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Efficacy and safety of melphalan, arsenic trioxide and ascorbic acid combination therapy in patients with relapsed or refractory multiple myeloma: a prospective, multicentre, phase II, single-arm study

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Summary

We assessed the safety and efficacy of melphalan, arsenic trioxide (ATO) and ascorbic acid (AA) (MAC) combination therapy for patients with multiple myeloma (MM) who failed more than two different prior regimens. Patients received melphalan (0·1 mg/kg p.o.), ATO (0·25 mg/kg i.v.) and AA (1 g i.v) on days 1–4 of week 1, ATO and AA twice weekly during weeks 2–5 and no treatment during week 6 of cycle 1; during cycles 2–6, the schedule remained the same except ATO and AA were given twice weekly in week 1. Objective responses occurred in 31 of 65 (48%) patients, including two complete, 15 partial and 14 minor responses. Median progression-free survival and overall survival were 7 and 19 months respectively. Twenty-two patients had elevated serum creatinine levels (SCr) at baseline, and 18 of 22 (82%) showed decreased SCr levels during treatment. Specific grade 3/4 haematological (3%) or cardiac adverse events occurred infrequently. Frequent grade 3/4 non-haematological adverse events included fever/chills (15%), pain (8%) and fatigue (6%). This steroid-free regimen was effective and well tolerated in this heavily pretreated group. These results indicate that the MAC regimen is a new therapeutic option for patients with relapsed or refractory MM.

Keywords: melphalan, arsenic trioxide, ascorbic acid, multiple myeloma, phase II trial.

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In 2005, multiple myeloma (MM) accounted for approximately 16% of new haematological cancer cases (1·2% of total new cancer cases) and 26% of haematological cancer deaths (2·0% of total cancer deaths) in the USA (American Cancer Society, 2005; Jemal *et al*, 2005). Although conventional combination chemotherapy regimens produce variable response rates in patients with MM, these regimens produce similar patient survival outcomes, with median overall survival of approximately 3 years (Myeloma Collaborative Trialists Group, 1998; Durie *et al*, 2003; Kyle *et al*, 2003). Current standard of care for relapsed or refractory MM includes glucocorticosteroids, salvage chemotherapy agents or combinations, thalidomide-based or bortezomib-based regimens or stem cell transplantation (National Comprehensive Cancer Network, 2006). Although survival has improved somewhat with these new treatment options, nearly all patients will develop resistant disease; thus, it is essential to provide patients with additional therapeutic options.

Recent reports have indicated that renal insufficiency is present in 20–48% of patients newly diagnosed with MM (Knudsen *et al*, 2000; Kyle *et al*, 2003; Blade & Rosinol, 2005). Patients with renal failure do not respond as well to conventional chemotherapy as patients with normal renal function (Blade *et al*, 1998; Blade & Rosinol, 2005). In addition, patients with renal failure have significantly shorter survival times than patients with normal renal function, especially if kidney function does not improve with antimyeloma treatment (Knudsen *et al*, 2000; Kyle *et al*, 2003; Blade & Rosinol, 2005). Thus, it is important to develop new therapeutic strategies for patients with MM that also improve renal function, and may result in a prolongation of their overall survival.

Arsenic trioxide (ATO) is an antineoplastic chemotherapeutic agent approved for the treatment of relapsed or refractory acute promyelocytic leukaemia (APL; Niu *et al*, 1999; Soignet *et al*, 2001). The use of ATO to treat MM is supported by preclinical studies in which this agent has been shown to inhibit growth, reduce viability and induce apoptosis in several myeloma cell lines at concentrations that can be safely achieved in patients (Park *et al*, 2000; Perkins *et al*, 2000; Hideshima *et al*, 2002; Campbell *et al*, 2004a). Early clinical studies of ATO for patients with advanced refractory MM have demonstrated that this drug produced significant, albeit minor, responses in approximately one-third of patients with daily dosing schedules (Munshi *et al*, 2002; Hussein *et al*, 2004). A more convenient twice-weekly dosing schedule produced similar response rates in a similar patient population (Berenson *et al*, 2005). Furthermore, as the antitumour activity of ATO is dependent upon the generation of reactive oxygen species (ROS), removing free glutathione (GSH), the molecule that eliminates ROS, with ascorbic acid (AA) enhances the anti-MM effects of ATO (Bahlis *et al*, 2002). However, another study has shown that AA may inhibit ATO antitumour effects *in vitro* under some conditions (Karasavvas *et al*, 2005).

