

Bortezomib, thalidomide and dexamethasone, with or without cyclophosphamide, for patients with previously untreated multiple myeloma: 5-year follow-up

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Summary

This follow-up extension of a randomised phase II study assessed differences in long-term outcomes between bortezomib-thalidomide-dexamethasone (VTD) and VTD-cyclophosphamide (VTDC) induction therapy in multiple myeloma. Newly diagnosed patients ($n = 98$) were randomised 1:1 to intravenous bortezomib (1.3 mg/m²; days 1, 4, 8, 11), thalidomide (100 mg; days 1–21), and dexamethasone (40 mg; days 1–4, 9–12), with/without cyclophosphamide (400 mg/m²; days 1, 8), for four 21-day cycles before stem-cell mobilisation/transplantation. After a median follow-up of 64.8 months, median time-to-next therapy was 51.8 and 47.9 months with VTD and VTDC, respectively. Type of subsequent therapy was similar in both arms. After adjusting for asymmetric censoring, median time to progression was not significantly different between VTD and VTDC [35.7 vs. 34.5 months; Hazard ratio (HR) 1.26, 95% confidence interval: 0.76–2.09; $P = 0.370$]. Five-year survival was 69.1% and 65.3% with VTD and VTDC, respectively. When analysed by minimal residual disease (MRD) status, overall survival was longer in MRD-negative *versus* MRD-positive patients with bone marrow-confirmed complete response (HR 3.66, $P = 0.0318$). VTD induction followed by transplantation provides long-term disease control and, consistent with the primary analysis, there is no additional benefit from adding cyclophosphamide. This study was registered at ClinicalTrials.gov (NCT00531453).

Keywords: multiple myeloma, minimal residual disease, transplantation.

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Trial registration: clinicaltrials.gov identifier: NCT00531453.

Bortezomib-based triplet combinations are among the established standards of care as induction therapy for previously untreated patients with multiple myeloma (MM) who are eligible for high-dose therapy with autologous stem cell transplantation (HDT-ASCT) (Anderson *et al*, 2013; Ludwig *et al*, 2014). Such combinations include bortezomib plus thalidomide and dexamethasone (VTD) – a regimen which has recently been approved in the European Union, Canada and Australia – and bortezomib plus cyclophosphamide and dexamethasone (VCD), both of which are effective in previously untreated MM (Cavo *et al*, 2010; Reeder *et al*, 2010; Moreau *et al*, 2011; Kumar *et al*, 2012; Rosiñol *et al*, 2012a). While the benefits of triplet combinations are proven, it has not been established whether the addition of a fourth agent might further improve the activity of these combinations (Kumar *et al*, 2012), despite some evidence indicating the potential of quadruplet regimens (Jakubowiak *et al*, 2011; Palumbo *et al*, 2014).

We conducted an open-label, randomised non-comparative phase II study to evaluate the efficacy and safety of VTD and VTD plus cyclophosphamide (VTDC) as induction therapy prior to HDT-ASCT in 98 patients with previously untreated MM (Ludwig *et al*, 2013). Results from the primary analysis conducted after a median follow up of 33.3 months showed that both VTD and VTDC are active induction regimens, resulting in bone marrow-confirmed complete response (CR) rates of 29% and 31% post-induction, and 57% and 61% post-HDT-ASCT, respectively. We also showed that 35% of VTD and 27% of VTDC patients achieved minimal residual disease (MRD)-negative status, which is a prognostic indicator of improved outcomes, particularly among patients achieving a ‘conventional’ CR (Korthals *et al*, 2012; Rawstron *et al*, 2013; Martinez-Lopez *et al*, 2014; Puig *et al*, 2014). At the time of the primary analysis, no significant differences in survival outcomes were seen, suggesting no benefit from the addition of cyclophosphamide to VTD. However, as outcomes data were not mature at this analysis, with only 21% of patients having progressed and 15% having died, we now report the findings from the protocol-specified, long-term extension follow-up phase of the study, which evaluated final time-to-event data after a median follow-up of more than 5 years. Experience from other trials (Mateos *et al*, 2014; Palumbo *et al*, 2014) indicates that subtle differences in outcome may only become detectable after long follow-up when the

treatment impact on good risk patients becomes evident. In addition, data on subsequent MM treatment are reported, together with analyses of outcomes according to MRD status and depth of response.

Methods

Patients and study design

The design of this randomised, non-comparative multicentre phase II study (clinicaltrials.gov identifier: NCT00531453) has been reported previously (Ludwig *et al*, 2013). Briefly, transplant-eligible patients aged 18–70 years with previously untreated, measurable MM and without grade ≥ 2 peripheral neuropathy or neuropathic pain [National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcae3.pdf)] were randomised [1:1, stratified by International Staging System (ISS) disease stage (Griep *et al*, 2005)] to receive initial treatment with four 21-day cycles of VTD or VTDC. Treatment comprised bortezomib 1.3 mg/m² intravenously on days 1, 4, 8 and 11, thalidomide 100 mg orally on days 1–21 and dexamethasone 40 mg orally on days 1–4 and 9–12, with or without cyclophosphamide 400 mg/m² intravenously on days 1 and 8. Patients who remained eligible for transplant then underwent stem cell mobilisation and single or double transplantation, while patients who had become transplant-ineligible or had achieved a CR post-induction could receive four additional cycles of VTD or VTDC.

