and most were mild to moderate and the rest (25.0%) being severe. The most common related TEAEs in Schedule B were fatigue (37.1%) and nausea (22.9%). The side effect profile of the 35 patient subset with multiple myeloma was similar to that of the all-patient Schedule B profile.

There were no grade 4 (life-threatening)-related AEs

For Schedule A, the related TEAEs that led to discontinuation included pruritus/fatigue/insomnia, loss of consciousness, abnormal coordination, and elevated mood/tremor in a total of 4 (10%) patients. For Schedule B, the related TEAEs that led to discontinuation occurred in 2 patients (5%) and were visual hallucinations, and fatigue/slurred speech/confusional state. Progression of disease was the cause of discontinuation in 29 (69%) for Schedule A and 32 (73%) for Schedule B.

Dose-limiting toxicity

In Schedule A, there were no DLTs in the dose-escalation stage at doses of 0.1 to 0.7 mg/m². The dose was escalated to 0.9 mg/m² and the first 2 patients had DLTs: 1 patient had gait disturbance and insomnia, and the other patient had visual hallucination and feeling drunk. The dose for the next cohort was reduced to 0.8 mg/m². At 0.8 mg/m², there was 1 DLT of dizziness and 1 of hallucination in 1 patient of the 3 patients in the cohort. The MTD was exceeded at the 0.9 mg/m² dose and because there was 1 patient with DLT in the 0.8 mg/m² dose cohort, and other AEs leading to dose reductions, the RP2D was considered to be 0.7 mg/m² infused over 10 minutes.

In Schedule B, 1 patient at 0.075 mg/m² (10-minute infusion) had grade 3 confusion that was a DLT, and the cohort was expanded to 6 patients without further DLTs. One patient at 0.4 mg/m² (10-minute infusion) had DLT of grade 3 intermittent expressive aphasia, and the cohort was expanded to 6 patients without further DLTs. One patient at 0.5 mg/m² (10-minute infusion) had DLT of grade 3 visual hallucinations and another had grade 3 cognitive disorder after cycle 1, so the cohort was expanded by the Safety Committee to 9 patients. At 0.6 mg/m² (2-hour infusion), a patient had DLTs of grade 1 visual disturbance and grade 3 cognitive impairment. Another patient developed grade 3 mental disorientation, unsteady gait, and auditory hallucinations that were considered DLTs, and enrollment into the cohort was halted. The dose of 0.6 mg/m² infused over 2 hours was considered as exceeding the MTD, and the MTD was determined to be the RP2D at 0.5 mg/m² infused over 2 hours.

Efficacy

Schedule A. Of the 14 patients with lymphoma and response evaluation treated on Schedule A, one patient with transformed

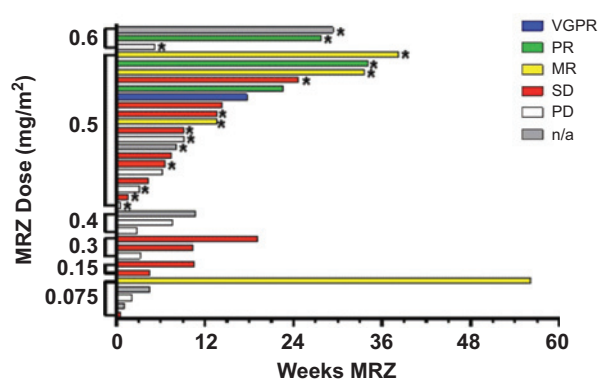


Figure 2. Response and time on therapy for the multiple myeloma patient subset. Infusion duration was 1 to 10 minutes (no asterisk), or 2 hours (*). MR, minimal response; MRZ, marizomib; response assessment not available (n/a); PD, progressive disease; SD, stable disease; VGPR, very good partial response.

marginal zone lymphoma had a complete response (RP2D cohort) lasting at least 10 cycles. There were no objective responses in the 24 patients with solid tumors, but 7 (29%) of these patients in Schedule A had stable disease of short duration. One of the 3 patients with leukemia in Schedule A had stable disease noted at cycle 2 and 3 and at the end of the study.

