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Bortezomib, cyclophosphamide, dexamethasone *versus* lenalidomide, cyclophosphamide, dexamethasone in multiple myeloma patients at first relapse

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Summary

Bortezomib- and lenalidomide-containing regimens are well-established therapies in multiple myeloma (MM). However, despite their extensive use, head-to-head comparisons have never been performed. Therefore, we compared bortezomib and lenalidomide in fixed-duration therapies. In this open-label, phase III study, we randomized MM patients at first relapse to receive either nine cycles of bortezomib plus cyclophosphamide plus dexamethasone (VCD) or lenalidomide plus cyclophosphamide plus dexamethasone (RCD). The primary endpoint was achievement of a very good partial response (VGPR) or better at six weeks after nine treatment cycles. From March 2011 to February 2015, 155 patients were randomized. VGPR or better was achieved by 12 patients (15%) in the VCD arm and 14 patients (18%) in the RCD arm ($P = 0{\cdot}70$). Median progression-free survival (PFS) was 16·3 (95% CI: 12·1–22·4) with VCD and 18·6 months (95% CI: 14·7–25·5) with RCD, and the two-year overall survival (OS) was 75% (95% CI: 66–86%) and 74% (95% CI: 64–85%) respectively. In subgroup analyses, no differences in PFS were observed in bortezomib- and lenalidomide-naïve patients, nor in patients who received a bortezomib-based regimen in first line. Adverse events were consistent with the well-established safety profiles of both drugs. Bortezomib and lenalidomide treatments were equally effective in terms of depth of response, PFS, and OS in MM patients at first relapse.

Keywords: myeloma, bortezomib, lenalidomide.

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Multiple myeloma (MM) is a plasma cell neoplasm still considered incurable. The clinical course is characterized by remission and followed by relapses requiring subsequent lines of treatment (Yee & Raje, 2016). Since the early 2000s, the therapeutic armamentarium has considerably increased with the introduction of immunomodulatory drugs and proteasome inhibitors (Kyle & Rajkumar, 2008), and, more recently, other classes of therapies, in particular monoclonal antibodies (Lokhorst *et al.*, 2015). The availability of several therapeutic options raises the issue of their optimal sequencing. Usually, first-line therapy in young patients consists of bortezomib-based triplets followed by single or double autologous stem cell transplantation (auto-SCT) and, more recently, lenalidomide maintenance (Cavo *et al.*, 2010; McCarthy *et al.*, 2017). Elderly patients are treated with non-transplant approaches, mainly consisting of bortezomib plus melphalan plus prednisone or lenalidomide plus desamethasone (Larocca & Palumbo, 2016). In second line, bortezomib- or lenalidomide-based regimens are commonly used depending on the type of previous therapy and patient's comorbidities (Bianchi *et al.*, 2015). In an attempt to improve their efficacy, both bortezomib and lenalidomide have been combined with cyclophosphamide (Kumar *et al.*, 2011; de Waal *et al.*, 2015). Although these agents were approved approximately 10 years ago, to date, no direct comparison of bortezomib *versus* lenalidomide has been conducted, preventing evidence-based tailoring of the second-line treatment.

To evaluate efficacy and safety of bortezomib *versus* lenalidomide, we conducted a phase III randomized trial comparing cyclophosphamide and dexamethasone plus bortezomib (VCD) or lenalidomide (RCD) in MM patients at first relapse. While bortezomib is usually administered for a limited number of cycles, lenalidomide therapy is continued until disease progression (PD) or intolerance. However, since treatment duration may impact on time-to-event outcome (Palumbo *et al.*, 2015), and alkylating agents can be administered only for a limited period of time, we planned a treatment schedule with a fixed number of cycles. We also believed that planning both regimens as fixed-duration therapy would have reduced the bias that is intrinsic when comparing a continuous treatment, such as lenalidomide-based regimen, with a therapy with a fixed number of cycles, such as bortezomib-based regimen. Here we report the final results of the study.

Patients and methods

Study design and patients

This is a randomized, open-label phase III trial recruiting patients at 16 Italian centres. Since we aimed at comparing bortezomib *versus* lenalidomide, both combined with dexamethasone, in a fixed-duration treatment, and we were aware that the use of these drugs was evolving from doublets to triplets, we decided to combine both with cyclophosphamide. Thus, this study consists in a comparison of VCD with RCD, both administered as fixed-duration therapies. MM patients at first symptomatic relapse were eligible. Key entry criteria were age $\geq$18 and $\leq$75 years, and measurable disease according to the International Myeloma Working Group (IMWG) criteria (Durie *et al.*, 2006). Patients who had received bortezomib or lenalidomide during their first-line treatment could be included in the study, provided that they had obtained at least a partial response (PR) lasting $\geq$12 months, had not received maintenance, and had no residual grade 3–4 peripheral neuropathy. Written informed consent was obtained from each patient. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Ethics committees at each study site reviewed and approved the protocol. This study is registered as EUDRACT 2010-021557-40.

