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REVIEW

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New therapies in multiple myeloma

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Abstract The melphalan-prednisone regimen has been considered as standard therapy for patients with multiple myeloma (MM) for many years. Recently, high-dose chemotherapy with stem-cell support has extended progression-free survival and increased overall survival, and it is now considered conventional therapy in younger patients. However, most patients relapse and the salvage treatment is not very effective. New active drugs, including immunomodulatory agents, thalidomide (Thal) and lenalidomide, and the proteasome inhibitor bortezomib, have shown promising anti-myeloma activity. These novel treatments are aimed at overcoming resistance of tumour cells to conventional chemotherapy, acting both directly on myeloma cells and indirectly by blocking the interactions of myeloma cells with their local microenvironment and suppressing growth and survival signals induced by autocrine and paracrine loops in the bone marrow. Thal has been widely studied, mostly in combination regimens in patients with relapsed MM and, more recently, in front-line therapy, showing efficacy in terms of response rate and event-free survival. Bortezomib has been found to possess remarkable activity, especially in combination with other chemotherapeutic agents, in relapsed/refractory and newly diagnosed MM, as well as in patients presenting adverse prognostic factors. Lenalidomide, in combination with dexamethasone, is showing high overall response rates in relapsed and refractory MM and promising results also in first-line therapy. In this paper, the results of the most significant trials with Thal, bortezomib and lenalidomide are reported. Several ongoing clinical studies will hopefully allow the identification of the most active combinations capable of improving survival in patients with MM.

Key words Multiple myeloma • Therapy • Thalidomide • Lenalidomide • Bortezomib

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Introduction

Multiple myeloma (MM) is an aggressive and incurable haematological neoplasia, characterised by expansion of malignant plasma cells, which accounts for an estimated 14,000 new cases per year in the USA [1]. For many years, the combination treatment with melphalan-prednisone (MP) has been its conventional chemotherapy, resulting in a median survival of about 3 years.

The frequency of remission, the disease-free survival (DFS) and overall survival (OS) have been improved in patients ≤65 years with the use of first-line high-dose chemotherapy, followed by autologous stem-cell transplantation (ASCT) [2, 3]. Indeed, two large randomised trials have compared this procedure with standard chemotherapy and the overall 5-year survival improved from 12% to 52% in one trial (P=0.03), while the median survival increased from 42 to 54 months in the second trial (P=0.04) [4, 5]. Even so, most patients relapse and further therapies are largely ineffective.

In the past 10 years, new advances have been gained into the understanding of the biologic and molecular mechanisms of MM pathogenesis. Several studies have indeed shown a critical role of the bone marrow microenvironment in the development of this tumour. The interactions of MM cells with stromal cells and extracellular

matrix trigger paracrine and autocrine loops of many cytokines involved in MM progression and activate intracellular signal pathways that promote bone destruction as well as survival, proliferation, drug resistance and genomic instability of myeloma cells [6–9].

The identification of these mechanisms has led to the development of novel therapeutic options to target specific pathways involved in the pathogenesis of disease in order to disrupt functional interactions between MM cells with bone marrow microenvironment and to block autocrine self-supporting circuits. These agents include the immunomodulatory drug thalidomide (Thal), the proteasome inhibitor bortezomib, and the Thal derivative lenalidomide. Clinical studies have shown encouraging results first in patients with relapsed/refractory MM, then in newly diagnosed patients. These drugs, alone and in combination, are now all approved treatment options for symptomatic MM.

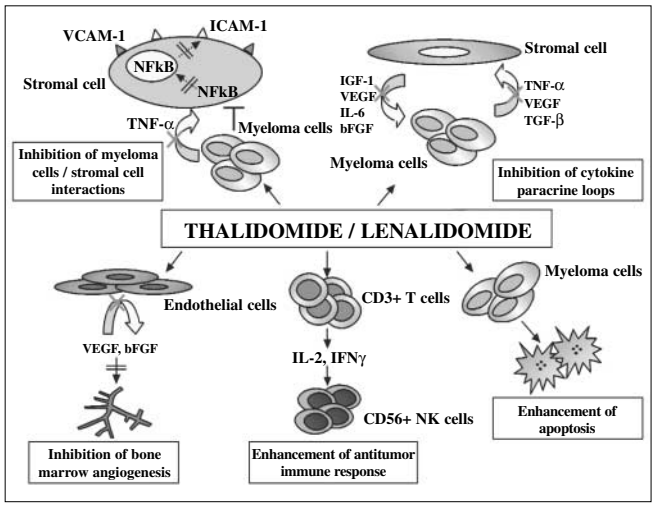

Fig. 1 Schematic representation of the effects of Thal and lenalidomide on myeloma cells, tumor microenvironment, and host immunity. *VCAM-1*= vascular cell adhesion molecule 1; *ICAM-1*= intercellular adhesion molecule 1; *TNF-α*= tumor necrosis factor alpha; *NFkB*= nuclear factor kappa B; *bFGF*= basic fibroblast growth factor; *VEGF*= vascular endothelial growth factor; *IGF*= insulin growth factor; *IL-6*= interleukin 6; *TGF-β*= transforming growth factor beta; *IL-2*= interleukin 2; *IFNγ*= interferon gamma

Thalidomide

Mechanism of action

Thal was introduced in the treatment of advanced MM in the late 1990s, because of its antiangiogenic properties. These were first described by D'Amato et al. [10], who showed that Thal and its metabolites inhibit basic fibroblast growth factor (bFGF)-induced angiogenesis in a rabbit cornea micropocket assay. The antiangiogenic activity of Thal metabolites was further confirmed by other Authors [11–13]. More recently, a significant decrease in bone marrow microvessel density was demonstrated in patients who responded to Thal [14] without reduction in the plasma level of angiogenic cytokines [15], thus suggesting that different mechanism(s) are involved in the antitumour effect of the drug.

