CEP-18770 (delanzomib) in combination with dexamethasone and lenalidomide inhibits the growth of multiple myeloma

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ABSTRACT

Preclinical and clinical studies have shown that proteasome inhibitors (PIs) have anti-MM activity in combination with dexamethasone or lenalidomide. However, no data exists on the anti-MM effects of combinations involving the PI delanzomib with dexamethasone and/or lenalidomide. Herein, we show that delanzomib in combination with dexamethasone and/or lenalidomide results in superior tumor reduction and extended tumor growth delays when compared to vehicle alone, these drugs alone, or the doublet of dexamethasone and lenalidomide. The favorable results obtained from the three xenograft studies suggest that delanzomib in combination with dexamethasone and lenalidomide should be explored for the treatment of MM.

1. Introduction

The immunomodulatory (IMiD) agents lenalidomide and thalidomide overcome drug resistance of human MM cells to conventional therapies and augment natural killer cell cytotoxicity against MM [1,2]. These IMiDs with dexamethasone show clinical efficacy but the neurotoxic effects of thalidomide have limited its clinical use [3–10]. Similarly, the proteasome inhibitor (PI) bortezomib also overcomes drug resistance in MM [11,12] and has been combined with dexamethasone and showed excellent clinical activity [13–15]. The combination of lenalidomide plus bortezomib has also shown efficacy for MM patients [16].

The vast majority of patients with this B-cell malignancy progress and become refractory to all treatments [17]; and, thus, it is imperative that more anti-MM regimens become developed for treating these patients. We previously evaluated the PI delanzomib alone and in combination with bortezomib or melphalan [18]. However, it has not been previously evaluated with lenalidomide and dexamethasone. Given that bortezomib combined with lenalidomide and dexamethasone has shown promising clinical activity [19], in this report we evaluated the anti-MM effect of delanzomib in combination with dexamethasone, lenalidomide and both drugs in vivo using our severe combined immunodeficiency (SCID)-hu models of MM and the human MM cell line RPMI 8226.

2. Materials and methods

2.1. Cell line

The MM cell line RPMI 8226 was obtained from the American Type Culture Collection (Rockville, MD, USA).

2.2. Reagents

Delanzomib (4 mg; Cephalon, Inc., Frazer, PA) stock solution was dissolved in 800 μl propylene glycol (Sigma–Aldrich, St. Louis, MO) and added to 3.2 ml of 5% mannitol (Sigma–Aldrich, St. Louis, MO) to generate a stock solution of 1 mg/ml. This was diluted to the appropriate doses in 5% mannitol (Sigma–Aldrich, St. Louis, MO).

Lenalidomide stock solution was prepared from donated patient pills or synthesized and diluted in 5% carboxymethylcellulose and 0.5% Tween 80 to the appropriate dose (30 mg/kg).

Dexamethasone stock (4 mg/ml) was obtained from the clinic and diluted to the appropriate dose (1.25 mg/kg) using 0.9% sodium chloride.

2.3. MM xenograft models

Eight-week old CB17 SCID mice were obtained from Charles River Laboratories (Wilmington, MA) and housed under sterile conditions. All animal studies were conducted according to protocols approved by the Institutional Animal Care and

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Use Committee. To establish the LAGκ-1A tumor, a BM biopsy was obtained from a female MM patient who had progressed on lenalidomide treatment but after obtaining this biopsy she responded to the combination of melphalan and bortezomib. The biopsy was implanted into a SCID mouse and the tumor has been passaged for many generations [20]. The LAGκ-1B tumor was established using this same protocol from the same patient after she had progressed while receiving treatment with melphalan and bortezomib. These MM tumors (40 mm³) were implanted into recipient mice. For the RPMI 8226 xenograft study, 5×10^6 RPMI 8266 cells were injected subcutaneously (s.c.) and mice randomized when established tumors were 200 mm³.

2.4. In vivo efficacy studies

The LAGκ-1A or LAGκ-1B tumors were allowed to grow in mice for 7 days and then drug treatments were initiated. Mice injected with RPMI 8226 cells grew for three weeks; animals were randomized into treatment groups and drugs started. Delanzomib was administered twice weekly (W, F at 1 mg/kg for the LAGκ-1A tumor; T, Th at 3 mg/kg for the LAGκ-1B tumor; W, F at 3 mg/kg for RPMI 8226) throughout the duration of the study via intravenous (i.v.) injection. The difference in doses between mice containing tumors was based on the previously established sensitivity or resistance of the tumor types to delanzomib [18]. Dexamethasone (1.25 mg/kg) was administered daily via intraperitoneal (i.p.) injection. The injection volume of delanzomib and dexamethasone was 100 μl. Lenalidomide (30 mg/kg) was administered daily via oral gavage injection using a volume of 200 μl.