Institutional review boards or independent ethics committees at all participating sites approved the study, which was conducted in accordance with the International Conference on Harmonisation for Good Clinical Practice and the Declaration of Helsinki. All patients provided written informed consent.

Assessments

Responses were determined by independent review per International Myeloma Working Group (IMWG) uniform response criteria (Durie *et al*, 2006), with the additional response categories of CR^{flc} [defined as CR with a normalised serum free light chain (FLC) ratio; used as a surrogate

for stringent CR due to the lack of routine κ/λ bone marrow staining] and near-CR (defined as absence of M-protein on electrophoresis and immunofixation-positive). Post-transplant, patients were followed every 12 weeks until disease progression, and then every 12 weeks for survival and subsequent therapies. The primary endpoint of the study was the combined rate of CR^{flc} plus CR and near-CR post-induction. Secondary endpoints included time to progression (TTP), progression-free survival (PFS) and overall survival (OS).

Additionally, an exploratory analysis of time-to-next therapy (TTNT; defined as the time from randomisation to the start of subsequent MM therapy or death prior to subsequent therapy) was conducted. Outcomes (PFS and OS) among patients achieving bone marrow-confirmed CR were also investigated according to MRD status. Among patients achieving MRD-negative status, outcomes were also investigated according to response (CR^{flc} versus other responses).

For MRD assessment, bone marrow aspirates were collected at suspected CR and, where possible, at screening. MRD status was assessed at a central laboratory in Salamanca by immunophenotyping using multiple staining combinations [CD38 (Alexa Fluor[®])/-/CD56-PE/CD45-AmCyan/CD19-PerCP-Cy5.5/CD138-APC, and CD38/ κ/λ -FITC/ κ/λ -PE/CD45-AmCyan/CD19-PerCP-Cy5.5/CD138-APC], with the aim of identifying, quantifying and characterising plasma cells. Data were acquired in a FACSCanto[™] II flow cytometer (BD Biosciences, San Jose, CA, USA), using FACS Diva[™] (BD Biosciences) software to acquire information, and Infinicyt[™] software (Cytognos, Salamanca, Spain) for data analysis by a central provider (Hospital Universitario de Salamanca, Spain). Samples were characterised as MRD-positive if clonal plasma cells were detected [based on increased expression of CD19 and/or CD45 and/or increased expression of CD56, together with immunoglobulin light chain restriction (cytoplasmaticK or cytoplasmaticL)], or MRD-negative if only normal and polyclonal plasma cells were detected.

Statistical analyses

The Kaplan–Meier method was used to estimate the distribution of time-to-event endpoints. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated based on a Cox's model stratified by ISS disease stage, and *P*-values for differences between treatment arms and patient groups were calculated using the log-rank test stratified by ISS disease stage.

For the primary analyses of TTP and PFS, patients who were lost to follow-up, withdrew consent or received subsequent MM therapy due to early indicators of progression, without fulfilling the standard criteria for disease progression, were censored. To mitigate the effect of asymmetric censoring between arms, sensitivity analyses of TTP and PFS were conducted in which patients who received subsequent therapy without meeting the standard IMWG criteria (Durie *et al*, 2006) for disease progression were regarded as having

had a progression event if reported by the investigator as having 'relapsed from CR' or having experienced 'clinical relapse'.

All analyses were undertaken by the sponsor. All authors had access to the primary clinical trial data.

Results

Patients and follow-up

As previously reported (Ludwig *et al*, 2013), a total of 98 patients were enrolled and randomised to receive VTD or VTDC ($n = 49$ in each arm) (see Fig S1). Median age was 57 years (range 35–65) and 58 years (range 33–68) in the VTD and VTDC arms, respectively, and 24%/45%/31% and 18%/47%/35% of patients, respectively, had ISS stage I/II/III disease. Other baseline characteristics were similarly well balanced between the arms (Ludwig *et al*, 2013).

All patients had completed VTD or VTDC treatment at the time of the initial report of the study (Ludwig *et al*, 2013). In both arms, patients received a median of four treatment cycles. Forty-eight (98%) patients in the VTD arm and 40 (82%) patients in the VTDC arm underwent HDT-ASCT.

The data cut-off for this final pre-specified, long-term extension analysis was 23 September, 2013; 5 years after the last patient was randomised. The overall median follow-up, calculated using reverse censoring, was 64.8 months in all 98 patients: 65.3 months in the VTD arm and 64.7 months in the VTDC arm. This represents an additional follow-up of approximately 32 months in each arm, based on the medians, when compared with the initial report of the study (Ludwig *et al*, 2013). At the time of data cut-off, 34 patients had died (15 VTD, 19 VTDC), 1 (2%) VTD patient was lost to follow-up and 1 (2%) VTD patient had chosen to withdraw from study data collection (see Fig S1). Deaths were primarily due to disease progression: 10 (20%) patients in the VTD arm and 15 (31%) patients in the VTDC arm.