Randomization

Patients were assigned to treatment on the basis of a computer-generated randomization schedule. Stratification criteria were: first-line treatment containing bortezomib *versus* lenalidomide, International Staging System (ISS) 1 vs. 2–3, and intention to proceed to stem cell transplantation after completion of the protocol *versus* no intention to proceed to transplant.

Treatment

Patients were randomly assigned (1:1) to receive induction treatment with cyclophosphamide 500 mg/m^2, iv, on day 1 and 8, and dexamethasone 20 mg, oral or iv, on days 1–2, 8–9, 15–16, and 22–23 in combination with either subcutaneous bortezomib 1·3 mg/m^2 on days 1, 8, 15, 22 (VCD) in six 35-day cycles, or oral lenalidomide 15 mg on days 1–21

(RCD) in six 28-day cycles. After the first six cycles, patients received consolidation with three further cycles of the assigned therapy, administered every two months. Patients who did not achieve at least a minimal response (MR) after the third cycle, or at least a PR after the sixth cycle, were allowed to go off study (Figure S1). During the first three cycles, RCD patients received low molecular weight heparin prophylaxis, and all patients received acyclovir 400 mg twice a day. Bisphosphonate treatment was allowed at physician's discretion. Patients were re-staged six weeks after the last cycle, and they could proceed to auto-SCT, according to the patient's and physician's choice.

Endpoint and disease assessment

Since we compared two fixed-duration therapies and we were mainly interested in discerning the depth of response obtained with the two treatments, we chose as primary endpoint the achievement of a very good PR (VGPR) or better at six weeks after the end of consolidation. At the time the trial was designed, depth of response endpoints were commonly used also in phase III trials (Attal *et al.*, 2003). It has been shown that the depth of response correlates with progression-free survival (PFS) (Lahuerta *et al.*, 2017). Pre-specified secondary endpoints included PFS, overall survival (OS), and treatment-related mortality.

Response was assessed according to the IMWG criteria, including MR category, characterized by a ≥25% and <50% reduction in the M-component (Table SI). Serum and 24-h urine samples were collected at screening, baseline, before the start of every cycle, and every three months after consolidation. Bone marrow aspirate and biopsy were performed at screening, after the sixth cycle and consolidation, then every six months. An X-ray skeletal survey was performed at baseline, after consolidation, then yearly. A spine and pelvis MRI scan was performed at baseline, after consolidation, then every six months. Best confirmed response was determined in response-evaluable patients with at least two post-baseline assessments.

Adverse events were assessed according to the National Cancer Institute's Common Terminology Criteria for Adverse Events v3.0.

Immunophenotypic analysis and immunoglobulin recovery

Number of total $CD3^+$ lymphocytes, $CD4^+$ helper and $CD8^+$ cytotoxic T cells in peripheral blood (PB) samples was determined using Trucount tubes containing fluorescent beads as internal standard (BD Biosciences) and the appropriate monoclonal antibodies. Staining of cells was performed at 4°C for 20 minutes in the dark in FACS staining buffer [1× PBS (phosphate-buffered saline) supplemented with 2% fetal bovine serum (FBS)]. Plasma cells were stained according to the European Myeloma Network guidelines (Rawstron *et al.*, 2008). Cell acquisition and data analysis were performed on a MACSQuant Analyzer (Miltenyi Biotec) using MACSQuantify Software 2.6 (Miltenyi Biotec) and FlowJo V10.2 (FlowJo LLC). The target for collection was $>5 \times 10^5$cellular events in each tube.

To evaluate the serological immune recovery, we analysed the myeloma-uninvolved immunoglobulin levels at 3, 6, 9, 12, 15, and 18 months after study entry.

Statistical analysis

The expected VGPR and complete remission (CR) rate in the VCD and RCD treatment groups were 40% and 20%, respectively. Allowing for a significance level (alpha) of 5%, and 85% power, a total number of 186 patients was required. The required sample size was increased up to 200 patients (100 for each arm) to account for about 5% drop-in and drop-outs. Statistical analysis was performed on an intention-to-treat (ITT) basis. OS was calculated as the time from the date of randomization to the date of death or to the last date the patient was known to be alive. PFS was calculated as the time from randomization to the date of first evidence of PD or death without evidence of PD or to the last date the patient was known to be progression-free. Median follow-up was calculated by the reverse Kaplan–Meier method. Survival distributions were estimated by the Kaplan–Meier method and compared by a log-rank test. A Cox proportional hazard regression model was used to estimate treatment effect by adjusting for known prognostic factors. Patients' age and sex, previous treatment, isotype, ISS stage and time to first relapse were considered for inclusion in the multivariate analysis. Subgroup analyses were also performed according to different levels of previous prognostic factors. Markers associated with antigen expression profiles (CD4, CD8 and CD56) were considered as well.

Crude cumulative incidence (CCI) for different causes of death was estimated with a suitable estimator in a competing risks framework. The incidence of the best response (achievement of a PR or better) over time was estimated in the same way. Statistical analyses were conducted using SAS (version 9.4; SAS Institute, Cary, NC, USA) and R (version 3.3.1; R Foundation for Statistical Computing, Vienna, Austria) software.