2.5. Statistical analysis

Tumors were measured at least once weekly using standard calipers and the formula for an ellipsoid volume was applied $(4/3\pi \times [\text{width}/2]^2 \times [\text{length}/2])$. Tumor growth and IgG curves were analyzed in terms of treatment group means and standard error ($n = 9$–10 mice/group). Statistical significance of differences observed was determined using a Student's t-test ($P < 0.05$). Efficacy of drug therapy against tumors was standardized to the time (t), in days, required for the treated and untreated control group tumors to grow to a determined size. This value can be represented as the growth delay between treated and control ($t_t - t_c$) [21]. Percent survival was determined by Kaplan–Meier curves using GraphPad Prism version 4.03, GraphPad Software, San Diego, CA.

2.6. Assessment of human (h) IgG levels

Serum levels of hIgG in LAGκ-1A tumor-bearing mice (LAGκ-1B and RPMI 8226 tumors do not secrete detectable amounts of paraprotein) were determined weekly with an enzyme-linked immunosorbent assay (ELISA). The human IgG ELISA kit (Bethyl Laboratories, Montgomery, TX) was used according to the manufacturer's specifications. Absorbance at 450 nm with a reference wavelength of 550 nm was determined on a μQuant microplate spectrophotometer with KC Junior software (Bio-Tek Instruments, Winooski, VT).

3. Results

3.1. Intravenous administration of delanzomib at 1 mg/kg alone and in combination with dexamethasone and/or lenalidomide reduced the growth of LAGκ-1A

At day 28 post tumor implantation, LAGκ-1A-bearing mice receiving drug treatment with delanzomib or dexamethasone resulted in statistically significant inhibition of tumor volume growth and IgG levels when compared to vehicle-treated mice (Fig. 1A and B, respectively). In contrast, lenalidomide alone did not. Delanzomib inhibited tumor growth more than lenalidomide (tumor volume; $P = 0.0020$: IgG; $P = 0.0007$) or dexamethasone (tumor volume $P = 0.0015$: IgG; $P = 0.0088$). The combination of all three drugs, and delanzomib with either dexamethasone or lenalidomide also significantly reduced tumor growth more than lenalidomide (tumor volume; $P = 0.0004$, $P = 0.0002$, $P = 0.0005$: IgG; $P < 0.0001$, $P < 0.001$: IgG; $P < 0.001$, respectively) or dexamethasone (tumor volume; $P < 0.0001$, $P < 0.0001$, $P < 0.0001$: IgG; $P = 0.0013$, $P = 0.0018$: IgG; $P = 0.003$, respectively). In contrast, lenalidomide plus dexamethasone only inhibited tumor growth more than lenalidomide (tumor volume; $P = 0.0331$: IgG; $P = 0.0444$) but not

more than dexamethasone. Furthermore, when comparing delanzomib to dexamethasone plus lenalidomide, a borderline significant reduction in tumor growth and IgG levels occurred ($P = 0.0544$; $P = 0.0557$, respectively). Importantly, delanzomib with lenalidomide or dexamethasone and the combination of all three drugs significantly inhibited tumor growth and IgG levels when compared to lenalidomide plus dexamethasone (tumor volume; $P = 0.0026$, $P = 0.0007$, $P = 0.0014$: IgG; $P = 0.0067$, $P = 0.0041$, $P = 0.0014$, respectively).