Subsequently, these mechanisms have been progressively disclosed. Thal has been shown to: (a) induce *in vitro* growth arrest during the G1 phase and apoptosis by either triggering activation of caspase-8 and/or increasing the MM cell sensitivity to Fas-induced apoptosis [16, 17]; (b) block the increased secretion of tumour necrosis factor alpha (TNF-α) by either enhancing TNF-α mRNA degradation or neutralising α1-acid glycoprotein, a stimulator of TNF-α secretion [18–20]; (c) modulate myeloma/stromal cell interaction by either decreasing the expression of adhesion molecules (ICAM-1, VCAM-1, E-selectin and L-selectin) or inhibiting the paracrine loops of cytokine secretion (particularly vascular endothelial growth factor (VEGF) and interleukin (IL)-6) [21, 22]; (d) enhance host immune response against MM, by the ability to increase anti-CD3+ T-cell-induced proliferation and cytokine secretion [IL-2 and interferon (IFN)-γ] in normal donors [23] and/or by increasing *in vivo* CD56+ NK cell proliferation [24]; and (e) interfere with intracellular growth signalling by down-regulating the constitutive activity of nuclear factor kappa B (NFkB) (i.e., through the block of insulin-like growth factor-1) [25, 26] as well as the expression of cellular inhibitor of apoptosis protein-2 (cIAP-2) and FLICE inhibitory protein (FLIP), two well known inhibitors of apoptosis [17] (Fig. 1).

The role of TNF-α is crucial, as indicated by Thompson et al. [27] and Neben et al [28]. The former investigators showed a poorer progression-free survival (PFS) of Thal-treated MM patients if their pretreatment serum TNF-α levels were elevated, while the second group demonstrated a correlation between TNF-α gene polymorphism and response to Thal.

Clinical studies

Relapsed/refractory myeloma

The efficacy of Thal as a single agent or in combination in the treatment of relapsed/refractory MM is described in Table 1 [29–36]. Singhal et al. were the first to demonstrate that Thal is effective in MM treatment [29]. In this phase II study and its update on 169 patients (most of whom had failed high-dose therapy) [30], a 30% partial response (PR) and a 14% near complete response (nCR) were observed. A 2-year follow-up showed 20% and 48% event-free survival (EFS) and OS rates respectively. Cytogenetic abnormalities were associated with a poor prognosis.

Table 1 Response and survival of refractory/relapsed myeloma patients following thalidomide-based therapy

Patients (no.)	Schedule			Response (%)			Survival (%/evaluation year)		Reference
	Drugs	Dose	Time	PR	CR+nCR	OR	EFS	OS	
84	Thal	200–800 mg/day	Continuous	–	–	32	22±5/1	58±5/1	[29]
169	Thal	200–800 mg/day	Continuous	30	14	44	20±6/2	48±6/2	[30]
83	Thal	400 mg/day	Continuous	35	13	48	78/1[a]	87/1[a]	[31]
77	Thal Dex	100 mg/day 40 mg/day	Continuous Days 1–4/month	23	18	41	1[b]	–	[32]
44	Thal Dex	200–400 mg/day 20 mg/m^2	Continuous Days 1–4, 9–12, 17–20, then 1–4	55	–	55	–	1.05[c]	[33]
71	Thal Cy Dex	200–800 mg/day 50 mg/day 40 mg/day	Continuous Continuous Days 1–4 every 3 weeks	55	2	57	57/2[b]	66/2	[34]
52	Thal Cy Dex	100–300 mg/day 300 mg/m^2 40 mg/day	Continuous 1 every week Days 1–4 every 4 weeks	62	17	79	34/2	73/2	[35]
50	Thal Doxil Dex	100 mg/day 40 mg/m^2 40 mg/day	Continuous Days 1 Days 1–4, 9–12	44	32	92	61/1	79/1	[36]

[a]Patients with favourable prognostic markers; [b]progression-free survival (PFS); [c]median OS in years
PR, partial response; *CR*, complete response; *nCR*, near complete response; *OR*, overall response; *EFS*, event-free survival; *OS*, overall survival ; *Thal*, thalidomide; *Dex*, dexamethasone; *Doxil*, pegylated liposomal doxorubicin; *Cy*, cyclophosphamide

Many other studies have demonstrated the efficacy of Thal as a single agent in advanced MM with response rates ranging from 25% to 64% [37–39].

Thal-Dexamethasone (Dex)

Preclinical studies have suggested synergistic activity of Thal-Dex and clinical results have confirmed major efficacy of this combination as compared with Thal alone [40, 41]. Dimopoulos et al. treated 44 patients with Thal (schedule is shown in Table 1) [33]. Twenty-four patients (55%) achieved a PR with a median time to response of 1.3 months. The median time to progression for responding patients was 10 months and the median survival for all patients was 12.6 months. Similar encouraging results have also been reported in another phase II study (Table 1) [32]. After 3 months of treatment, 41% of patients reported objective response rates (18% complete response (CR) and nCR). After a median follow-up of 8 months, median PFS was 12 months.

Thal-Dex-pegylated liposomal doxorubicin (Doxil) or Thal-Dex-cyclophosphamide (Cy)

The addition of Doxil to Thal-Dex increased the rate of objective responses up to over 70% [42]. In a recent prospective multicentre phase II study, 50 patients received Thal-Dex and Doxil (40 mg/m^2) (Table 1). The overall response (OR) rate was 92%, with 26% CR, 6% nCR and 44% PR [36]. The median PFS, EFS and OS were 22 months, 17 months and not reached, respectively.