Results

Patient characteristics

In total, 159 patients were enrolled from March 2011 until February 2015. The study was prematurely closed due to regional regulatory issues, leading the National Healthcare System to stop the free supply of bortezomib and lenalidomide for the study. Data cut-off date was February 2017. Demographic and clinical characteristics of study patients are reported in Table I. Among patients enrolled, 155 were

randomized to receive VCD (n = 76) or RCD (n = 79), and were included in the ITT analysis. Overall, 83 patients (53·5%) were female, mean age was 63 years (SD 8·1) and was similar in both groups. Median PFS with first-line therapy was three years (range 10–51 months). Most of the patients (n = 108, 69·7%) presented with IgG MM isotype, and 73 (47·1%) had ISS stage I. Seventy-nine patients (51%) had previously received bortezomib-based regimen, 62 (40%) conventional chemotherapy, 14 (9%) lenalidomide-based treatment, and 123 (80%) had received auto-SCT (Table I). Conventional chemotherapy mainly consisted in VAD (vincristine, doxorubicin, and dexamethasone), or VAD-like regimens.

Treatment compliance

Among 159 patients enrolled, four patients were not randomized due to death (n = 1), patient refusal (n = 1) or screening failure (n = 2). All randomized patients started the assigned treatment, apart from one patient in the VCD arm, who was excluded from the safety analysis. Twenty-three VCD, and 18 RCD patients did not proceed beyond the third cycle due to failure to achieve at least a MR (20 with VCD and 13 with RCD), for medical reasons (two with VCD and four with RCD) and toxicity (one with RCD), or due to patient refusal (one with VCD). Moreover, 12 VCD and 12 RCD patients did not proceed beyond the sixth cycle due to failure to achieve at least a PR (nine with VCD and nine with RCD), for medical reasons (one with VCD and two with RCD), loss to follow-up (three with RCD), and due to patient refusal (two with VCD). Another 10 VCD and six RCD patients did not complete the nine assigned cycles for PD (six with VCD and six with RCD), were lost to follow-up (three with VCD), and for medical reasons (one with VCD). Overall, 31 VCD and 43 RCD patients completed the nine cycles of therapy (Fig 1). Although patients could proceed to auto-SCT upon completion of the assigned treatment, only one patient per arm proceeded to transplant.

Efficacy

The distribution of the best response achieved during treatment and the final response according to ITT are reported in Table II. The same distribution was also reported stratified for previous treatment distinguishing among bortezomib- and lenalidomide-naïve patients.

The overall response rate was 64·5% (95% CI: 53·3–74·3%) in the VCD group, and 79·7% (95% CI: 69·6–87·1%) in the RCD group (P = 0·03) (Table II). Best responses in VCD and RCD included: stringent CR (sCR) 7% and 4%, CR 9% and 8%, VGPR 9% and 20%, PR 39% and 48%, less than PR 34% and 19% respectively. Best response could not be assessed in one patient in each arm. The mean time to response was 88 days in the VCD group and 42 days in the RCD group; among patients reaching a PR, the median duration of response was 14·5 and 17·2 months respectively. At sixweeks after the ninth cycle, 12 (15·7%) VCD and 14 (17·7%) RCD patients achieved at least a VGPR (P = 0·70). The distribution of time to a PR or better response, stratified for previous treatment, is reported in Figure S4.

In the 21 patients attaining CR (sCR and CR), the phenotypic aberrancies detected by flow cytometry in plasma cells at study entry were used as patient-specific probes for residual disease assessment. None of these patients achieved minimal residual disease (MRD)-negativity (Flanders *et al.*, 2013), since residual cells were detected at a median frequency of 0·077% of tumour cells out of the total analyzed events (range 0·005–0·45%). Longitudinal plasma samples were available in 12 patients: in these patients, residual disease at CR was also evaluated using a recent next-generation sequencing approach on cell-free DNA (Biancon *et al.*, 2018). A significant positive correlation between frequencies of the clonotypic *IGH* sequences in plasma (median 0·3025%, range 0·00143–6·5%) and of plasma cells (0·1%, range 0·004–8·05%) was found at CR time points (r = 0·8156, P = 0·00122, Pearson's correlation test) (Figure S6).

Survival

Median follow-up was 34 months (IQR 26–45·5), specifically 34 months (IQR 25–45) in the VCD group, and 32 months (IQR 24–45) in the RCD group. One-year PFS was 60% (95% CI: 50–72%) and 64% (95% CI: 53–75%), two-year PFS was 34% (95% CI: 25–47%) and 40% (95% CI: 30–53%), and median PFS was 16·3 (95% CI: 12·1–22·4) and 18·6 (95% CI: 14·7–25·5), in the VCD and in RCD arms respectively (Fig 2). No statistically significant differences in PFS were observed with VCD and RCD according to age (<65 or ≥65 years), first-line therapy (chemotherapy or bortezomib-based regimen), ISS stage (I vs. II–III), and time-to-progression with first-line therapy (>3 years vs. ≤3 years) (Fig 3).