At day 35, mice receiving delanzomib, dexamethasone, or dexamethasone plus lenalidomide showed a reduction in tumor growth and IgG levels compared with mice receiving vehicle (tumor volume; $P = 0.0005$, $P = 0.0205$, $P = 0.0047$: IgG; $P = 0.0026$, $P = 0.0168$; $P = 0.0103$, respectively). Lenalidomide alone had no significant anti-MM effect, in terms of IgG levels or tumor volumes. Mice receiving the three drug combination, delanzomib plus lenalidomide, and delanzomib plus dexamethasone showed lower hIgG levels compared to mice receiving vehicle ($P = 0.0023$, $P = 0.0027$, $P = 0.0015$, respectively). Delanzomib with either dexamethasone or lenalidomide, and in combination with both of these agents markedly reduced tumor volume growth compared with mice receiving vehicle, lenalidomide or dexamethasone. A P-value cannot be calculated because all mice receiving the delanzomib combination regimens had undetectable tumor volumes; and, thus, a value from the t-test was unable to be calculated. As can be seen in Fig. 1A and B, the tumors were undetectable beginning at day 35 until the termination of the study (day 91). The three drug combination was superior to dexamethasone plus lenalidomide (IgG; $P = 0.0004$; tumor volume; cannot be calculated because all mice receiving the three drug combination had undetectable tumor volumes), but was not significantly different, in terms of IgG levels or tumor volumes, than delanzomib with either dexamethasone or lenalidomide. Similarly, delanzomib with either dexamethasone or lenalidomide markedly reduced tumor volume growth compared with mice receiving dexamethasone plus lenalidomide. A P-value cannot be calculated because all mice receiving the delanzomib combinations had undetectable tumor volumes.

Delanzomib markedly reduced hIgG levels compared to lenalidomide or dexamethasone ($P < 0.0001$, $P = 0.004$, respectively). Animals receiving delanzomib with dexamethasone or lenalidomide showed lower hIgG levels than mice treated with lenalidomide or dexamethasone alone or, both drugs together ($P < 0.0001$, $P = 0.001$, $P = 0.0003$, and $P < 0.0001$, $P = 0.0005$, $P = 0.0015$, respectively). Additionally, the three drug combination was superior to lenalidomide ($P < 0.0001$) or dexamethasone ($P = 0.0002$) alone but not compared to delanzomib alone. However, with longer follow up treatment, delanzomib plus dexamethasone and/or lenalidomide combinations proved to be superior to delanzomib alone (Fig. 1A and B).

At study termination, body weights in mice receiving the triplicate combination were measured and mice gained weight (Fig. 1C) similar to what was observed in the other treatment groups (data not shown). Death due to toxicity was favorable and similar between the different regimens. All mice (10/10) were alive in the groups receiving delanzomib alone or in combination with dexamethasone, or dexamethasone alone. Nine of ten mice were alive in groups receiving delanzomib plus lenalidomide or all three drugs together . Eight of ten mice were alive in the single agent lenalidomide group. Survival, as assessed by the number of mice within treatment groups which did not have tumor volumes at ≥2000 mm³, was only favorable in mice receiving combinations of delanzomib with either lenalidomide or dexamethasone or all three drugs together (Fig. 1D).

3.2. Delanzomib at 3 mg/kg alone and in combination with dexamethasone and/or lenalidomide inhibited the growth of LAGκ-1B