A regimen that included cyclophosphamide (Cy) with Thal-Dex showed objective responses in 79% of the 52 patients (17% of them reaching a CR) [35]. This combination can induce a stable plateau phase and prolong stable disease.

Newly diagnosed MM

The efficacy of Thal as salvage treatment has led to the use of this agent in newly diagnosed MM patients and to its proposal as front-line therapy in preparation for subsequent ASCT [43, 44].

For many years, vincristine, doxorubicin and Dex (VAD) has been used as standard pretransplantation induction therapy [45]. However, VAD has some disadvantages, including the risk of catheter-related infection with related thrombosis and toxicity (particularly, cardiac and neuropathic), which limit the subsequent use of Thal or bortezomib in advanced phases of the disease. In a comparative case-control study of 200 patients, the superiority of Thal-Dex over VAD was reported with a significantly higher response rate (76% *vs.* 52%; $P<0.001$) [46] (Table 2) [46–52]. In each of the 2 treatment groups, 91% of patients proceeded to peripheral blood stem-cell mobilisation.

Recently, in a phase 3 randomised study, Thal-Dex combination therapy has been compared to Dex alone in 207 patients eligible for ASCT [48]. The OR was signif-

Table 2 Response and survival of newly diagnosed myeloma patients following Thal-based therapy in comparative trials

Patients (no.)	Schedule			Response (%; *P*)			Survival (%; *p*/evaluation-year)		Reference
	Drugs	Dose	Time	PR	CR+nCR	OR	EFS	OS	
200	Thal Dex[a]	100–200 mg/day 40 mg	Continuous Days 1–4, 9–12, 17–20 then 1–4	63 *vs.* 39; NA[b]	13 *vs.* 13; NA	76 *vs.* 52; <0.001	–	–	[46]
668	Thal high-dose therapy[c]	100–400 mg/day	Continuous	–	62 *vs.* 43; <0.001	62 *vs.* 43; <0.001	56 *vs.* 44; =0.01/5	65 *vs.* 65; =0.09/5	[47]
207	Thal Dex[d]	200 mg/day 40 mg	Continuous Days 1–4, 9–12, 17–20	–	4	63 *vs.* 41; =0.0017	–	–	[48]
255	Thal Melphalan Prednisone (MPT)[e]	100 mg/day 4 mg/m^2 40 mg/m^2	Continuous Days 1–7 Days 1–7	60.4 *vs.* 45.2; <0.0001	27.9 *vs.* 7.2; <0.0001	76 *vs.* 47.6; <0.0001	54 *vs.* 27; =0.0006/2	80 *vs.* 64; =0.19/3	[49]
50	Thal Melphalan Dex	300 mg/day 8 mg/m^2 20 mg/m^2	Days 1–4, 17–20 Days 1–4 Days 1–4, 17–20	62	10	72	–	–	[50]
597	Thal Pamidronate[f]	100 mg/day 90 mg/m^2	Continuous Day 1 every 4 weeks	30 *vs.* 37 *vs.*37; =0.001	67 *vs.* 57 *vs.* 55; =0.001	97 *vs.* 97 *vs.* 92; =0.001	52 *vs.* 37 *vs.* 36; <0.009/3	87 *vs.* 74 *vs.* 77; <0.04/4	[51]
112	Thal[g]	200 mg/day	Continuous	–	–	–	–	5.4 *vs* 3.8; =0.09[h]	[52]

[a]*vs.* VAD; [b]*NA*, not applicable; [c]*vs.* no Thal; [d]*vs.* Dex; [e]*vs.* MP; [f]*vs.* pamidronate *vs.* no maintenance; [g]maintenance in post-transplantation; [h]median OS

icantly higher with Thal-Dex than Dex alone (63% *vs.* 41% respectively, *P*=0.0017), with CR in 4% of patients. Disease progression was noted in 2% of patients with Thal-Dex and 5% of patients with Dex alone. Stem-cell harvest was successful in 90% of patients in each arm. However, combination therapy was associated with more severe nonhaematologic toxicity ≥ grade 3 (67% *vs.* 43%). Thromboembolic events, especially deep venous thrombosis (DVT), occurred more frequently in the Thal-Dex arm (17% *vs.* 3%, *P*<0.001). The evaluation of OS was not an endpoint in this study, because the trial was intended to assess the efficacy of pretransplantation induction therapy.

The capacity to further prolong survival and to improve the outcome of patients with MM has been evaluated in a randomised phase III study. Patients (668) were enrolled to receive two cycles of high-dose melphalan supported by ASCT either with Thal (400 mg daily during induction chemotherapy, 100 mg daily between transplantations, 200 mg daily with consolidation therapy, 100 mg daily during the first year of maintenance therapy and 50 mg on alternate days) or not [47]. After a median follow-up of 42 months, the CR rate in the Thal group was 62% *vs.* 43% in the control group (*P*<0.001), and the 5-year EFS was 56% *vs.* 44% (*P*=0.01). Even so, the five-year OS did not statistically differ between the two groups (65% in both groups, *P*=0.90) and the median survival after relapse was lower in the Thal group (1.1 years) than in the control group (2.7 years) (*P*<0.001).

The occurrence of adverse events (DVT and pulmonary embolism) was higher in the Thal group (30% *vs.* 17% of the control group). The addition of low-molecular-weight

heparin given prophylactically, starting after three years from the beginning of the study, did not reduce the risk. Moreover, the incidence of debilitating peripheral neuropathy (>grade 2) was more frequent in the Thal group than in the control group (27% *vs.* 17%, $P<0.001$). These results indicate that: (a) Thal failed to improve OS; (b) it was associated to severe toxic effects; and (c) CR was not necessarily correlated to a longer survival. When the relapsed patients received salvage therapies, the response rate was higher and the survival longer in the patients who did not receive Thal, suggesting that the drug may also induce resistance to following treatments in relapsed patients.