At study closure, 30 patients in the VCD and 27 in RCD arm had died. Two-year OS was 75% (95% CI: 66–86%) in the VCD arm and 74% (95% CI: 64–85%) in the RCD arm. The three-year OS was 48% (95% CI: 36·1–65·9%) and 51% (95% CI: 37·2–69·0%) in VCD and RCD patients respectively (Fig 2).

In multivariable analysis, the only prognostic factor significantly influencing both OS and PFS was shorter first-line PFS for patients relapsing in <3 years vs. ≥3 years (HR 0·44; 95% CI: 2·37–8·20; P < 0·0001) (Table SII).

The majority of patients in both study groups (n = 42) died from relapse or progression: 25 (CCI = 47·9%, 95% CI = 32·7–63·1%) in the VCD and 17 (CCI = 30·4%, 95% CI = 15·9–44·9%) in the RCD arm respectively. Among others, nine patients died for other causes: two (CCI = 3·1%, 95% CI = 0–7·4%) in the VCD arm and seven (CCI = 14·4%, 95% CI = 3·5–25·2%) in the RCD arm respectively. For six patients (three in each arm) the cause of death is unknown.

Table I. Baseline characteristics of the intention-to-treat population.

Variables	VCD (76 patients)	RCD (79 patients)	Missing
Sex			
Male	28 (36·8%)	44 (55·7%)	
Female	48 (63·2%)	35 (44·3%)	
Age* (range)	65·0 (60·0–69·1)	63·6 (55·6–69·4)	
Previous treatment			
Chemotherapy	32 (42·1%)	30 (38·0%)	
Lenalidomide	7 (9·2%)	7 (8·9%)	
Bortezomib	37 (48·7%)	42 (53·2%)	
Previous thalidomide			
No	41 (54·0%)	48 (60·8%)	
Yes	35 (46·1%)	31 (39·2%)	
Previous autologous transplant			
No	16 (21·6%)	16 (20·2%)	
Yes	60 (78·4%)	63 (79·7%)	
Single	*31 (51·7%)*	*32 (50·8%)*	
Double	*29 (48·3%)*	*31 (49·2%)*	
Best response to the first line			1
PR	16 (22·3%)	19 (26·3%)	
VGPR	31 (40·8%)	28 (35·9%)	
CR	23 (30·3%)	25 (32·0%)	
sCR	5 (6·6%)	5 (6·4%)	
Time to first recurrence (years)*	3·06 (2·3–4·2)	2·93 (2·3–4·1)	
≤1 year	1 (1·3%)	2 (2·5%)	
1–3 years	36 (47·4%)	29 (36·7%)	
3–5 years	25 (32·9%)	32 (40·5%)	
>5 years	14 (18·4%)	16 (20·3%)	
ECOG			
0	38 (50·0%)	39 (49·4%)	
1	37 (48·7%)	38 (48·1%)	
2	1 (1·3%)	2 (2·5%)	
Isotype			
Bence Jones	10 (13·2%)	10 (12·7%)	
IgA	11 (14·5%)	14 (17·7%)	
IgD	2 (2·6%)	0 (0·0%)	
IgG	53 (69·7%)	55 (69·6%)	
ISS stage			
I	37 (48·7%)	36 (45·6%)	
II	21 (27·6%)	28 (35·4%)	
III	18 (23·7%)	15 (19·0%)	
Plasmacytomas			
No	71 (93·4%)	77 (97·5%)	
Yes	5 (6·6%)	2 (2·5%)	
Estimated creatinine clearance*,†	72·4 (53·5–95·8)	84·8 (61·2–101·9)	1
CD4	210·8 (133·2–301·5)	188·3 (115·9–263·9)	99
CD8	246·3 (160·2–365·2)	276·3 (188·7–337)	99
CD56	169·3 (90·7–288·4)	95·8 (72·8–157·5)	106

PR, partial response; VGPR, very good partial response; CR, complete response; sCR, stringent complete response; Ig, immunoglobulin; ISS, International Staging System; VCD, bortezomib plus cyclophosphamide plus dexamethasone; RCD, lenalidomide plus cyclophosphamide plus dexamethasone.

*Median (interquartile range).

†Estimated with CKD-EPI formula, accounting for sex, age and race (not African American).