At day 28 post implantation, LAGκ-1B-bearing mice receiving delanzomib or dexamethasone plus lenalidomide did not produce significant inhibition of tumor growth compared to vehicle-treated mice (Fig. 1E). However, at days 28 and 35, the three drug combination ($P = 0.0001$ for both time points) and the combination of delanzomib with either dexamethasone ($P = 0.0007$ and $P < 0.0001$ for days 28 and 35, respectively) or lenalidomide ($P = 0.0004$ and $P < 0.0001$) produced markedly smaller tumor
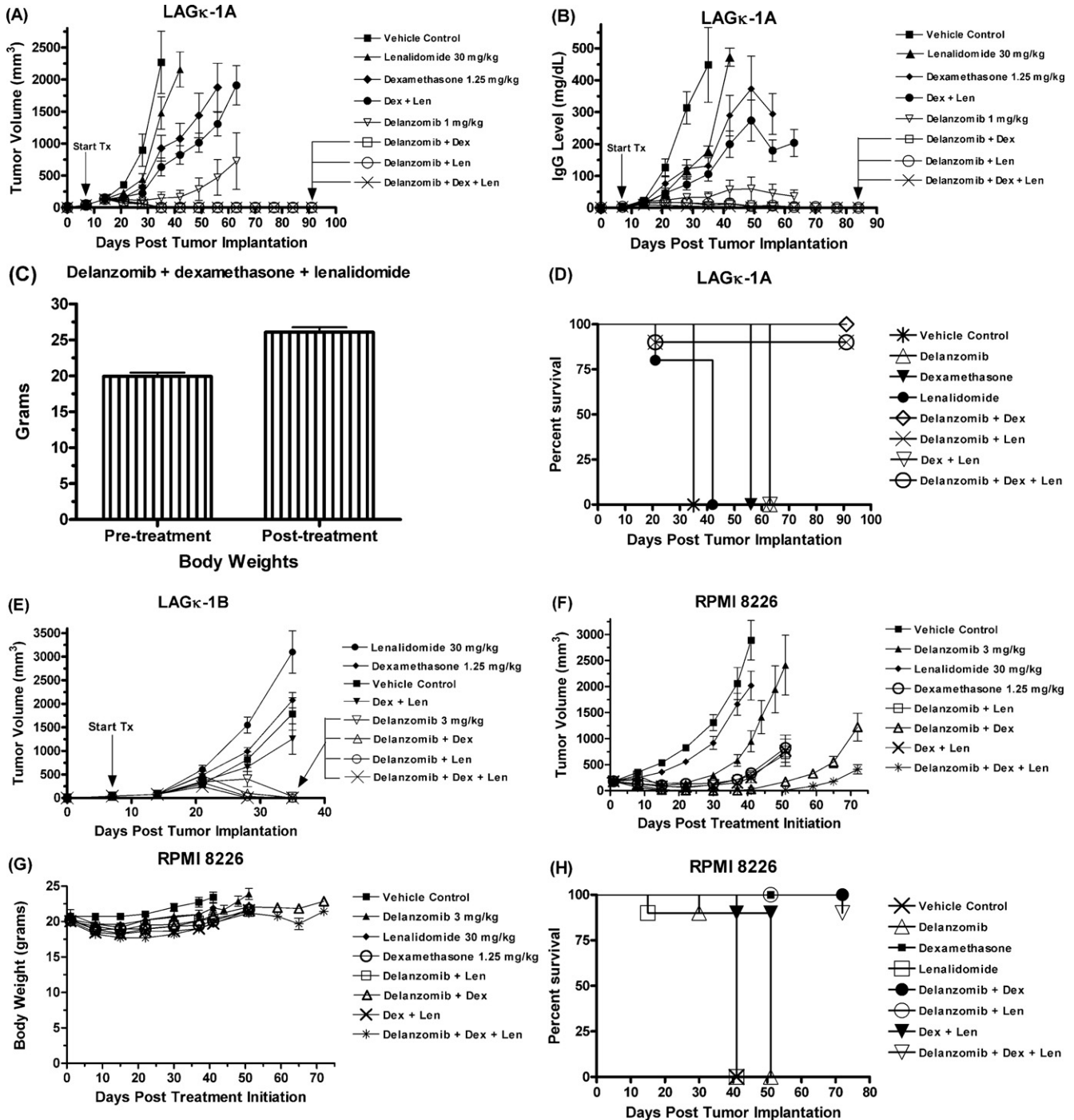

Fig. 1. (A) A significant reduction in tumor volume was observed following delanzomib administration either alone or in combination with dexamethasone and/or lenalidomide, when compared to vehicle-treated mice, single agent treatment groups and the combination of dexamethasone and lenalidomide. (B) Similarly, hIgG levels following delanzomib administration showed similar anti-MM effects as those obtained from assessing tumor volume. (C) At study termination, mice receiving delanzomib in combination with dexamethasone and lenalidomide gained weight when compared to their pre-treatment weights. (D) Survival among mice which had tumor volumes at 2000 mm³ was not favorable for mice which did not receive delanzomib combination therapies. (E) A significant reduction in tumor volume was observed among LAGκ-1B-bearing mice receiving doses of delanzomib (3 mg/kg, twice weekly via i.v. injection) alone or in combination with dexamethasone and/or lenalidomide when compared to vehicle-treated mice. (F) The RPMI 8226 xenograft model was used to assess response to therapy and tumor volume is significantly less compared to vehicle-treated mice, following treatment with delanzomib (at 3 mg/kg twice weekly via i.v. injection) in combination with dexamethasone or lenalidomide or all three drugs together. (G) Body weight was used to assess toxicity of treatment and, at study termination, mice gained weight when dosed with single agents or combination therapy. (H) Survival among mice which did not have tumor volumes at 2000 mm³ was favorable for mice which received delanzomib combination therapies.

volumes compared to vehicle-treated mice (Fig. 1E). By day 35, mice in the groups receiving delanzomib either alone or in combination showed significant anti-MM effects. Overall, treatment with delanzomib alone or with dexamethasone was well tolerated with 10/10 mice in each group alive at study termination. Similarly, treatments with delanzomib plus lenalidomide and the three drug combination were also well tolerated with 9/10 mice alive at study termination.