The combination of ATO with the cytotoxic agent melphalan has been shown to help to overcome the resistance to melphalan both *in vitro* and in severe combined immunodeficient-human (hu) murine models of human myeloma (Campbell *et al*, 2004a, b). Adding AA to this combination regimen enhanced the anti-MM effects of the melphalan/ATO combination both *in vitro* and *in vivo* (Campbell *et al*, 2004a). A small pilot study involving 10 patients with relapsed or refractory MM showed that treatment with melphalan, ATO and AA (MAC) was well tolerated and produced significant reductions in paraprotein levels in the majority of patients as well as improvement in renal function among the five patients with azotaemia at baseline (Berenson *et al*, 2004; Borad *et al*, 2005).

This multicentre phase II study investigated the efficacy and safety of MAC combination therapy in a larger cohort of patients with relapsed or refractory MM. Because MM is often associated with renal insufficiency, the effects on renal function were also specifically evaluated.

Methods

Study design

This study was a phase II, prospective, multicentre, open-label, single-arm study. Approvals were obtained by all participating sites from the Western Institutional Review Board (Olympia, WA, USA), and informed consent was obtained from all patients prior to entry into the study. Patients received a maximum of six treatment cycles. Each cycle was 6 weeks in duration, comprising 5 weeks of therapy followed by 1 week of no therapy (Table I). In the first treatment cycle, patients received oral melphalan (0·1 mg/kg) and intravenously administered ATO (0·25 mg/kg) and AA (1 g) on days 1–4 of week 1 of the cycle. During weeks 2–5 of cycle 1, patients received ATO and AA twice weekly. In treatment cycles 2–6, patients received oral melphalan on days 1–4 of week 1, and ATO and AA twice weekly in weeks 1–5 of each cycle.

Patients who showed disease progression at any time during the study were eligible to have corticosteroids (prednisone 100 mg p.o. once daily on days 1–4 and 22–25 of each 6-week cycle) added to their therapy. Patients who continued to show disease progression after the addition of corticosteroids were withdrawn from the study.

Patients who achieved either stable disease or responsive disease at the completion of cycle 6 continued to receive intravenous ATO (0·25 mg/kg) and AA (1 g) once weekly. Patients who had complete responses (CRs) at the end of cycle 6 were discontinued from treatment at the discretion of the investigator.

Patient selection

Men or women ≥18 years of age with measurable disease, defined as a monoclonal immunoglobulin spike of ≥10 g/l on serum electrophoresis and/or urine monoclonal immunoglobulin spike of ≥0·2 g/24 h, were enrolled in this study. At the time of study entry, patients had to have relapsed following a

Table I. Treatment schema.

Cycle	Week	Melphalan (0·1 mg/kg)	Arsenic trioxide (0·25 mg/kg)	Ascorbic acid (1 g)
1	1	Daily (days 1–4)	Daily (days 1–4)	Daily (days 1–4)
	2–5	–	Twice a week	Twice a week
	6	Rest	Rest	Rest
2–6	1	Daily (days 1–4)	Twice a week	Twice a week
	2–5	–	Twice a week	Twice a week
	6	Rest	Rest	Rest

response to their previous anti-MM treatment, and/or were refractory to their most recent anti-MM therapy. Patients had to have failed at least two different anti-MM regimens prior to study entry. Karnofsky performance status had to be ≥60. Baseline platelet count had to be $\geq 50 \times 10^9$/l (if the bone marrow was extensively infiltrated, $\geq 30 \times 10^9$/l), haemoglobin ≥8·0 g/dl and absolute neutrophil count $\geq 1{\cdot}0 \times 10^9$/l. Exclusion criteria included: POEMS (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy and skin defects) syndrome; non-secretory myeloma; plasma cell leukaemia; major surgery within 4 weeks of screening visit; active infection (including human immunodeficiency virus and hepatitis B or C); New York Hospital Association class III or IV heart failure; prolonged QT_c interval (>500 ms) with serum potassium ≥4 mmol/l and serum magnesium ≥0·74 mmol/l; severe hypercalcaemia; chemotherapy within 3 weeks or nitrosoureas within 6 weeks of study enrolment; immunotherapy, antibody therapy or radiation therapy within 4 weeks of study enrolment; uncontrolled hypertension, diabetes mellitus or other serious medical or psychiatric illness; history of grand mal seizures; and history of allergic reaction to compounds of similar chemical or biological composition to melphalan, ATO or AA.