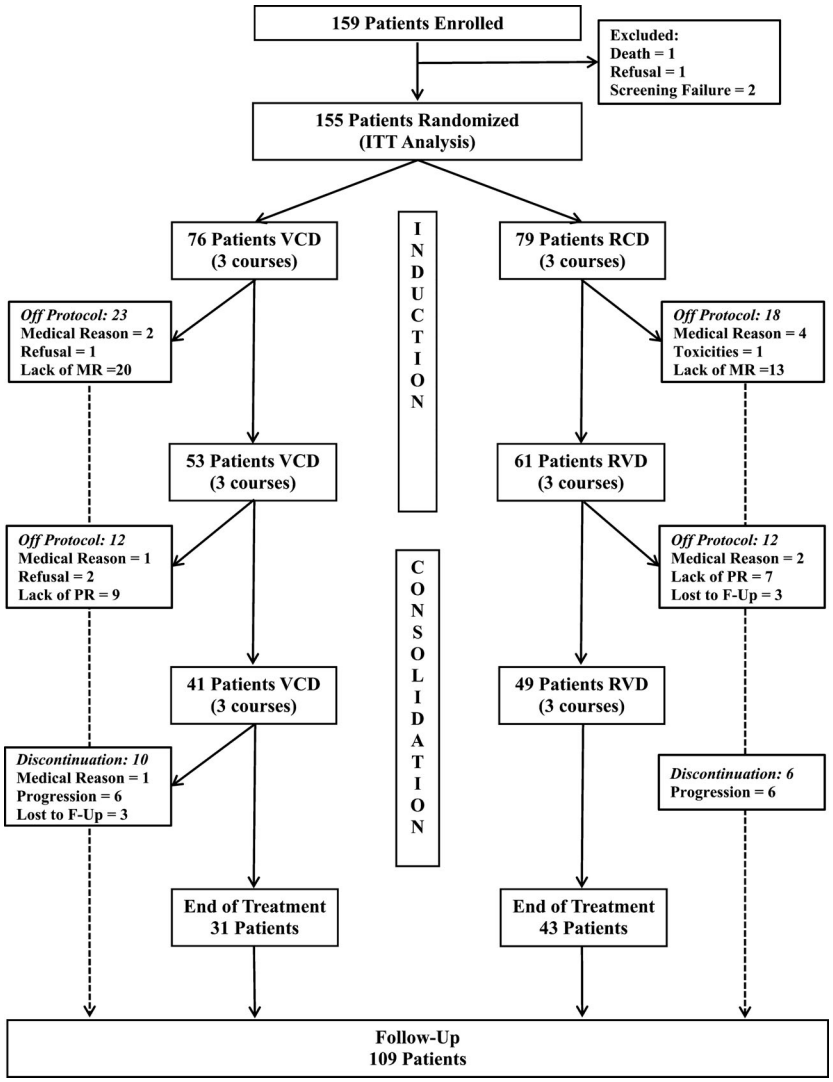

Fig 1. CONSORT diagram. VCD, bortezomib plus cyclophosphamide plus dexamethasone; RCD, lenalidomide plus cyclophosphamide plus dexamethasone; MR, minimal response; PR, partial response; F-Up, follow-up.

Safety

A total of 52 patients (33·5%), 26 in each arm (32·9% in RCD and 34·2% in VCD), experienced at least one toxicity. Grade 3–4 haematologic toxicity was observed in six (8%) VCD and in nine (11%) RCD patients. Grade 3–4 non-haematologic toxicity was observed in 10 (13%) VCD and in four (5%) RCD patients. Toxicities (any grade) of special interest were peripheral neuropathy, occurring in 12 (16%) VCD and four (5%) RCD patients; and neutropenia, reported in four (5%) VCD and 17 (22%) RCD patients. Infections of any grade were observed in 16 (21%) VCD patients and in 19 (24%) RCD patients. One patient in the VCD group experienced a second primary malignancy. One patient (1%) in the VCD arm discontinued treatment for toxicity (Table III).

Immunological reconstitution

CD4, CD8 and CD56 levels were evaluated during the treatment period. The levels of all lymphocyte subpopulations did not differ between the two treatment groups, and there were no differences between enrolment and end of treatment (Figure S2).

During the first nine cycles, no substantial differences in myeloma-uninvolved immunoglobulin levels were observed between VCD and RCD patients. After the end of treatment, IgA and IgM increased in the RCD group; at 15 months after enrolment, mean IgA levels were 66 mg/dl (SE 16 mg/dl) and 134 mg/dl (SE 50 mg/dl), and IgM were 42 mg/dl (SE 7 mg/dl) and 63 mg/dl (SE 12 mg/dl) in VCD and RCD patients respectively. IgG levels were not different between the two arms, but the number of evaluable patients was consistently lower (Figure S3).