Long-term outcomes

At data cut-off for this protocol-specified final analysis, per investigator assessment in the intent-to-treat population, 20/49 (41%) patients in the VTD arm and 32/49 (65%) in the VTDC arm had disease progression events in the TTP analysis, and 24/49 (49%) and 34/49 (69%) patients had PFS events (disease progression or death). Median TTP in this primary analysis was not reached with VTD versus 39.3 months with VTDC [HR 1.55 (95% CI: 0.88–2.72), $P = 0.125$], and 5-year progression-free rates were 54.1% and 30.6%, respectively (Fig 1A). Median PFS was 56.3 vs. 36.3 months with VTD versus VTDC [HR 1.37 (95% CI: 0.81–2.31), $P = 0.244$], and the respective 5-year PFS rates were 47.8% and 29.1% (Fig 1C).

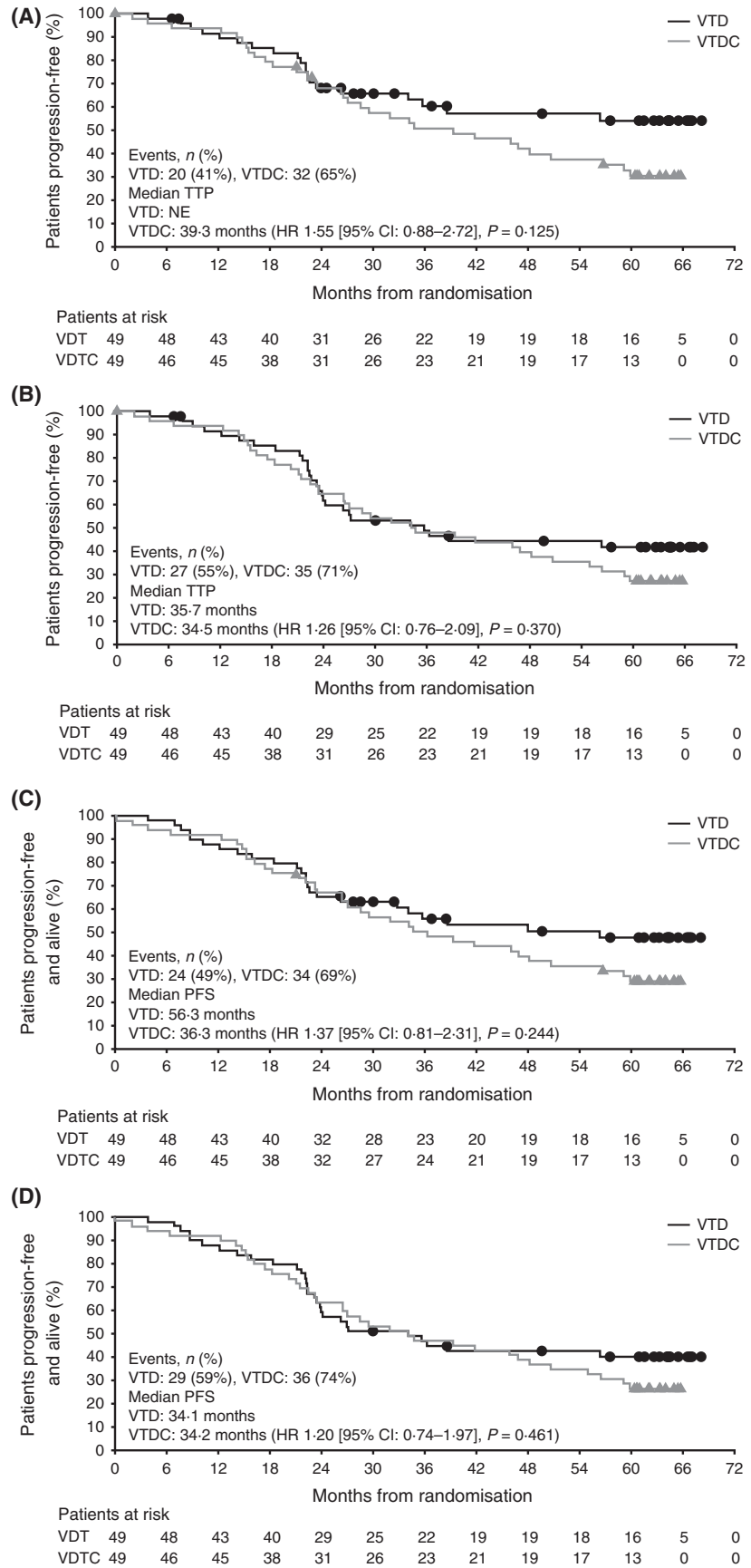


Fig 1. Time to progression (TTP) and progression-free survival (PFS) with VTD and VTDC. (A) TTP per the primary analysis, with asymmetric censoring between arms. (B) TTP per the sensitivity analysis. (C) PFS per the primary analysis. (D) PFS per the sensitivity analysis. CI: confidence interval; HR: hazard ratio; NE: not estimatable; VTD, bortezomib-thalidomide-dexamethasone; VTDC, bortezomib-thalidomide-dexamethasone plus cyclophosphamide.