Schedule B. Of the 35 patients with RRMM who were enrolled in Schedule B, 27 had response evaluations according to IURC. There were 4 (14.8%) objective responses. One patient had a very good partial response at 0.5 mg/m² (10-minute infusion), and 3 patients had partial responses: 1 at 0.5 mg/m² (10-minute infusion), 1 at 0.6 mg/m² (2-hour infusion), and 1 in the RP2D Cohort (0.5 mg/m² by 2-hour infusion) with a median duration of response of 27 weeks. In addition, there were 4 minimal responses: 1 at 0.075 mg/m² (1 minute infusion) and 3 in the RP2D Cohort. There were 12 patients with stable disease across the cohorts starting at 0.075 mg/m² (1 minute infusion). For the 10 patients with sufficient information in the RP2D Cohort, median progression-free survival (95% confidence interval) was 20.4 (2.43–37.0) weeks. One patient with leukemia treated on Schedule B (0.075 mg/m² cohort) had stable disease and received 18 cycles of treatment. None of the 6 patients with lymphoma in Schedule B had a response. The overall response and time on therapy of the patients treated at the RP2D are shown in Figure 2.

Pharmacokinetics

The pharmacokinetic parameter estimates were comparable between the two treatment days in Schedule A (days 1 and 15) and Schedule B (days 1 and 11). Schedule B showed lower mean clearance on treatment day 11 as compared with day 1. However, the high variability associated with the clearance estimates and the limited dataset, limited the interpretation of this observation. The mean C_{max} ranged from 2.76 to 57.75 ng/mL (% CV, 6%–133%) on treatment day 1 and 5.19 to 34.53 ng/mL (%CV, 30%–138%) on treatment day 15 on Schedule A, and from 2.59 to 12.90 ng/mL (% CV, 24%–155%) on treatment day 1 and 1.89 to 28.35 ng/mL (%CV 7%–

101%) on treatment day 11 on Schedule B. The individual C_{max} values were similar between day 1 and day 15 for each dose level except for a few potential outliers at doses 0.3, 0.55, and 0.8 mg/m² in Schedule A, while the individual and mean C_{max} values were higher on day 11 as compared with day 1 especially at doses greater than 0.4 mg/m² on Schedule B. Given the same dose, a shorter duration infusion would be expected to have a higher C_{max} value compared with the value resulting from a longer duration infusion; however, this was not consistently observed, due to high interpatient variability and limited data set. The mean half-life of MRZ was very short, ranging across the entire study was 2.52 to 33.33 minutes (excluding the two potential outlier half-life estimates of >100 minutes on Schedule B). The mean V_d was 14,975 to 415,766 mL and the mean CL was 3,769–16,320 mL/minute in Schedule A patients, while the mean V_d was 17,823 to 129,266 mL and the mean CL was 895–22,311 mL/minute in Schedule B patients. Schedule A showed a statistically higher mean clearance in males (about 41% higher with $P = 0.039$) as compared with females, while no significant gender specific differences in clearance were observed with Schedule B. Dose proportionality assessment (AUC_{last} and AUC_{inf} vs. dose) indicated a general increase in drug exposure with dose in both the dosing schedules. These results indicated that MRZ exhibits a linear pharmacokinetics within the dose range under study with comparable pharmacokinetic parameter estimates on both the treatment days in both Schedules implying no time-dependent changes in pharmacokinetics and minimal accumulation. Marizomib has a very high V_d indicating wide tissue distribution and/or binding to blood components, and a very high clearance suggesting the involvement of extra hepatic clearance mechanisms.

Discussion

Proteasome inhibitors have been validated as effective therapy for patients with RRMM with bortezomib and carfilzomib approved for that indication in multiple regions. Proteasome inhibitors are used in combination with dexamethasone and may be combined with an immunomodulatory drug, such as thalidomide, lenalidomide, or pomalidomide, or chemotherapeutic agents, such as cyclophosphamide or melphalan (18).

The RP2D of the weekly dosing regimen (day 1, 8, and 15) in 4-week cycles was determined to be 0.7 mg/m² infused over 10 minutes in patients with solid tumors and other advanced malignancies such as lymphoma and leukemia. This dose was identical to that determined in the first phase I trial of marizomib (NCT00396864) in a similar patient population. In both studies, DLTs at doses higher than the RP2D often involved symptoms related to reversible dysfunction of the CNS. From preclinical studies, marizomib is known to cross the blood-brain barrier. A study of weekly marizomib in combination with bevacizumab is now underway to explore the safety and preliminary activity in patients with recurrent World Health Organization (WHO) grade IV malignant glioma (NCT02330562).