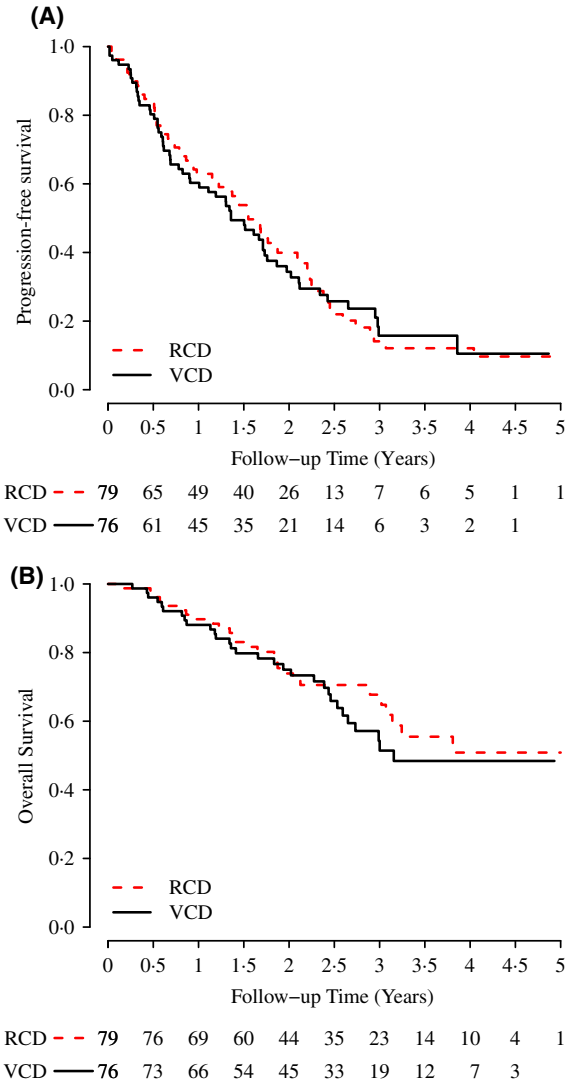

Fig 2. Kaplan–Meier estimates of progression-free survival (A) and overall survival (B) in the treatment arms. VCD, bortezomib plus cyclophosphamide plus dexamethasone; RCD, lenalidomide plus cyclophosphamide plus dexamethasone. [Colour figure can be viewed at wileyonlinelibrary.com]

Discussion

In this randomized, controlled trial, fixed-duration treatment with bortezomib or lenalidomide, both in combination with cyclophosphamide and dexamethasone in MM patients at first relapse, showed similar efficacy. It is important to note that our results should be considered with caution, since, for regulatory issues on use of drugs in clinical trials, we enrolled only 159 patients, in spite of at least 186 patients being necessary to keep a statistical power of 85%. After nine cycles of therapy, 12 VCD and 14 RCD patients achieved a VGPR or better ($P = 0{\cdot}70$); thus the primary endpoint of the study was not met. Median PFS was 16·3 months in the VCD group *versus* 18·6 months in the RCD group ($P = 0{\cdot}8$), with no differences in all the analyzed subgroups. Our data are in line with previous studies. Indeed, in one study in bortezomib-naïve, relapsed MM patients, a slightly different variant of VCD regimen induced 33% of VGPR or better and a median PFS of 18 months (de Waal *et al.*, 2015). In another study — although in newly diagnosed MM patients — a more intense schedule of RCD with a continuous approach led to 47% of VGPR or better, and a median PFS of 28 months (Kumar *et al.*, 2011).

In our study, only patients at first relapse were enrolled. A high proportion (50%) had already received bortezomib at first line, while only a small number of patients (9%) had already been exposed to lenalidomide. However, there were no differences in efficacy between bortezomib or lenalidomide in the 77 patients who had received bortezomib as their first-line treatment. This is consistent with previous data showing a high response rate in case of bortezomib rechallenge (Petrucci *et al.*, 2013). More interestingly, also in the 61 patients who were bortezomib- and lenalidomide-naïve, VCD and RCD showed to be equally effective, with no differences within the analyzed subgroups. In multivariate analysis, a PFS >3 years with first-line therapy was the only factor associated with an improvement in PFS. This is in line with previous studies showing that a prolonged response was a surrogate marker of chemosensitivity and a prognostic factor for long-term disease control (Durie *et al.*, 2004; Barlogie *et al.*, 2008; Lonial & Anderson, 2014).

It seems reasonable to compare the outcome of VCD and RCD with the corresponding doublets bortezomib plus dexamethasone (VD) or lenalidomide plus dexamethasone (RD). The median PFS of 16·3 months obtained with VCD in our study compares favourably with the Castor trial comparing daratumumab plus bortezomib plus dexamethasone *versus* bortezomib plus dexamethasone, where the median PFS in the bortezomib plus dexamethasone in patients at first relapse was 7·9 months (Spencer *et al.*, 2018).

Likewise, the median PFS of 18·6 months with our RCD fixed-dose nine-cycle regimen was similar to that of 19·6 months obtained with continuous RD in patients at first relapse enrolled in the Pollux trial, that compared daratumumab plus lenalidomide plus dexamethasone *versus* lenalidomide plus dexamethasone (Dimopoulos *et al.*, 2018). Likewise, in three other trials assessing continuous treatment with RD as control arm, the median PFS of this doublet ranged between 14·7 and 18·4 months (Lonial *et al.*, 2015; Stewart *et al.*, 2015; Moreau *et al.*, 2016).

In our study, we observed an acceptable rate of toxicities, since grade 3–4 haematologic toxicities were 9·2% with VCD and 17·7% RCD, and grade 3–4 non-haematologic toxicities were 11·8% and 3·8%, respectively. The higher incidence of haematologic toxicities, in particular neutropenia, in the RCD arm did not translate into an increased incidence of infections, probably due to the use of granulocyte colony-stimulating factor. The slight increase in grade 3–4 myelotoxicity with RCD reinforces the concept of the additive