Asymmetric censoring was seen between 24 and 54 months in the primary analysis of TTP/PFS. In the VTD arm, 10 patients were censored in the analysis of TTP (Fig 1A) and 8 in the analysis of PFS (Fig 1C) during this follow-up period, compared with no patients in the VTDC arm. Of the 10 VTD patients censored in the TTP analysis, 2 were censored due to study data cut-off and 1 was lost to follow-up; the other 7 patients were censored due to starting subsequent therapy following a recorded 'relapse from CR' or 'clinical relapse' in the absence of investigator-documented disease progression per IMWG criteria (Durie *et al*, 2006). Therefore, to mitigate the impact of this asymmetric censoring, a sensitivity analysis of TTP and PFS was conducted in which these patients were considered as having an event at the time of recorded 'relapse from CR' or 'clinical relapse'. Results from the sensitivity analysis are summarised in Table I. Using this approach, median TTP was 35.7 vs. 34.5 months with VTD and VTDC, respectively [HR 1.26

(95% CI: 0.76–2.09), $P = 0.370$; Fig 1B], and median PFS was 34.1 vs. 34.2 months [HR 1.20 (95% CI: 0.74–1.97), $P = 0.461$; Fig 1D].

To further evaluate the impact of asymmetric censoring on apparent differences in long-term outcomes between arms, an exploratory analysis of TTNT was conducted (Table I; Fig 2A). In this analysis, median TTNT was 51.8 vs. 47.9 months with VTD *versus* VTDC [HR 1.21 (95% CI: 0.71–2.05), $P = 0.484$], with 26 (53%) and 30 (61%) patients, respectively, having received subsequent therapy or died due to disease progression prior to receiving subsequent therapy at data cut-off. Eight (16%) and 6 (12%) patients received at least three more lines of therapy, and 14 (29%) and 12 (25%) received at least two more lines of therapy in the VTD and VTDC treatment groups, respectively. The agents most commonly received as part of subsequent therapy included dexamethasone, lenalidomide, bortezomib, thalidomide and cyclophosphamide (Table II). The type of

Table I. Time to progression (TTP) and progression-free survival (PFS) per investigator assessment (sensitivity analysis), time-to-next therapy (TTNT) and overall survival (OS) in the VTD and VTDC arms, based on Kaplan–Meier product limit estimates.

Outcome	VTD (<i>n</i> = 49)	VTDC (<i>n</i> = 49)	HR (95% CI)	<i>P</i> *
TTP (sensitivity analysis)				
Events, <i>n</i> (%)	27 (55)	35 (71)		
Median (95% CI), months	35.7 (23.9–NE)	34.5 (23.5–50.6)	1.26 (0.76–2.09)	0.370
3-year rate, % (95% CI)	48.8 (33.9–62.1)	47.9 (33.3–61.1)	NA	NA
5-year rate, % (95% CI)	41.8 (27.5–55.5)	27.1 (15.5–40.0)	NA	NA
PFS (sensitivity analysis)				
Events, <i>n</i> (%)	29 (59)	36 (74)		
Median (95% CI), months	34.1 (23.5–NE)	34.2 (23.5–48.2)	1.20 (0.74–1.97)	0.461
3-year rate, % (95% CI)	46.8 (32.4–59.9)	46.9 (32.6–60.0)	NA	NA
5-year rate, % (95% CI)	40.1 (26.2–53.5)	26.5 (15.2–39.3)	NA	NA
TTNT				
Events, <i>n</i> (%)	26 (53)	30 (61)		
Median (95% CI), months	51.8 (31.9–NE)	47.9 (28.7–NE)	1.21 (0.71–2.05)	0.484
OS				
Events, <i>n</i> (%)	15 (31)	19 (39)		
Median (95% CI), months	NE (NE–NE)	NE (64.3–NE)	1.15 (0.58–2.27)	0.692
3-year rate, % (95% CI)	79.6 (65.4–88.5)	83.7 (70.0–91.5)	NA	NA
5-year rate, % (95% CI)	69.1 (54.1–80.1)	65.3 (50.3–76.8)	NA	NA

HR, hazard ratio; CI, confidence interval; NE: not estimable; NA: not applicable; VTD, bortezomib-thalidomide-dexamethasone; VTDC, bortezomib-thalidomide-dexamethasone plus cyclophosphamide.

*Based on a stratified log-rank test.