Pretreatment and safety assessments

At a screening visit held within 14 d of study entry (baseline), patients underwent a complete physical examination; they were evaluated for Karnofsky performance status, height and weight and had a 12-lead electrocardiogram (ECG) and posteroanterior and lateral chest X-rays. Baseline clinical laboratory tests included routine haematology and clinical chemistry, including serum magnesium, total protein, liver tests, albumin, urinalysis and serum pregnancy test for women of childbearing potential. Disease was assessed at baseline with a skeletal survey, bone marrow aspirate and biopsy, serum β_2-microglobulin, C-reactive protein, and serum and urine protein electrophoresis with quantitation of immunoglobulins as well as 24-h urine protein and immunofixation. Adverse events were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0 (National Cancer Institute, 2003).

During the study, patients had complete blood counts performed weekly during the first 5 weeks of each cycle and serum creatinine (SCr) levels measured weekly during the study. Disease assessments except bone marrow examination and skeletal survey were performed every 6 weeks just before the start of the next treatment cycle. ECG was performed at weeks 1, 2 and 4 of each treatment cycle unless a conduction abnormality was present that could affect electrolytes or QT prolongation (QT_c > 460 ms). Complete clinical chemistry laboratory tests were performed on days 1 and 22 of each cycle. Serum potassium and magnesium were measured weekly unless the QT interval was prolonged (QT_c > 460 ms) or another event was present that could affect electrolytes or QT interval. Serum potassium and magnesium were maintained at ≥4·0 and ≥0·74 mmol/l respectively. If the QT_c was >500 ms, then the study treatment was withheld until it was <460 ms. Adverse events data were collected on day 1, weekly for the first 10 weeks, then every 6 weeks until study completion. Patients who experienced a first or second grade 3 adverse event or haematological toxicity had their study medications withheld and underwent twice-weekly assessments of cell counts. Upon reversal of the haematological toxicity, patients were re-initiated on MAC therapy, with the exception that the melphalan dose was reduced by 33% of the last dose. Those patients who experienced a third haematological dose-related toxicity were withdrawn from the study. Serious adverse events were followed until resolution or until clearly determined to be due to an unrelated chronic condition or to intercurrent illness.

At the completion of the study, patients underwent a complete physical examination, clinical laboratory tests and disease assessment. In addition, a skeletal survey was performed to assess lesion status. If the patient showed a CR, a bone marrow biopsy was performed. After discontinuing study treatment, patients were followed (via visit or telephone) for survival status every 3 months.

Response criteria

Patients were monitored for response every 6 weeks, before day 1 of each cycle, by quantification of serum immunoglobulins, serum protein electrophoresis and immunofixation, and collection of a 24-h urine specimen for total protein, electrophoresis and immunofixation. CR was defined as negative immunofixation test for myeloma protein in serum and in urine on at least two determinations for a minimum of

6 weeks, no increase in the size of lytic bone lesions, and <5% plasma cells in the marrow. Partial response (PR) was defined as a ≥50% reduction in the level of serum M-protein on at least two determinations 6 weeks apart, reduction in 24-h urinary light-chain excretion by either ≥90% or to <0·2 g (if present), ≥50% reduction in size of soft tissue plasmacytomas and no increase in size or number of lytic bone lesions. Minor response (MR) was defined as 25–49% reduction in serum M-protein, 50–89% reduction in urine M-protein (if present), 25–49% reduction in size of plasmacytomas and no increase in size or number of lytic bone lesions. Progressive disease (PD) was defined as >25% increase in serum M-protein or in 24-h urinary light-chain excretion, increase in size or development of new existing lytic bone lesions or plasmacytomas or development of hypercalcaemia.