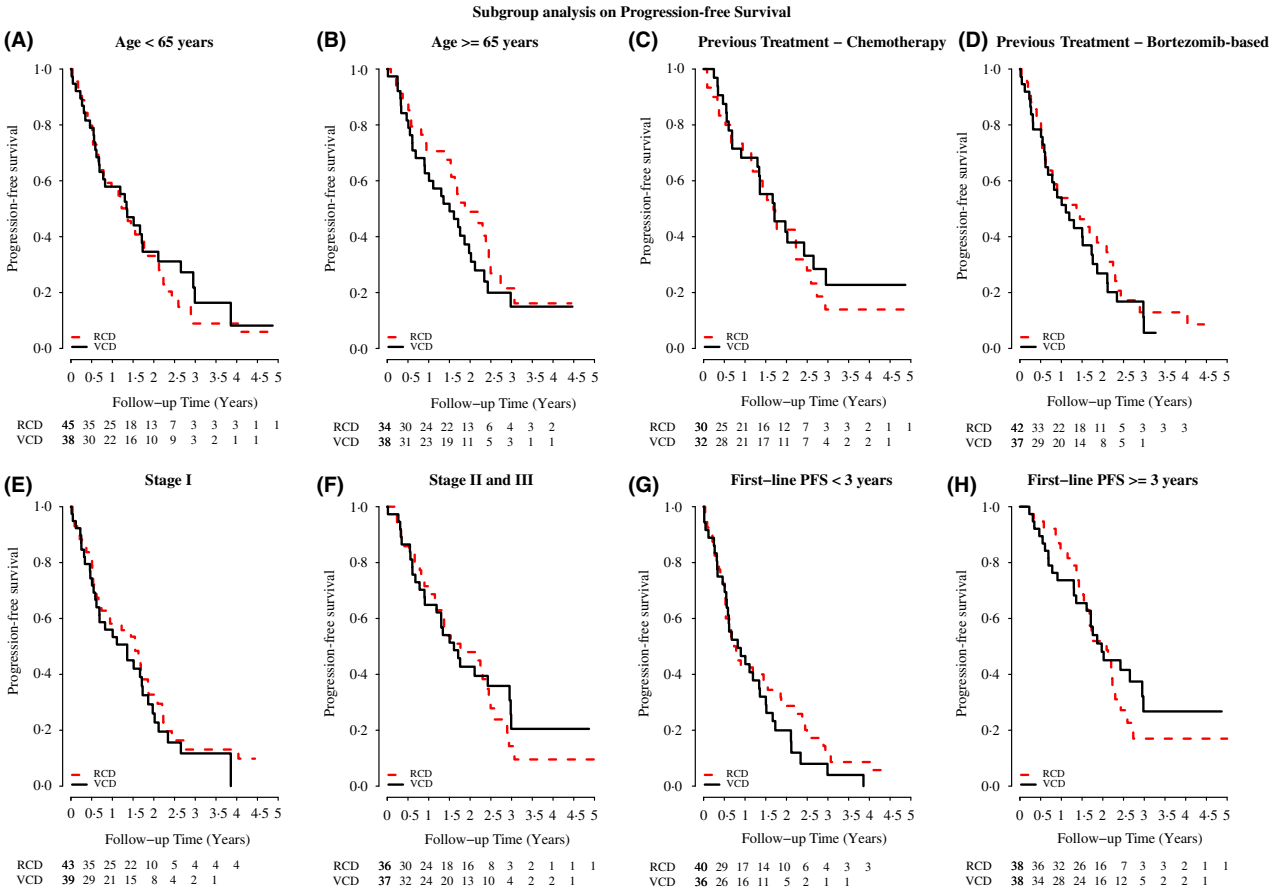

Fig 3. Kaplan–Meier estimates of progression-free survival (PFS) in the treatment arms in specific subgroups. (A) Age <65 years; (B) age ≥65 years; (C) previous chemotherapy; (D) previous bortezomib; (E) ISS I; (F) ISS II–III; (G) first-line PFS <3 years; (H) first-line PFS ≥3 years. VCD, bortezomib plus cyclophosphamide plus dexamethasone; RCD, lenalidomide plus cyclophosphamide plus dexamethasone. [Colour figure can be viewed at wileyonlinelibrary.com]

myelotoxic effect with lenalidomide combined with alkylating agents. Interestingly, the rate of any grade neuropathy was 16% in our VCD arm, and this figure compares favourably with the 66% observed with VCD in the study by Reeder *et al.* (2009). Our low toxicity rate is probably related to several factors. First, no heavily pretreated patients could be enrolled in our trial; second, we designed the VCD and RCD treatments in order to minimize toxicity, choosing once-weekly bortezomib, lenalidomide at 15 mg, and with the last three cycles administered every two months. Thus, only one patient went off study for toxicity.

Although bortezomib and lenalidomide have been widely used in the relapse setting for more than 10 years, they have never been formally compared as fixed-duration regimens, with a head-to-head trial design. More recent triplet regimens including new-generation agents, such as new proteasome inhibitors and monoclonal antibodies, are available today and results are certainly more impressive. However, the combinations tested in our trial are cheaper and more easily accessible. In fact, cost of therapy is not a negligible factor when choosing treatment, and this is of great importance both in western countries with increasing health-care deficit, and in emerging countries with limited economic resources. For instance, bortezomib and lenalidomide are available in the Chinese and Indian markets, which — if combined — represent a population larger than 2.6 billion.