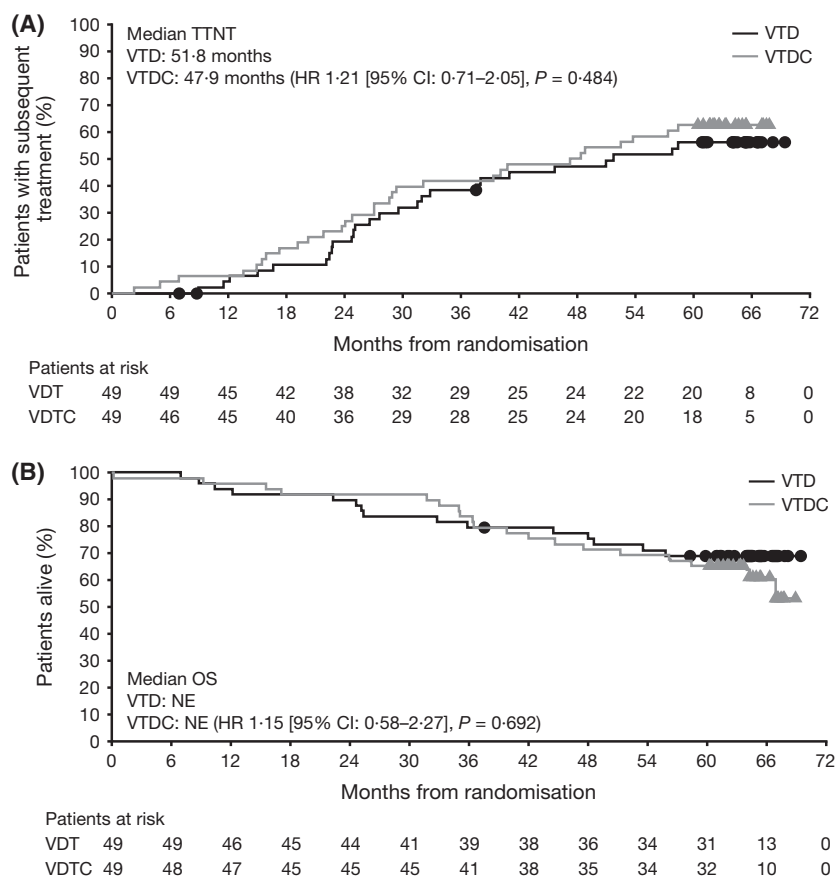


Fig 2. Time-to-next therapy (TTNT) and overall survival (OS) with VTD and VTDC. (A) TTNT. (B) OS. CI: confidence interval; HR: hazard ratio; NE: not estimatable; VTD, bortezomib-thalidomide-dexamethasone; VTDC, bortezomib-thalidomide-dexamethasone plus cyclophosphamide.

subsequent therapy was similar in both treatment groups; 15 (31%) and 16 (33%) patients were retreated with bortezomib, and 9 (18%) and 8 (16%) patients were retreated with thalidomide.

Median OS was not reached in either arm (Table I; Fig 2B). Five-year survival rates were 69.1% with VTD and 65.3% with VTDC.

Outcomes by depth of response and minimal residual disease status

To assess the relationship between depth of response and long-term outcomes, PFS and OS were analysed in patients achieving CR^{flc} (CR with normal FLC ratio confirmed by bone marrow plasma cells, but without bone marrow immunohistochemistry; n = 32) at any point in the study versus \geq very good partial response (VGPR) but excluding the 32 patients with CR^{flc} (n = 48) versus <VGPR (n = 18). This analysis was pooled across the VTD and VTDC arms. As expected, there was a trend for better PFS and OS in the true CR (CR^{flc}) versus \geq VGPR versus <VGPR groups. Across both arms combined, median (95% CI) PFS was 56.3 months (35.7–not estimable) in the CR^{flc} group, 38.6 months (26.3–not estimable) in the \geq VGPR group and 26.0 months (10.2–34.7) in the <VGPR group. In the sensitivity analysis, median (95% CI) PFS was 46.9 months (23.9–not estimable) in the

Table II. Agents commonly received (\geq 10% of patients overall) as part of subsequent therapy following VTD or VTDC.

Agent	VTD (n = 49)		VTDC (n = 49)	
	n	%	n	%
Any subsequent therapy	24	49	28	57
Dexamethasone	20	41	21	43
Lenalidomide	17	35	16	33
Bortezomib	15	31	16	33
Thalidomide	9	18	8	16
Cyclophosphamide	6	12	10	20
Melphalan	3	6	8	16
Doxorubicin	4	8	6	12

VTD, bortezomib-thalidomide-dexamethasone; VTDC, bortezomib-thalidomide-dexamethasone plus cyclophosphamide.

CR^{flc} group, 30.6 months (23.2–not estimable) in the \geq VGPR group and 26.0 months (10.2–34.7) in the <VGPR group. Median (95% CI) OS was not reached in the CR^{flc} or \geq VGPR groups, but was 66.9 months (35.0–not estimable) in the <VGPR group (Fig 3A).