Statistical analysis

The primary endpoints were overall objective response rate (ORR) to MAC therapy and progression-free survival for the intent-to-treat population. The secondary endpoints were time to response, overall survival and change in renal function as determined by serum creatinine. In addition, safety analyses were performed on all patients who received at least one dose of the study drug regimen.

Time to response was defined as the time from first treatment until at least MR was observed. Progression-free survival was defined as the time during and after treatment that a patient maintained a response or stable disease. The actuarial durations of progression-free survival and overall survival were plotted according to the method of Kaplan and Meier (1958).

Survival for this study was determined from the start of treatment. This report is based on follow-up data collected as of 31 December 2005, which was the time of the planned final analysis. All patients were monitored according to their treatment group even if treatment was discontinued owing to toxicity or non-compliance.

Results

Patient characteristics

Sixty-five patients were enrolled in this study and comprised the intent-to-treat population. Patient demographics and baseline characteristics are summarised in Table II. These patients had been heavily pretreated, having failed a median of 4 (range 1–8) prior therapies. All 65 patients were evaluable for efficacy and safety. Nine patients discontinued prematurely during cycle 1 because of PD or death ($n = 5$), patient decision ($n = 1$), sepsis and intracranial haemorrhage ($n = 2$) and rash and vomiting ($n = 1$). All of these nine patients were included in efficacy analysis and treated as non-responders for tumour response evaluation in order to avoid selection bias.

Table II. Patient demographics and baseline characteristics ($n = 65$).

Median age (range), years	66 (29–89)
Median number (range) of failed therapies	4 (1–8)
Number of patients by failed therapies	
Melphalan	25
Thalidomide/lenalidomide	40
Bortezomib	21
Peripheral stem cell transplant	11
Serum M-protein (g/l)*	
Mean (range)	38 (1·0–1·10)
Urine M-protein (g/24 h)†	
Mean (range)	4·4 (0·01–100·6)
β_2-microglobulin (mg/dl)‡	
Mean (range)	5·5 (0·5–25·9)

All patients had active progressive disease.
*$n = 49$ (patients with measurable serum M-protein).
†$n = 40$ (patients with measurable urine M-protein).
‡$n = 61$.

Response to and efficacy of MAC therapy

Objective responses to MAC therapy were observed in 31 of 65 evaluable patients, yielding an ORR of 48% (Table III), and were determined prior to the addition of prednisone, which was administered to only seven (11%) patients. Specifically, CR was observed in two patients (3%), PR in 15 patients (23%) and MR in 14 patients (22%). The ORR was determined for patients who had failed prior treatment with one or more of the following: melphalan, thalidomide/lenalidomide, bortezomib or peripheral stem cell transplant. For patients who had failed one or more of these specific prior therapies, ORRs ranged from 44% to 57% (Table III). Median follow-up among living patients was 12 months (range 5–27 months). The median progression-free survival for the intent-to-treat population ($n = 65$) was 7 months (range 0·25–25+ months)

Table III. Patient responses to melphalan, arsenic trioxide and ascorbic acid combination therapy ($n = 65$).

Objective responses in evaluable patients	*n* (%)
Overall response	31 (48)
Complete response	2 (3)
Partial response	15 (23)
Minimal response	14 (22)
Objective responses by failed prior therapies	
Melphalan	11 (44)
Thalidomide/lenalidomide	22 (55)
Bortezomib	12 (57)
Peripheral stem cell transplant	5 (46)
Progression-free survival (months)	
Median (range)	7 (0·25–25+)
Time to response (months)	
Median (range)	1·5 (1–6)
Duration of response	
Median (range)	12 (1–16+)