However, some studies showed encouraging results with the use of Thal as maintenance therapy following high-dose chemotherapy/ASCT [53, 54]. In a randomised phase III study, conducted by the InterGroupe Francophone du Myélome, 597 patients were assigned to receive maintenance therapy with Thal (100 mg daily) plus pamidronate (PAM), maintenance with PAM alone or no maintenance [51]. A CR or very good partial response (VGPR) was achieved by 67% of patients in the group receiving Thal-PAM, 57% in the group of PAM alone and 55% in the group with no maintenance. Thal-PAM increased 3-year EFS compared to the other two groups (52% *vs.* 37% and 36%, respectively; $P<0.009$) as well as the probability of 4-year OS (87% *vs.* 74% and 77%; $P<0.04$).

In a recent retrospective study on 112 patients undergoing ASCT, it has been demonstrated that patients receiving Thal-based maintenance treatment had a higher median survival (65.5 months) as compared with patients (44.5 months) who did not receive Thal ($P=0.09$) [52]. Based on these results, the post-transplant use of low-dose (50–100 mg) Thal as maintenance treatment appears to be promising, because of the lower incidence of adverse events (thromboembolic risk and peripheral neuropathy) and of developing drug resistance.

MP and Thal (MPT)

In patients over 60, who are not eligible for bone-marrow transplantation, the results of a phase III randomised trial conducted by Palumbo et al. showed that the addition of Thal to MP in elderly patients is more active than standard MP [49]. One hundred and twenty-nine out of 255 enrolled patients received Thal (100 mg daily until progression or relapse) and oral MP for six 4-week cycles. These patients had higher response rates and longer EFS than patients treated with MP alone. Combined CR and PR rates were 76% for MPT and 47.6% for MP alone, and the nCR or CR rates were 27.9% and 7.2%, respectively. Two-year EFS was 54% for MPT and 27% for MP ($P=0.0006$). Three-year survival rates were 80% for MPT and 64% for MP ($P=0.19$). The grade 3 or 4 adverse events of MPT compared with those of MP were 48% *vs.* 25%. The addition of enoxaparin prophylaxis reduced the rate of thromboembolism from 20% to 3% ($P=0.005$).

Thus, therapy with Thal is effective, even if not curative, in the patients with symptomatic MM and can offer the advantage of oral administration.

Bortezomib

Mechanism of action

Bortezomib is representative of a class of peptide boronate proteasome inhibitors, which target the 26S proteasome, a multicatalytic proteinase complex involved in intracellular protein degradation [55].

A variety of proteins that regulate cell-cycle progression, signal transduction, gene expression, apoptosis, immune response and angiogenesis are tagged for degradation by polyubiquitin chains. The ubiquitin-tagged proteins entering the proteasome are stripped of their ubiquitin and cleaved. Bortezomib has high affinity, specificity and selectivity for catalytic activity of the proteasome. It ultimately inhibits the activation of the transcription factor NF-kB by protecting its inhibitor (IkB) from degradation by the proteasome complex (Fig. 2).

NF-kB is a transcription factor constitutively active in MM and, when it is bound to its inhibitor IkB, it is retained in the cytoplasm [8, 56, 57]. Degradation of IkB by proteasome activates NF-kB, which moves to the nucleus and up-regulates transcription of proteins that promote cell survival and growth, reduce susceptibility to apoptosis, influence the expression of adhesion molecules and induce drug resistance in myeloma cells. Bortezomib can also directly induce apoptosis of primary and drug-resistant myeloma cell lines by interfering with the caspase-dependent pathway, by down-regulating IL-6 and up-regulating p53 and the cell-cycle inhibitor p27 [58, 59].

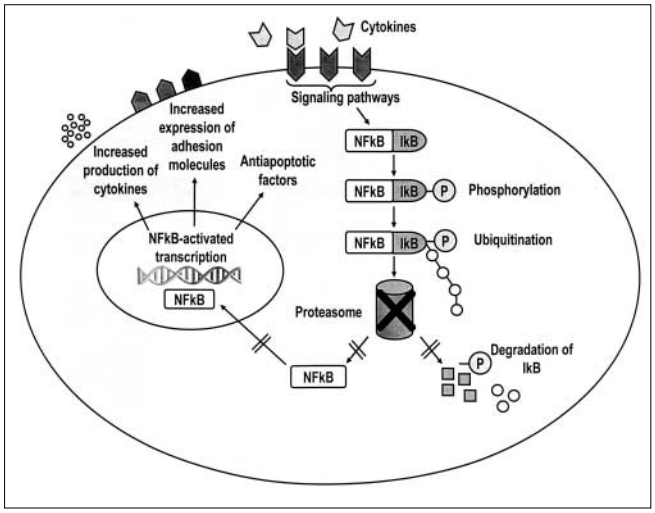

Fig. 2 Mechanism of action of bortezomib. *P*, phosphorylated protein; *IkB*, nuclear factor kB inhibitory protein

Furthermore, bortezomib acts in the bone marrow microenvironment by inhibiting the binding of MM cells to bone marrow stromal cells, bone marrow-triggered angiogenesis and/or cytokine (particularly IL-6) transcription/secretion involved in the growth, survival and migration of myeloma cells and in the bone marrow angiogenesis [58, 60].

The results of recent studies suggest that bortezomib inhibits osteoclastic bone resorption and increases osteoblastic function [61–64], thus counteracting the lytic processes triggered by myeloma cells.