A total of 42 patients with bone marrow-confirmed CR were available for analysis of outcomes (PFS and OS) according to MRD status: 34 were MRD-negative and 8 were

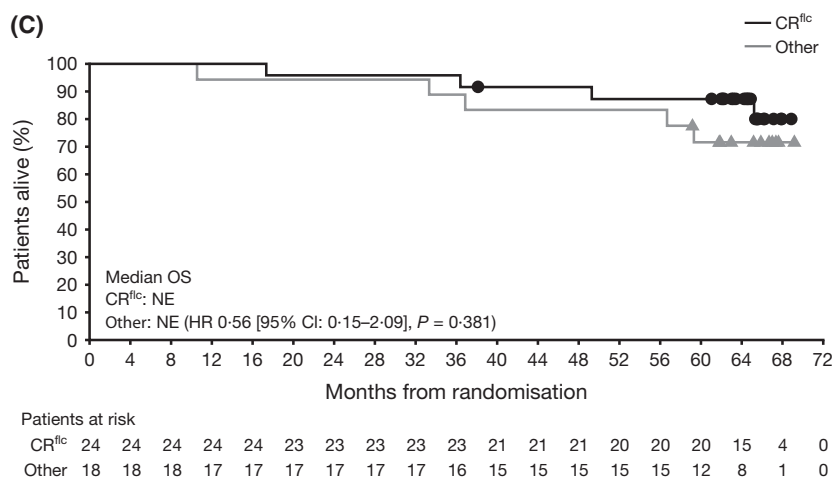
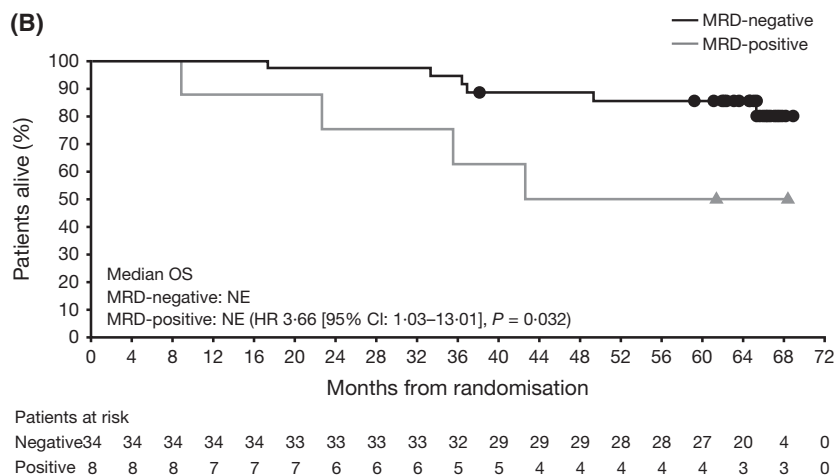
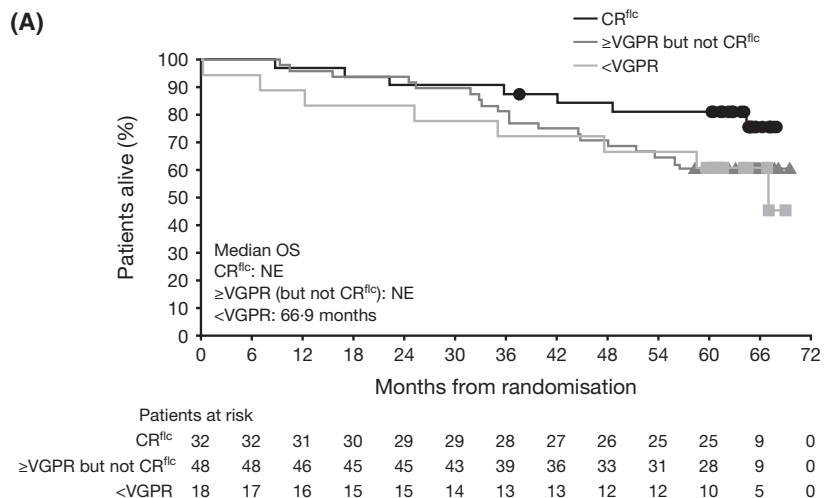


Fig 3. Overall survival (OS) according to minimal residual disease (MRD) status and response (pooled across VTD and VTDC arms). Kaplan-Meier analyses of OS in: (A) patients who achieved CR^{flc}, ≥VGPR but excluding the CR^{flc} patients, or <VGPR; (B) patients with bone marrow-confirmed CR who were MRD-negative or MRD-positive; (C) MRD-negative patients who achieved CR^{flc} or other responses. CI: confidence interval; CR: complete response; CR^{flc}: CR with normalized serum free light chain ratio; HR: hazard ratio; NE: not estimable; VGPR: very good partial response; VTD, bortezomib-thalidomide-dexamethasone; VTDC, bortezomib-thalidomide-dexamethasone plus cyclophosphamide.

MRD-positive by multiparameter flow cytometry. In the primary analysis of PFS, median was not reached in MRD-negative patients *versus* 38.6 months in MRD-positive patients [HR 2.29 (95% CI: 0.87-6.04), P = 0.085]. Per the PFS sensitivity analysis, respective medians were 46.9 *versus* 38.6 months [HR 1.51 (95% CI: 0.61-3.77), P = 0.373]. OS was longer in MRD-negative *versus* MRD-positive patients

[median not reached in either group; HR 3.66 (95% CI: 1.03-13.01), P = 0.032; Fig 3B].