This study also explored a twice-weekly treatment (day 1, 4, 8, and 15) in 3-week cycles with a schedule similar to that of bortezomib. The RP2D of this trial of 0.5 mg/m² infused over 2 hours in combination with dexamethasone was the same as

another clinical trial (NCT00461045) of marizomib with the same schedule in patients with RRMM. In both studies, the infusion length was increased to 2 hours to ameliorate CNS toxicities observed with a 10-minute infusion that are thought to be related to the maximum concentration that occurs at the end of the infusion. DLTs in Schedule B in this study were primarily CNS toxicities and were observed at doses of 0.075, 0.4, and 0.5 mg/m² when the drug was infused over 1 to 10 minutes. With a 2-hour infusion, CNS toxicities were noted in 2 of 4 patients at 0.6 mg/m² and none of 3 patients in the dose escalation phase, and there were no grade 3 or 4 CNS toxicities related to marizomib in the 10 patients in the RP2D cohort (0.5 mg/m² as a 2-hour infusion). In both studies, dexamethasone was added to potentially prevent toxicities related to immunoglobulin deposition, while dexamethasone may have some antimyeloma activity, patients in this study were heavily pretreated and had been exposed to multiple dexamethasone-containing regimens. Hydration was added before and after the infusion to prevent acute renal injury. With these measures, the number and severity of CNS AEs decreased, and acute renal injury did not appear to be a further issue.

Pharmacokinetic data indicate that the half-life is short with rapid clearance and a large volume of distribution. Exposure increases linearly with dose without indications of accumulation or induction of metabolism. Because it binds irreversibly to all three proteasome subunits, marizomib maintains a long duration of inhibition despite its short exposure and overcomes compensatory hyperactivation of caspase-like (C-L) and trypsin-like (T-L) subunits seen when inhibiting the chymotrypsin-like (CT-L) subunit alone (19). Marizomib does not appear to induce the common bortezomib- and carfilzomib-associated toxicities of peripheral neuropathy, thrombocytopenia, and neutropenia, despite reaching levels of proteasome inhibition that equal or exceed those reported with bortezomib (data not shown).

As a highly potent proteasome inhibitor, marizomib was generally well tolerated and demonstrated activity in heavily pretreated RRMM patients, providing a rational platform for

combinatorial studies in the future. This study determined the RP2D of marizomib in patients with multiple myeloma to be 0.5 mg/m² on days 1, 4, 8, and 11 in 3-week cycles infused over 2 hours with dexamethasone given on the day before and day of marizomib dosing. Marizomib at this RP2D is now being explored in another study in combination with pomalidomide and dexamethasone in patients with refractory multiple myeloma (ClinicalTrials.gov identifier NCT02103335) with the same schedule, with a plan to explore dosing marizomib on a weekly schedule.

Disclosure of Potential Conflicts of Interest

P. Mainwaring reports receiving speakers bureau honoraria from Astellas, Janssen, Novartis, and Roche and is a consultant/advisory board member for Astellas, Janssen, and Novartis. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: P. Mainwaring, M.J. Millward, A. Spencer

Development of methodology: T. Price

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S.J. Harrison, P. Mainwaring, T. Price, M.J. Millward, P. Padrik, C.R. Underhill, P.K. Cannell, A. Spencer

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S.J. Harrison, P. Mainwaring, T. Price, M.J. Millward, P. Padrik, M. Trikha, A. Spencer

Writing, review, and/or revision of the manuscript: S.J. Harrison, P. Mainwaring, T. Price, M.J. Millward, P. Padrik, C.R. Underhill, S.D. Reich, M. Trikha, A. Spencer

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Trikha

Study supervision: S.J. Harrison, P. Mainwaring, T. Price, P.K. Cannell, A. Spencer

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received December 9, 2015; revised March 21, 2016; accepted March 24, 2016; published OnlineFirst April 26, 2016.