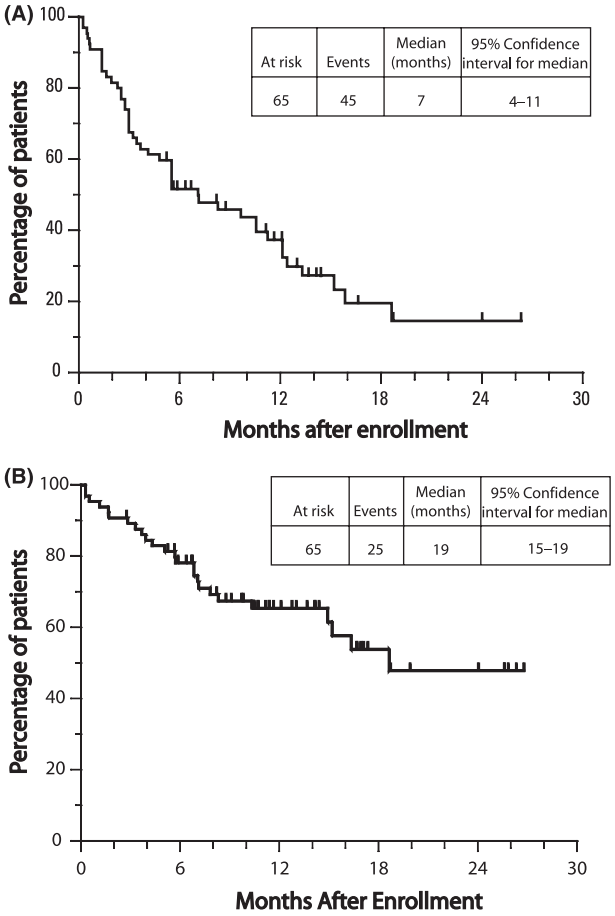

Fig 1. Kaplan–Meier estimates of survival among the patients who received melphalan, arsenic trioxide and ascorbic acid combination therapy ($n = 65$). Estimates of progression-free survival (A) and overall survival (B).

(Fig 1A). The median time to response was at the time of their first assessment for response which was at 1·5 months (range 1·5–6 months), and the median duration of response was 12 months (range 1–16+ months) (Table III). The median overall survival time was 19 months (range 2–27+ months) (Fig 1B). Among the seven patients who received the addition of prednisone per protocol to the MAC regimen at the time of PD, no additional benefit was observed.

Notably, 15 patients with objective responses to MAC and three patients with stable disease continued on maintenance therapy consisting of ATO and AA given once weekly for 2·5–16 months. To date, 11 of these 18 patients have remained on maintenance therapy, one has discontinued maintenance therapy and is stable, five have progressed and one has died.

Serum creatinine levels during MAC therapy

Weekly SCr measurements were available for 58 of 65 patients (89%). At baseline, 22 of 58 patients (38%) had SCr levels ≥132·6 μmol/l, suggesting that these patients had some degree of renal insufficiency (Table IV). During the study, 18 of these 22 patients (82%) showed a reduction in SCr levels, and 12 (55%) had SCr levels reduced to <132·6 μmol/l at some point during the study (Table IV). The median percentage change in SCr levels from baseline to best SCr response was −17% (range 0% to −54%) for patients with available baseline SCr measurements ($n = 58$), −24% (range 0% to −44%) for those with baseline SCr levels between 132·6 and 167·9 μmol/l ($n = 12$) and −30% (range 0% to 54%) for those with baseline SCr levels ≥176·8 mmol/l ($n = 10$). Of the 22 patients with elevated baseline SCr levels, 14 (64%) had objective responses to therapy, including one CR, three PR and 10 MR. Notably, six of eight uraemic patients without responses to MAC therapy showed a reduction in SCr levels.

Adverse events

All patients ($n = 65$) were evaluable for safety and tolerability. Four incidences of serious haematological adverse events (grade ≥3) occurred (Table V). Among these four cases, only one patient required a dose reduction of melphalan. The most common serious non-haematological adverse events occurring in >5% of patients were fever/chills ($n = 10$; 15%), pain ($n = 5$; 8%) and fatigue ($n = 4$; 6%) (Table V). Non-serious adverse events included occurrences of mild cytopenias, which were reversible, and episodes of fluid retention, which responded quickly to diuretics and/or a short course of steroids. Two patients had single occurrences of prolonged QT_c interval (498 and 502 ms) resulting in a brief delay in ATO administration, but continued ATO dosing was not accompanied by any further episodes of QT_c prolongation. One patient developed unstable bradycardia without a prolonged QT_c following the first ATO infusion and was removed from the study.