Including those patients who achieved <CR, 42 patients achieved MRD-negative status across the VTD and VTDC groups. Twenty-four of these patients had a best response of CR^{flc}, 14 had a response of CR (bone marrow-confirmed) and 4 had a response of <CR [1 near complete response

(nCR), 1 VGPR, 2 PR]. PFS and OS were evaluated in the 24 MRD-negative CR^{flc} patients *versus* the 18 MRD-negative patients achieving \leq CR. In the primary analysis of PFS, median was not reached *versus* 59.9 months in MRD-negative CR^{flc} *versus* \leq CR patients, respectively [HR 1.01 (95% CI: 0.40–2.57), $P = 0.982$]; in the sensitivity analysis, median PFS was 46.9 *versus* 55.8 months [HR 0.97 (95% CI: 0.44–2.14), $P = 0.941$]. Median OS was not reached in either group of MRD-negative patients [HR 0.56 (95% CI: 0.15–2.09), $P = 0.381$; Fig 3C].

Safety

There were no new adverse events reported in the study database since the initial report of the study. In addition, no second primary malignancies were reported during the long-term extension phase.

Discussion

At a median follow-up of 64.5 months, equating to nearly 3 years of additional follow-up compared with the primary analysis, data from this phase II extension study (Ludwig *et al*, 2013) demonstrate that VTD or VTDC induction followed by HDT-ASCT provides long-term disease control for patients with previously untreated MM. Across both treatment arms, median time until patients required second-line therapy was approximately 4 years and approximately two-thirds of patients were alive at 5 years post-randomisation. Consistent with the primary analysis (Ludwig *et al*, 2013), there were no statistically significant differences in long-term outcomes between the two treatment arms, and outcomes (TTP and PFS) were numerically very similar once asymmetric censoring had been accounted for. A total of 42 (43%) patients achieved MRD-negativity, highlighting the high activity of the VTD and VTDC induction protocols used here. In the prognostic analyses, patients who achieved MRD-negativity had particularly promising outcomes, with 5-year OS rates of approximately 80% and significantly better median OS (HR 3.66, $P = 0.032$) than patients who remained MRD-positive. Achievement of CR^{flc} *versus* 'standard' CR or less, however, did not confer additional prognostic significance in MRD-negative patients. The current analysis thus suggests that MRD-negativity (*versus* MRD-positivity) may be a stronger prognostic marker for OS than bone marrow-confirmed CR^{flc} [*versus* other responses (CR/nCR/VGPR/PR)].

Despite a lack of statistically significant differences, the numerical differences in TTP and PFS between the two treatment arms prompted us to check for potential confounding factors. It was observed that there was a marked difference between the two arms in the number of patients who were censored between 24 and 54 months' follow-up. This 'asymmetric censoring' was caused predominantly by VTD patients receiving subsequent therapy due to 'relapse from CR' or 'clinical relapse' before they had been recorded as having progressive

disease per IMWG criteria (Durie *et al*, 2006). To account for this censoring imbalance, and potential limitation of the study, TTP and PFS analyses were re-run so that these patients were considered as having an event at the time of recorded 'relapse from CR' or 'clinical relapse'. Using this approach, the sensitivity analyses revealed very similar median TTP and PFS durations in the VTD and VTDC treatment arms [median TTP, 35.7 vs. 34.5 months (HR 1.26, $P = 0.370$); median PFS 34.1 vs. 34.2 months (HR 1.20, $P = 0.461$), respectively]. The findings of the exploratory analysis of TTNT [median 51.8 vs. 47.9 months (HR 1.21, $P = 0.484$)] were also consistent both with those seen in the sensitivity analyses of TTP and PFS, and with the lack of difference in OS between arms, supporting the finding of no meaningful differences in long-term outcomes between the two treatment arms. Additionally, the number and type of subsequent lines of therapy were similar in both treatment groups.

Our findings of high activity of VTD induction (Ludwig *et al*, 2013) that persists over the long term are consistent with results reported for the VTD regimen in the Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA) MMY-3006 (Cavo *et al*, 2010, 2012, 2013) and Programa para el Estudio de la Terapéutica en Hemopatías Malignas/Grupo Español de MM (PETHEMA/GEM) phase III (Rosñol *et al*, 2012a,b) trials in newly diagnosed MM, and confirm VTD as one of the most clinically active regimens in this setting. This is supported by a recent meta-analysis showing significant superiority of VTD over VCD, both in terms of activity and of tolerance (Leiba *et al*, 2014), and by a retrospective comparison of the VTD and VCD arms of large European trials (Cavo *et al*, 2014). High activity has also been reported with other proteasome inhibitor-based triplet induction regimens, such as bortezomib-doxorubicin-dexamethasone (PAD) (Sonneveld *et al*, 2012, 2013), bortezomib-lenalidomide-dexamethasone (VRD) (Kumar *et al*, 2012), and carfilzomib-lenalidomide-dexamethasone (Jakubowiak *et al*, 2012). In the present extension study, addition of a fourth agent, cyclophosphamide, to the VTD triplet did not result in any improvement in efficacy, which is in accordance with the primary analysis data (Ludwig *et al*, 2013). This observation is also supported by evidence from a previous study, in which a quadruplet regimen incorporating lenalidomide instead of thalidomide [VRD-cyclophosphamide (VRDC)] was found to yield similar outcomes to triplet induction therapy (VCD or VRD) in previously untreated, transplant-eligible patients with MM (Kumar *et al*, 2012). In contrast, data from a randomised study conducted in newly diagnosed, transplant-ineligible patients suggested that the quadruplet regimen of VMP-thalidomide (VMPT) may be associated with better outcomes (CR rate, PFS, TTNT and OS) than VMP alone (Palumbo *et al*, 2014). However, not only were these findings in transplant-ineligible patients, it should also be noted that VMPT induction was followed by maintenance with bortezomib-thalidomide, whereas no maintenance was used in the VMP arm, and the induction responses were not consolidated with transplantation. Furthermore, when the