Clinical studies

Relapsed/refractory myeloma

Bortezomib has been approved for the treatment of relapsed/refractory MM in the USA and Europe on the basis of results of two phase 2 clinical trials, namely SUMMIT and CREST, and of the multicentre randomised phase 3 APEX study [65–67]. This trial, conducted on 669 patients who relapsed after the first-, second- or third-line therapies, compared bortezomib with standard high-dose Dex and demonstrated a higher efficacy of bortezomib than Dex in terms of response rate, time to progression (primary endpoint) and OS. The results of these trials are summarised in Table 3 [65–69]. An APEX subgroup analysis demonstrated the efficacy of bortezomib also in elderly high-risk patients (β2-microglobulin level >2.5 mg/l, >65 years of age, refractory to previous therapy and >1 previous line of therapy). Furthermore, bortezomib was more effective in patients who received the drug as second- than as third- and fourth-line treatment (45% *vs.* 26% P=0.004). At 8.3-months median follow-up, the median duration of response was significantly longer in first relapsed patients treated with bortezomib than in those with Dex (8.1 *vs.* 6.2 months, respectively).

More recently, an updated analysis of the APEX trial confirmed significant benefits of bortezomib after a longer follow-up (median 22 months) as far as time to progression and duration of response were concerned. The OR (CR and PR) improved from 38% to 43% and the CR and nCR rate increased to 16% [70]. In this trial, the most prominent bortezomib-dependent adverse events were grade 3–4 (8% of patients) and grade 1–2 (28% of patients) peripheral neuropathy. Gastrointestinal disorders were: grade 1–2 diarrhoea (50%), vomiting (35%), nausea (57%), constipation (42%) and grade 1–2 fatigue (37% of patients). Herpes zoster infection was common during the treatment (13% *vs.* 5% of patients of the Dex group; P<0.001). Grade 3–4 thrombocytopenia was also more frequent (30% *vs.* 6% of Dex group), but reversible in a short time.

Bortezomib-Dex

Of the 256 patients enrolled in the SUMMIT (n=202) and CREST (n=54) studies, 106 patients (41%) who had a suboptimal response, in addition to bortezomib, received Dex at the third or fourth cycle (20 mg on the day of and the day after each dose of bortezomib) [71]. Thirteen (18%) patients from SUMMIT and 9 (33%) from CREST had improved responses to the combined treatment, including the 8 patients (6 out of 13 and 2 out of 9 from SUMMIT and CREST study respectively) who had been refractory to previous Dex regimen alone. Nevertheless, the median time to progression in this subset of patients was shorter than in the whole population of patients both in SUMMIT and CREST studies (5.3 *vs.* 6.9 months and 6.8 *vs.* 10.6 months, respectively).

Bortezomib-melphalan

To improve survival of patients with relapsed or refractory MM and overcome chemotherapy resistance, additional combinations have been considered.

In a phase I/II trial on 35 patients, which included the association of bortezomib plus low doses of oral melphalan, CR and PR were achieved in 47% of them (6% of CR and 9% of nCR) [68]. Median PFS was 8 months (Table 3). The addition of Dex to this combined therapy improved response rates up to 80% [72].

Bortezomib-Cy and bortezomib-Doxil

The combination bortezomib, Dex and Cy was shown to be significantly more effective than bortezomib alone or bortezomib and Dex (OR rates were 64% *vs.* 30% and 47% in 42 evaluable patients) [73].

In a phase 1 study on 22 evaluable patients, the association of bortezomib and Doxil demonstrated significant antitumour activity in advanced MM, with an OR rate of 73%, including 36% of CR or nCR [74]. An additional follow-up on all patients revealed a median time to progression of 9.3 months, and a median OS of 38.3 months [75]. These findings have suggested the possibility that this combination treatment may be very effective in relapsed/refractory MM. Actually, a multicentre randomised phase 3 study on 646 patients comparing the combination of Doxil and bortezomib *vs.* bortezomib alone has confirmed these encouraging results [76].

Bortezomib-MP-Thal (VMPT)

Among the bortezomib-based combinations, the addition of immunomodulatory drugs is also being investigated. In a recent multicentre phase I/II study, Palumbo et al. evaluated the efficacy and tolerability of VMPT on 30 patients to identify the most appropriate and effective dose of bortezomib in the MPT regimen [69] (Table 3). The maximum tolerated dose of bortezomib was 1.3 mg/m^2. Sixty-seven percent of patients achieved a PR and 43% of these had a VGPR. A CR was observed in the subset of patients who received this reg-

Table 3 Response and survival of refractory/relapsed myeloma patients following bortezomib-based therapy

Patients (no.)	Schedule				Response (%; *P*)			Survival (median in months)		Reference
	Drugs	Dose	Time (days)	Cycles (no.)	PR	CR+nCR	OR	PFS	OS	
202	Bortezomib Dex[a]	1.3 mg/m^2 20 mg	1,4,8,11 every 3 weeks; on day and day after bortezomib	8	18	10	28	12[b]	16	[65]
54	Bortezomib Dex[a]	1 or 1.3 mg/m^2 20 mg	1,4,8,11 every 3 weeks; on day and day after bortezomib	8	–	–	30 38 50[c]	–	–	[66]
669	Bortezomib[d]	1.3 mg/m^2	1,4,8,11[e] 1,8,15,22[f]	8 3	25 *vs* 16; <0.001	13 *vs* 2; <0.001	38 *vs* 18; <0.001	6.22[b]	80[g]	[67]
35	Bortezomib Melphalan	0.7–1 mg/m^2 0.025–0.25 mg/kg	1,4,8,11 then 1–4 every 4 weeks	8	32	15	47	8	–	[68]
30	Bortezomib Melphalan Prednisone Thal (VMPT)	1–1.6 mg/m^2 6 mg/m^2 60 mg/m^2 50 mg	1,4,15,22 1–5 1–5 1–35	6	50	17	67	61[g]	84[g]	[69]

[a]Administered in patients with suboptimal response (progressive disease or stable disease after two or four cycles respectively); [b]time to progression in months; [c]results with both doses of bortezomib; [d]*vs.* high-dose Dex; [e]induction; [f]maintenance; [g]% at 1 year

imen as second-line treatment (36%). The 1-year PFS and 1-year OS were 61% and 84%, respectively.