3.3. Delanzomib at 3 mg/kg alone and in combination with dexamethasone and/or lenalidomide decreased the growth of the MM cell line RPMI 8226

We also evaluated these delanzomib combination treatments in vivo using the RPMI 8226 MM cell line. On days 15, 22, 30, 37, and 41, significant anti-MM activity, when compared to mice receiving vehicle, was observed following treatment with delanzomib alone ($P < 0.0001$, $P < 0.0001$, $P < 0.0001$, $P = 0.0005$, $P = 0.0004$, respectively), dexamethasone alone, delanzomib with dexamethasone and/or lenalidomide, and dexamethasone plus lenalidomide ($P < 0.0001$ for all these remaining groups and time points). In contrast, lenalidomide alone produced significant anti-MM activity only on days 15 and 22 ($P = 0.0044$ and $P = 0.0091$, respectively) when compared to mice receiving vehicle. As single agents, delanzomib also produced more anti-MM activity on days 15, 22, 30, 37, and 41 ($P = 0.0005$, $P < 0.0001$, $P = 0.0003$, $P = 0.0004$ and $P = 0.0067$, respectively) than mice receiving lenalidomide. From days 30 to 51, dexamethasone alone or with lenalidomide, as well as delanzomib with dexamethasone and/or lenalidomide produced significant anti-MM activity when compared to delanzomib as a single agent (Fig. 1F). On days 37, 41 and 51, when comparing these latter treatment groups to each other, delanzomib plus dexamethasone showed smaller tumors than animals treated with dexamethasone alone ($P < 0.0001$, $P = 0.0004$ and $P = 0.0017$), delanzomib plus lenalidomide ($P = 0.0001$, $P < 0.001$ and $P = 0.0042$), and dexamethasone plus lenalidomide ($P = 0.0141$, $P = 0.0292$, but not significant on day 51, Fig. 1F). Similarly, at these same time points, the combination of all three drugs showed smaller tumors than animals treated with dexamethasone alone ($P < 0.0001$, $P = 0.0004$ and $P = 0.0003$), delanzomib plus lenalidomide ($P = 0.0002$, $P < 0.0001$ and $P = 0.0006$), and dexamethasone plus lenalidomide ($P = 0.0213$, $P = 0.0261$ and $P = 0.0223$, Fig. 1F). Importantly, the three-drug combination also proved to be superior to delanzomib plus dexamethasone following a longer duration of treatment, specifically on days 51, 59, 65, and 72 ($P = 0.0048$, $P = 0.0075$, $P = 0.0083$, $P = 0.0135$, respectively; Fig. 1F).

Body weight changes were used to determine tolerability of the treatments, and were similar between all treatment groups with no significant body weight loss observed (Fig. 1G). Death due to toxicity was also similar between the different regimens. All 10 mice survived treatment with vehicle, dexamethasone alone, and delanzomib with either dexamethasone or lenalidomide. Ninety percent (9/10) of mice survived in the groups treated with delanzomib alone, lenalidomide alone, dexamethasone plus lenalidomide and the three-drug combination. Survival, as assessed by mice within treatment groups which had tumor volumes at ≥ 2000 mm^3 at study termination, was worse among mice receiving vehicle control, delanzomib alone and lenalidomide alone (Fig. 1H).