We also performed a biological study to evaluate whether specific lymphocyte subpopulations correlate with outcome, considering the different mechanism of action of bortezomib and lenalidomide and the specific immune stimulation activity of the latter agent (Quach *et al.*, 2010). During the treatment period, we did not observe any difference between the two arms in terms of CD4, CD8 and CD56 lymphocyte subpopulations. Moreover, considering the possible superior immunostimulatory effect of lenalidomide compared with bortezomib, we evaluated the trend of non-involved immunoglobulin values in the two study arms. We observed that, after the end of treatment, IgA and IgM increased particularly in the RCD group, suggesting that lenalidomide may have immunostimulating effects (Montefusco *et al.*, 2014). However, by evaluating lymphocyte subpopulations, we did not observe

Table II. Treatment responses in the intention-to-treat population.

	Overall		Velcade-naïve		Revlemid-naïve	
Best response to treatment	VCD (n = 76)	RCD (n = 79)	VCD (n = 39)	RCD (n = 37)	VCD (n = 69)	RCD (n = 72)
Not assessable	1	1	1	–	1	1
PD	4	1	1	0	3	1
SD	22	14	13	4	20	12
PR	30	38	9	22	28	35
VGPR	7	16	6	6	6	14
CR	7	6	5	5	6	6
SCR	5	3	4	–	5	3
Off protocol*	45	36	21	13	40	33
Not assessable	2	2	2	–	2	2
PD	4	6	1	2	4	5
SD	3	1	3	–	3	1
PR	10	20	3	13	9	19
VGPR	3	8	3	5	3	6
CR	5	5	3	4	4	6
Scr	4	1	3	–	4	1
≥PR	22	34	12	22	20	32
≥VGPR	12	14	9	9	11	13

PD, progressive disease; SD, stable disease; PR, partial response; VGPR, very good partial response; CR, complete response; sCR, stringent complete response; VCD, bortezomib plus cyclophosphamide plus dexamethasone; RCD, lenalidomide plus cyclophosphamide plus dexamethasone.
*Among these, one patient in the VCD arm did not start the treatment.

Table III. Adverse events in the safety population.

Toxicities	VCD G3–G4	RCD G3–G4
Haematologic		
Neutropenia	4 (20·0)	9 (50·0)
Thrombocytopenia	3 (15·0)	5 (27·8)
Anaemia	0	0
Non-haematologic		
Infections	9 (45·0)	3 (16·7)
Diarrhoea	0	0
Peripheral sensory neuropathy	0	0
Nausea	0	0
Rash	0	0
Pain	0	0
Constipation	0	0

VCD, bortezomib plus cyclophosphamide plus dexamethasone; RCD, lenalidomide plus cyclophosphamide plus dexamethasone; G, grade.

significant differences between the two groups, nor changes at different timepoints. However, unlike immunoglobulins, we did not study immunophenotype beyond the ninth cycle. Interestingly, exactly from that timepoint, differences in immunoglobulin levels started to appear, suggesting that the combination with cyclophosphamide and dexamethasone might have blunted the immunostimulatory effects of lenalidomide during treatment.

In conclusion, our study represents a first academic effort to perform a head-to-head comparison of two major anti-myeloma drugs. With the limitations of a study that did not meet the enrolment target, we showed that bortezomib and lenalidomide, both combined with cyclophosphamide and dexamethasone in a fixed-duration therapy, are equally effective in MM patients at first relapse, with reasonably low costs. The choice of treatment should therefore be based on patients' characteristics and preferences, residual toxicities, and drug class sequencing.

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Conflict of interest

VM has received honoraria and travel grants from Janssen, Celgene, Bristol-Myers Squibb, Amgen. MG has received honoraria and travel grants from Janssen, Celgene, Bristol-Myers Squibb. AMC has received honoraria and travel grants from Janssen and Celgene. FP has received honoraria and travel grants from Janssen and Celgene. PC has received honoraria from advisory board and speaking fees from Celgene, Janssen, Novartis, Roche, Takeda, Sanofi, Abbvie, Amgen, Gilead, and Klowa Kirin. The other authors have no competing interests.

Authors contribution

PC was involved in the conception and design of the study; VM, AC, MG, SP, CCarniti, FP, FG, RZ, SS, MM, AN, CCrippa, AMC and LB collected and assembled the data; VM, IA and PC analyzed and interpreted the data; CCarniti performed the biological study; all the authors were involved in the writing and final approval of the manuscript.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Treatment outline.

Figure S2. Immunophenotypic analysis.

Figure S3. Uninvolved immunoglobulin serum levels.

Figure S4. Time to response.

Figure S5. Progression-free survival according to relevant clinical features.

Figure S6. Comparison between IGH cfDNA and plasma cell frequencies at complete-remission timepoints.

Table SI. International Myeloma Working Group (IMWG) response criteria.

Table SII. Univariate and multivariate analysis. (A) Univariate analysis of progression-free survival. (B) Univariate analysis of progression-free survival on immunophenotypic markers. (C) Multivariable analysis of progression-free survival.

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