Other bortezomib-based combination regimens

Many other combination regimens including Thal and lenalidomide have been considered, such as bortezomib-Doxil-Thal or bortezomib-Thal-Dex or bortezomib–lenalidomide [77–79]. Most phase I or II studies reported response rates of about 60%, with improvement of median PFS and OS compared to standard regimens of chemotherapy. These results are promising and raise the suggestion that bortezomib, in association with either conventional or novel active agents, may offer a valid treatment option in relapsed and refractory patients presenting adverse prognostic factors such as increased β2-microglobulin, cytogenetic abnormalities (chromosome 13 deletion) and/or renal failure.

Newly diagnosed MM

Bortezomib is currently being evaluated as a first-line treatment in previously untreated patients either ineligible or candidates for ASCT in ongoing clinical trials. In the front-line setting, bortezomib was studied as a single agent in a multicentre phase 2 study in 63 patients (46 of them evaluable for response) with a median age of 60 years [80]. Eleven, 20 and 28% of patients obtained a complete, partial and minimal response respectively, giving an OR rate of 59%. The most common adverse events were peripheral neuropathy (55% treatment-related) of mild to moderate severity, and fatigue, rash, nausea, constipation and infection by Varicella zoster virus.

Bortezomib-MP

Mateos et al. performed a phase 1–2 study involving 60 untreated patients aged ≥65 (median age 74 years) with the aim of determining efficacy and safety of bortezomib plus standard MP [81]. The administration schedule and response rates are indicated in Table 4 [81, 82]. Responses were not conditioned by cytogenetic abnormalities, such as retinoblastoma gene deletion, IgH translocations and t(11; 14), t(4; 14), t(14; 16). Principal adverse effects ≥grade 3 were haematologic (thrombocytopenia 46% and neutropenia 39%), gastrointestinal (23%), peripheral neuropathy (15%) and infections (14%). The results seem to be superior to those of historical data with MP, suggesting this combination as a valid option to standard MP in elderly patients who are not eligible to transplantation.

Presently, an international phase 3 randomised trial (VISTA) comparing VMP with standard MP is ongoing to determine if this combined therapy could be a standard of care in replacing MP.

Bortezomib and ASCT

The rationale behind the use of bortezomib as part of induction therapy stems from the observation that the drug does not have meaningful toxic effects on haematopoietic stem cells and that a successful mobilisation and quality of the haematopoietic graft could be obtained following its administration. Indeed, the combination of bortezomib-Dex has been shown to be a very effective induction therapy. In a phase II trial, when dexamethasone was added to bortezomib in 36 of 48 patients who had achieved less than a PR after 2 cycles of treatment with bortezomib as monotherapy or less than a CR after 4 cycles, the response to bortezomib improved in 64% of patients [83]. At the end of treatment, the OR rate was 88% (CR and nCR 25% and minimal response 8%). After a median follow-up of 24 months, the median time to alternative therapy for patients not undergoing high-dose therapy was 22 months and 1-year survival rate was 80%. Post-transplantation 1-year survival rate was 90%. A very similar rate of CR (21%) was obtained in another multicentre open-label phase II trial with 52 patients, when Dex was administered from the beginning of the study (schedule is shown in Table 4) [82]. The stem cell collection was successful in most patients (88%) and the toxicity was manageable.

Table 4 Response and survival of newly diagnosed myeloma patients following bortezomib-based therapy

Patients (no.)	Schedule				Response (%)			Survival (%/evaluation months)		Reference
	Drugs	Dose	Time (days)	Cycles (no.)	PR	CR+nCR	OR	EFS	OS	
60	Bortezomib	1.3 mg/m^2	1,4,8,11,22, 25,29,32	4	46	43	89	83/16	90/16	[81]
			1,8,15,22	5						
	Melphalan	9 mg/m^2	1–4	9						
	Prednisone	60 mg/m^2	1–4	9						
52	Bortezomib	1.3 mg/m^2	1,4,8,11	4	45	21	66	–	–	[82]
	Dex	40 mg	1–4, 9–12	2						
			then 1–4	2						

Although it has not been shown that achieving a better response is associated with overall improved survival after transplantation, these results provide the rationale for the ongoing phase III randomised trial planning to enrol 480 patients to compare bortezomib plus Dex regimen with standard induction treatment VAD [84]. The results from the interim analysis, presented at the American Society of Hematology meeting in 2006, are promising (response rate of bortezomib-Dex *vs.* VAD: 20% *vs.* 10%), though not conclusive.

Bortezomib-doxorubicin-Dex (PAD)
In an initial trial, the PAD regimen induced overall CR rates after 4 cycles of therapy of 29% and CR and VGPR rates of 62% [85]. More recently, this combination has been studied to determine if it can improve the quality of response in patients who obtained a plateau PR after induction therapy [86]. The association is able to break this plateau and to induce further cytoreduction, also achieving a cytogenetic CR in 3/8 patients with chromosome 13 deletion. The efficacy of doxorubicin in a steroid-free regimen is being evaluated by Orlowski et al. in 63 patients who are candidates for ASCT [87]. Preliminary response data available for 57 patients and presented at the American Society of Hematology meeting in 2006 showed 16% CR or nCR and 58% PR. Among 29 patients who completed the therapeutic programme, the CR and nCR were 28%, with an OR rate of 79%. Furthermore, this therapeutic regimen allowed an adequate collection of stem cells and was well tolerated.