data were first published after a median follow-up of 23.2 months, no difference in OS was noted (Palumbo *et al*, 2010). Survival curves diverged significantly only after prolonged follow-up (median 54 months) (Palumbo *et al*, 2014), indicating the importance of long-term observation for conclusive evaluation of the impact of a treatment strategy. VMPT was also less well tolerated than VMP (Palumbo *et al*, 2014).

Multiparameter flow cytometry is a highly sensitive technique for evaluation of MRD in MM that seems to offer similar sensitivity to the polymerase chain reaction (Hart *et al*, 2012; Martinez-Lopez *et al*, 2014; Puig *et al*, 2014). The flow-MRD assay used here benefited from a 6-colour approach that confirmed the clonal nature (through light-chain restriction) of phenotypically aberrant plasma cells. Using this technique, we showed that MRD-negative patients had significantly longer OS than those who were MRD-positive, a finding supported by data from other recent studies in the literature (Korthals *et al*, 2012; Rawstron *et al*, 2013; Martinez-Lopez *et al*, 2014; Puig *et al*, 2014). Current efforts are now underway to develop an automated flow-MRD method based on 10-colour approaches, with similar sensitivity to next-generation sequencing techniques. The similar PFS or OS among MRD-negative patients achieving CR^{fc} versus lower responses confirms the superiority of MRD as a marker for long-term outcomes in newly diagnosed MM over achievement of a true CR, which requires negative immunofixation. As the detection limit of this technique is around 150 mg/l (Tate *et al*, 2009), the presence of a substantial number of myeloma cells may be missed. Alternatively, M-protein may be detectable due to the unusually long half-life of certain M-proteins, with recycling of IgG by IgG FcRn receptors (Mead *et al*, 2004; Paiva *et al*, 2011) being one potential cause of this phenomenon.

In summary, our long-term follow-up data support the notion that three-drug bortezomib-based induction regimens are the most appropriate therapies for previously untreated, transplant-eligible MM. They also confirm VTD as a highly active regimen, providing high response rates and notable long-term outcome data following a limited period of induction therapy of only four cycles. Lastly, these analyses support the prognostic benefit of achieving MRD-negative status and the importance of this as a goal of first-line therapy in MM.

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Authorship contributions

Contribution: HL and HvDV designed the research; HL, RG, TM, IS, OS, RH, AD, BP, M-BV, GE, AMS, DR, SC, OA, CE, HB, HvDV and LV performed the research; HL, RG, TM, IS, OS, RH, AD, BP, M-BV, GE, AMS and LV collected data; SC, OA and HF performed the statistical analysis; HL, BP, DR, SC, OA, CE, HB and HvDV analysed and interpreted the data; and HL, SC, OA, HF and HvDV wrote the manuscript. All authors reviewed the draft manuscript, and approved the final version for submission.

Conflicts of interest

HL: Honoraria and research funding (Janssen-Cilag, Celgene, Millenium). RG: Consultant/advisory role (Mundipharma); honoraria (Celgene, Mundipharma). TM: Board membership (Janssen-Cilag). IS: Board membership (Celgene), consultancy (Celgene, Janssen-Cilag), research funding (Celgene), honorarium (Celgene, Janssen-Cilag, Novartis, Amgen). OS: Nothing to disclose. RH: Consultancy (Celgene, Merck, Janssen). AD: Honoraria (Janssen-Cilag). BP: Honoraria (Millennium, Janssen, Celgene, and The Binding Site). M-BV: Nothing to disclose. GE: Consultancy (Janssen, Celgene); honoraria (Janssen, Celgene). AMS: Nothing to disclose. DR: Employment (Janssen Global Services, LLC). SC: Employment (Janssen Research & Development, LLC), holds stock (Johnson & Johnson). OA: Employment (Janssen-Cilag Limited). CE: Employment (Janssen Research & Development, LLC), holds stock (Johnson & Johnson). HF: Employment (Janssen Research & Development, LLC). HvDV: Employment (Janssen Pharmaceutica NV), holds stock (Johnson & Johnson). LV: Nothing to disclose.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig S1. Updated CONSORT diagram showing patient flow through the study.

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