4. Discussion

Although bortezomib has transformed the treatment of MM, many patients do not respond to this drug and those who do respond eventually relapse, whether it is administered alone or in combination therapies [22,23]. Like bortezomib, delanzomib is a reversible PI in the peptide boronic acid class and the inhibitory concentration at 50% of delanzomib and bortezomib, and the ability to block proteasome activities in MM cell lysates were found to be similar [24,25]. Although several different PIs (delanzomib, bortezomib, MLN9708 or MLN2238, carfilzomib, and ONX 0912) mostly target the chymotrypsin-like subunits of proteasomes, preclinical studies have suggested different effects and differential anti-tumor activity between PIs [26]. The combination of the PI NPI-0052 and lenalidomide has shown marked anti-MM activity in a mouse model [27]. ONX 0912 plus dexamethasone or lenalidomide or bortezomib has been shown to induce synergistic or additive anti-MM activity in the MM1S cell line [28]. MLN2238 was shown to induce apoptosis in bortezomib resistant MM cells [29]. Carfilzomib plus NPI-0052 was shown to induce cell death in MM cells derived from bortezomib-refractory patients [30,31]. Our laboratory has shown in vivo that delanzomib can produce anti-MM effects in bortezomib-resistant MM and the combination of delanzomib plus bortezomib, resulted in enhanced anti-MM activity compared to either agent alone [18]. NPI-0052 has also been shown to synergize with bortezomib in vitro and in vivo [32,33]. These findings suggest that although these PIs primarily target a similar target, the proteasome, they show different anti-MM effects and can overcome resistance to other PIs in ways that are not currently understood. These drugs are potent inhibitors of proteasome activity in vitro but show differences in binding kinetics, which might affect their pharmacology and result in different efficacy and safety profiles [26]. This has also now been confirmed from clinical studies. Responses have been observed with carfilzomib among patients who have relapsed from or are refractory to bortezomib [34]. Clinically, results from a phase I/II trial show that MM patients refractory to bortezomib in combination with dexamethasone, alkylating agents, pegylated liposomal doxorubicin, or IMiDs often respond to the same therapeutic regimen when carfilzomib replaces bortezomib in the regimen [35]. Currently, a phase I/II study of carfilzomib plus lenalidomide and dexamethasone for patients with newly diagnosed MM is being conducted with early promising results [36]. Several PIs are now in clinical development and with time it is likely that more clinical studies will demonstrate their anti-MM differences, despite these agents sharing a similar drug target.

The rationale for the evaluation of PIs alone and in combination with conventional anti-MM therapies was first demonstrated with bortezomib [22,37]. Preclinically and clinically, bortezomib enhances the activity of MM therapies [11,12,38–49]. However, as patients will inevitably fail bortezomib-based therapies [22,23], additional treatment options are needed. On the basis of encouraging preclinical and clinical studies, which have shown the efficacy of bortezomib in combination with lenalidomide and/or dexamethasone as well as the ability of another PI, carfilzomib, to overcome resistance to bortezomib using the same dexamethasone or IMiD-containing combination to which the patient failed bortezomib [35], we evaluated the in vivo anti-MM effects of the PI delanzomib in combination with dexamethasone and/or the IMiD lenalidomide in three human MM models in vivo. Although LAGκ-1A tumors eventually grew in mice after single agent treatment with delanzomib, dexamethasone, or lenalidomide or the combination of lenalidomide plus dexamethasone, these tumors did not reappear among mice which received the combinations of delanzomib with either dexamethasone or lenalidomide or all three drugs together. These tumors remained undetectable beginning at day 28 and throughout the study until its termination (day 91). Additionally, reduction of serum IgG levels mirrored the effects observed on tumor volume.

Evaluation of these therapies using a different in vivo model, RPMI 8266, showed similar anti-MM effects of delanzomib in combination with dexamethasone and/or lenalidomide. Although the

initial anti-MM effects were similar between single agent dexamethasone and the doublet combinations of lenalidomide with dexamethasone or delanzomib, animals treated with delanzomib plus dexamethasone or in combination with both dexamethasone and lenalidomide showed more marked anti-MM effects than these other groups with longer treatment. These results were similar to those observed using the LAGκ-1A xenograft model with longer treatment showing the superiority of delanzomib combination therapy over single agent delanzomib, dexamethasone or lenalidomide, or dexamethasone in combination with lenalidomide. Although in the LAGκ-1B model, delanzomib as a single agent showed similar anti-MM effects as the combination treatments at day 35 but the study was terminated at that early point; thus, it is unknown whether further treatment would have resulted in the emergence of superiority of delanzomib combination therapy over single agent PI that was observed in both the LAGκ-1A and RPMI 8226 models with longer treatment.

The significance of the findings from these in vivo studies is that mice treated with delanzomib combination therapies experienced little or no tumor progression with long term therapy whereas single agent treatment failed to provide ongoing protection from tumor regrowth. Our results are also in accord with other preclinical studies demonstrating that combination regimens involving PIs with dexamethasone and/or lenalidomide are synergistic [27–31] and can overcome bortezomib resistance in the clinical setting [34–36]. Collectively, the promising results from these experiments provide evidence for a potentially favorable therapeutic outcome when delanzomib is combined with dexamethasone and/or lenalidomide, and the rationale for the clinical development of these combination treatments for MM patients.

Conflict of interest statement

Acknowledgements

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