Additional trials including bortezomib alone or in combination are ongoing to evaluate its efficacy as front-line therapy and the results seem promising, but many more studies are required to provide clinical guidance, to define how to use this agent and, finally, to establish if initial efficacy can be translated into a real prolonged survival.

Lenalidomide

Mechanism of action

Lenalidomide is a Thal derivative, which has been developed to overcome the toxic nonhaematologic profile of Thal, including its teratogenicity. It differs from Thal for an amino group and is a more powerful inhibitor of TNF-α activity. Similarly to Thal, lenalidomide is an anti-angiogenic agent, inhibits the adhesion of myeloma cells to bone marrow stromal cells, reduces the secretion of growth and survival factors, induces direct apoptosis of myeloma cells and promotes the cytotoxic activity of natural killer and T-cells against human myeloma cells by stimulating their proliferation and the secretion of IL-2 and IFN-γ [17, 88, 89] (Fig. 1). It also down-regulates the activity of NFkB. These observations prompted the introduction of lenalidomide for MM treatment.

Clinical studies

Relapsed/refractory MM

In phase I studies of patients with relapsed/refractory MM, 25 mg of lenalidomide daily was fixed as the maximum tolerated dose [90]. The major dose-limiting toxic effect was myelosuppression. Lenalidomide was evaluated in a multicentre, open-label, randomised phase II trial of 70 patients by Richardson et al. [91]. The different schedules of administration are shown in Table 5 [91–94]. Treatment with lenalidomide alone resulted in OR, including CR and PR or minor response, of 25%; median OS in the two groups was similar (28 and 27 months, respectively), while median PFS was higher in the first high-dose group (7.7 months *vs.* 3.9 months). Among 68 patients who received Dex, response occurred in 29% of them. Myelosuppression of grade 3 or 4 (thrombocytopenia and neutropenia) occurred more frequently in patients who received 15 mg twice daily (41% *vs.* 13%); peripheral neuropathy and DVT were observed in 3% of the patients.

Two randomised phase 3 trials (MM-009 and MM-010) have compared the combination lenalidomide plus high-dose Dex *vs*. high-dose Dex alone, achieving similar response rates in patients treated with the combination [95, 96]. In both studies, the group receiving lenalidomide and Dex therapy reported remarkably higher OR rates (CR and PR) than the Dex group (MM-009 61% *vs.* 20.5%, $P<0.001$; MM-010 59.1% *vs.* 24%, $P<0.001$, respectively), time to progression was better for combination therapy than for Dex alone (MM-009: 11.1 *vs.* 4.7 months, $P<0.001$; MM-010: 11.3 *vs.* 4.7 months, $P<0.001$, respectively) and the same applied for OS (MM-009 29.6 *vs.* 20.5 months, $P<0.001$). The most common adverse event was myelosuppression with thrombocytopenia and neutropenia of grade 3 or 4, but the risk of venous thromboembolism was much higher when lenalidomide was associated to Dex. Differently from Thal, the incidence of peripheral neuropathy, sedation and constipation was very low.

Lenalidomide–Doxil and lenalidomide–Cy
Lenalidomide has also been studied in combination with other chemotherapeutic agents. Recently, the efficacy and safety of a regimen comprising Doxil was studied in a phase I/II trial of 62 patients with a median age of 62 years (Table 5) [92]. After a median follow-up of 7.5 months, the OR rate was 75%, and the rate of CR and nCR was 29%, with a maximum tolerated dose of lenalidomide of 10 mg. The median PFS was 12 months.

Also, the addition of an alkylating agent such as Cy has been demonstrated to increase the response rate. In a retrospective analysis, 21 patients of median age 59 years, pretreated with various lines of chemotherapy (comprising Thal, high-dose melphalan, bortezomib and allogeneic bone marrow transplantation) were included [93]. The

Table 5 Response and survival of refractory/relapsed and newly diagnosed myeloma patients following lenalidomide-based therapy

Patients (no.)	Schedule				Response (%; *P*)			Survival (median in months; *P*)		Reference
	Drugs	Dose	Time (days)	Cycles (no.)	PR	CR+nCR	OR	PFS	OS	
70	Lenalidomide Dex[a]	30 or 15x2 mg 40 mg	1–21 every 4 weeks 1–4		12 *vs.* 14[b]; NR[c]	6 *vs.* 0[b]; NR	24 *vs.* 29[b]; NR	7.7 *vs.* 3.9[b]; =0.2	28 *vs.* 27[b]; NR	[91]
62	Lenalidomide Doxil Vincristine Dex	25 mg 40 mg/m^2 2 mg 40 mg	1–21 every 4 weeks 1 1 1–4		46	29	75	12	–	[92]
21	Lenalidomide Cy Dex	25 mg 500 mg 40 mg	1–21 1,8,15,21 1–4, 12–15 every 4 weeks	9	–	–	65	–	–	[93]
34	Lenalidomide Dex	25 mg 40 mg	1–21 1–4, 9–12, 17–20 every 4 weeks	4	53	38	91	–	–	[94]

[a]Administered in patients with progressive or stable disease after two cycles; [b]once-daily *vs.* twice-daily cohort; [c]NR, non-reported

observed OR rate was 65% (CR and PR), with a toxicity profile especially characterised by neutropenia (38%) and DVT (14%).