References

1. Chauhan D, Catley L, Li G, Podar K, Hideshima T, Velankar M, et al. A novel orally active proteasome inhibitor induces apoptosis in multiple myeloma cells with mechanisms distinct from Bortezomib. *Cancer Cell* 2005;8:407-19.
2. Fenical W, Jensen PR, Palladino MA, Lam KS, Lloyd GK, Potts BC. Discovery and development of the anticancer agent salinosporamide A (NPI-0052). *Bioorg Med Chem* 2009;17:2175-80.
3. Chauhan D, Hideshima T, Anderson KC. A novel proteasome inhibitor NPI-0052 as an anticancer therapy. *Br J Cancer* 2006;95:961-5.
4. Einsele H. Bortezomib. Recent results in cancer research *Fortschritte der Krebsforschung Progres dans les recherches sur le cancer* 2014;201:325-45.
5. Palumbo A, Anderson K. Multiple myeloma. *N Engl J Med* 2011;364:1046-60.
6. Dimopoulos MA, Moreau P, Palumbo A, Joshua D, Pour L, Hájek R, et al. Carfilzomib and dexamethasone versus bortezomib and dexamethasone for patients with relapsed or refractory multiple myeloma (ENDEAVOR): a randomised, phase 3, open-label, multicentre study. *Lancet Oncol* 2016;17:27-38.
7. Kortuem KM, Stewart AK. Carfilzomib. *Blood* 2013;121:893-7.
8. Kumar SK, LaPlant B, Roy V, Reeder CB, Lacy MQ, Gertz MA, et al. Phase 2 trial of ixazomib in patients with relapsed multiple myeloma not refractory to bortezomib. *Blood Cancer J* 2015;5:e338.
9. Richardson PG, Moreau P, Laubach JP, Gupta N, Hui AM, Anderson KC, et al. The investigational proteasome inhibitor ixazomib for the treatment of multiple myeloma. *Future Oncol* 2015;11:1153-68.
10. Chauhan D, Das DS, Ray A, Richardson PG, Trikha M, Anderson KC. Effect of combination of proteasome inhibitor marizomib and immunomodulatory agent pomalidomide on synergistic cytotoxicity in multiple myeloma. *J Clin Oncol* 2014;32:8588.
11. Di K, Gong X, Curticiu DM, Palladino M, Bota DA. Marizomib (NPI-0052) activity as a single agent in malignant glioma. *Cancer Res* 2014;74:1809.
12. Chauhan D, Singh AV, Ciccarelli B, Richardson PG, Palladino MA, Anderson KC. Combination of novel proteasome inhibitor NPI-0052 and lenalidomide trigger *in vitro* and *in vivo* synergistic cytotoxicity in multiple myeloma. *Blood* 2010;115:834-45.
13. Therasse P, Arbus SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of The United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000;92:205-16.
14. Blade J, Samson D, Reece D, Apperley J, Bjorkstrand B, Gahrton G, et al. Criteria for evaluating disease response and progression in patients with multiple myeloma treated by high-dose therapy and

- haemopoietic stem cell transplantation. Myeloma Subcommittee of the EBMT. European group for blood and marrow transplant. *Br J Haematol* 1998;102:1115-23.
15. Durie BG, Harousseau JL, Miguel JS, Blade J, Barlogie B, Anderson K, et al. International uniform response criteria for multiple myeloma. *Leukemia* 2006;20:1467-73.
 16. Cheson BD, Horning SJ, Coiffier B, Shipp MA, Fisher RI, Connors JM, et al. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI sponsored international working group. *J Clin Oncol* 1999;17:1244.
 17. Cheson BD, Bennett JM, Grever M, Kay N, Keating MJ, O'Brien S, et al. National Cancer institute-sponsored working group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. *Blood* 1996;87:4990-7.
 18. National Comprehensive Cancer Network Guidelines (NCCN). Multiple Myeloma Guidelines Version 4; 2015.
 19. Levin N, Spencer A, Harrison SJ, Chauhan D, Anderson K, Reich S, et al. Marizomib irreversibly inhibits proteasome to overcome compensatory hyperactivation in multiple myeloma and solid tumor patients. *Br J Haematol* 9 May 2016, DOI: 10.1111/bjh.14113.