Newly diagnosed MM

Lenalidomide–Dex

A phase 2 study evaluated a lenalidomide–Dex regimen as induction therapy in 34 patients of median age 64 years [94]. The treatment schedule is shown in Table 5. An objective response was obtained in 91% of patients, and 6% of them achieved a CR, 53% a PR and 32% a VGPR. Forty-four percent of patients proceeded to ASCT with an adequate collection of stem cells. In 47% of patients, non-haematologic toxicity of grade 3 or 4 was observed, including fatigue (15%), muscle weakness (6%), pneumonitis (6%), anxiety (6%) and rash (6%). The incidence of thromboembolic events was very low (3%), probably because all patients initiated aspirin prophylaxis from the very beginning.

Lenalidomide–MP

In patients ineligible for transplantation, lenalidomide-MP has been evaluated in 54 patients aged ≥65 years [97]. The OR rate was up to 85% with 23.8% of CR. Toxicity mainly included myelosuppression, with neutropenia and thrombocytopenia of grade 3–4 (66% and 34%, respectively). Thromboembolic events were observed in 3 patients, though all patients received prophylaxis with aspirin.

These combination regimens are promising and may represent a valid alternative to Thal-based treatment, mainly because there are fewer non-haematologic adverse events observed with lenalidomide.

Adverse events

Thalidomide

Besides the advantages of oral treatment for Thal and lenalidomide, generally these drugs are well tolerated and show a predictable and manageable toxicity profile. Nevertheless, several side effects are related to their administration, though with different severity.

Most of the adverse events correlate with dose and duration of treatment. With doses lower than 400 mg daily, their severity is mild to moderate. In addition to known teratogenicity, frequently observed adverse events are: sedation, somnolence, constipation, nausea, fatigue, cutaneous rash, bradycardia, hypothyroidism and oedema. The most serious complications are peripheral neuropathy and venous thromboembolism [98–101]. The incidence of the former is very high (up to 50%) and seems to be related to long-term use (generally over 6 months) and to a pretreatment neuropathy [102]. The most frequent neuropathy-related symptoms were numbness, paraesthesia or burning sensation with involvement of hands and feet. Its definite correlation with daily/cumulative dose [103] or with duration of treatment [104] has been confirmed. Thus, before receiving Thal, patients should undergo neurological evaluation to identify those at higher risk for peripheral neuropathy. In the initial phases of neuropathy, drug withdrawal can increase the probability of recovery.

The incidence of thromboembolic events, especially DVT and less commonly pulmonary embolism, seems to be higher in patients with either newly diagnosed MM (30% *vs.* 17% of control group) [47] and/or in Thal-steroids combination regimens (17% *vs.* 3% of control group) [48, 105]. On the basis of this evidence, the Authors recommend routine prophylaxis with low-molecular-weight heparin or warfarin or, in the patients with high bleeding risk, aspirin. However, the efficacy of low-molecular-weight heparin is an open question, because some, but not all, Authors reported a considerable reduction in the risk of DVT. Nevertheless, the generally held opinion is that prophylaxis is recommended.

Lenalidomide

Lenalidomide has a better safety profile than Thal and the most common adverse event is myelosuppression, particularly neutropenia and thrombocytopenia ≥3 grade. But lenalidomide is also associated with a high risk of DVT when it is used with other agents, especially high-dose Dex [106]. Dimopoulos et al. and Weber et al. reported high rates of thromboses (9% and 17.5% respectively) in patients receiving this combination therapy and the rate was higher among patients who received concomitant erythropoietic growth factors [107]. However, administration of aspirin or salicylates seems to reduce the risk of DVT [94, 108]. Therefore, aspirin or other antithrombotic drugs should regularly be used in patients receiving this combination therapy and the concomitant use of erythropoietic agents should be considered with caution.

Bortezomib

The toxicity profile of bortezomib is mainly characterised by peripheral neuropathy and thrombocytopenia. Clinical manifestations include paraesthesias, numbness and pain affecting especially the lower extremities. Overall, the investigators reported that 37% of patients had developed a dose-related peripheral neuropathy of any grade, and

14% of grade ≥3 within the first five cycles of treatment [109]. The baseline neuropathy and previous therapies with other neurotoxic agents, Thal, vincristine or platinum, did not appear to affect the incidence of peripheral neuropathy. Complete resolution or improvement of neuropathic symptoms was observed in 71% of patients during treatment by reducing the dose without compromising the efficacy of therapy or completion of treatment.

In the APEX study, bortezomib was associated with thrombocytopenia of grade ≥3 in 30% of patients, but this effect was transient and the platelet count returned to normal values between cycles with short time of recovery and without cumulative effect. The cause and kinetics of bortezomib-induced thrombocytopenia were different from those seen with standard cytotoxic agents, given that bortezomib does not induce cytotoxic effects on marrow megakaryocytes [110]. Generally, thrombocytopenia is characterised by a mean reduction in platelet counts from a baseline of approximately 60%. Therefore, the most important predictor of severe thrombocytopenia is the initial platelet count. Grade 3 or 4 thrombocytopenia occurs in patients who have low platelet counts at baseline. Approximately 15% of patients treated with bortezomib in the APEX study required transfusions of platelets and this requirement peaked during the first 2 cycles and decreased with increasing cycles of treatment. Moreover, the incidence of serious bleeding episodes associated with grade 3 thrombocytopenia was very low.

Conclusions

The use of targeted therapies exhibiting efficacy in MM is certainly opening a new scenario in this tumour and is showing promise to improve the outcome of these patients, even though many randomised phase 3 studies are still required. Despite impressive advances, the most important challenge remains a better understanding of the disease biology, and to identify additional and more specific targets either within molecular genetics, especially gene expression profile, involved in myelomagenesis or within bidirectional interactions between myeloma cells and their microenvironment, which promote not only growth and survival of malignant cells, but also bone resorption and drug resistance.

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