Discussion

Nearly all MM patients will eventually develop chemoresistance. Traditional chemotherapeutic agents for chemoresistant relapsed and/or refractory MM typically only achieve response rates of 10%–30% generally lasting only several months (Buzaid & Durie, 1988; reviewed in Blade & Esteve, 2000). The mechanisms of chemoresistance (reviewed in Yang *et al*, 2003 and Dalton, 2002) have been proposed to be expression of drug efflux pumps (Dalton *et al*, 1986), changes in apoptotic threshold (Klein *et al*, 1995; Tu *et al*, 1998), increased ability to detoxify or metabolise chemotherapeutic agents via GSH redox pathway (Gupta *et al*, 1989) and activation of nuclear factor-κB (NF-κB) (Wang *et al*, 1999; Ma *et al*, 2003). Therefore, effective therapies for chemoresistant MM must be able to overcome these adaptive cellular changes.

Arsenic trioxide has been shown to be a promising antineoplastic agent for MM. Changes in cellular redox status, as measured by increases in free ROS, are a common feature of

Table IV. Baseline and best serum creatinine (SCr) levels during melphalan, arsenic trioxide and ascorbic acid combination therapy ($n = 58$).

Patient no.	Baseline SCr (mg/dl)	Best SCr (mg/dl)	% Change	Best response
026–002	556·9	424·3	−24	PR
010–003	309·4	176·8	−43	PD
010–001	282·9	265·2	−6	PD
010–012	256·4	203·3	−21	PD
004–009	229·8	106·1	−54	MR
026–001	229·8	114·9	−50	PR
021–001	212·2	212·2	0	PD
010–014	203·3	168·0	−17	MR
004–012	194·5	123·8	−36	MR
017–003	185·6	114·9	−38	CR
004–004	168·0	123·8	−26	SD
021–002	168·0	132·6	−21	MR
010–010	159·1	106·1	−33	PR
012–001	159·1	88·4	−44	PD
010–005	150·3	150·3	0	MR
010–006	150·3	88·4	−41	MR
010–016	150·3	132·6	−12	MR
042–001	141·4	79·6	−44	MR
002–002	132·6	123·8	0	PD
004–001	132·6	79·6	−40	SD
015–002	132·6	132·6	0	MR
020–001	132·6	114·9	−13	MR
004–007	123·8	123·8	0	PD
010–009	123·8	106·1	−14	SD
024–003	123·8	123·8	0	PD
042–003	123·8	123·8	0	PD
010–004	114·9	88·4	−23	PD
017–002	114·9	79·6	−31	PR
042–004	114·9	97·2	−15	SD
010–011	106·1	88·4	−17	MR
015–001	106·1	61·9	−42	PR
017–001	106·1	79·6	−25	PR
024–002	106·1	53·0	−50	CR
025–003	106·1	97·2	−8	PD
002–001	97·2	61·9	−36	MR
010–018	97·2	88·4	−9	SD
042–002	97·2	79·6	−18	MR
002–004	88·4	79·6	−10	PR
004–002	88·4	61·9	−30	PR
006–001	88·4	53·0	−40	PD
006–002	88·4	70·7	−20	SD
018–001	88·4	88·4	0	PD
002–003	79·6	70·7	−11	MR
003–001	79·6	61·9	−22	PR
004–005	79·6	70·7	−11	PR
004–006	79·6	79·6	0	PD
004–010	79·6	61·9	−22	PR
015–008	79·6	70·7	−11	PR
024–001	79·6	70·7	−11	PD
025–004	79·6	70·7	−11	SD
010–002	70·7	44·2	−38	PR
010–015	70·7	61·9	−13	PD
010–017	70·7	61·9	−13	SD
015–005	70·7	53·0	−25	PR
020–002	70·7	61·9	−13	SD
010–013	61·9	61·9	0	SD
004–003	53·0	53·0	0	SD
015–004	53·0	53·0	0	PR

PR, partial response; PD, progressive disease; MR, minor response; CR, complete response; SD, stable disease.

Table V. Grade 3 or 4 adverse events.

	Total number of patients*
Haematological	
Anaemia	3
Neutropenia	1
Non-haematological	
Fever/chills	10
Pain	5
Fatigue	4
Dyspnoea	3
Nausea	2
Vomiting	2
Pancreatitis	2
Herpes zoster	2
Hernia/bowel obstruction	2
Sepsis	2
Cholecystitis	1
Prolonged QT_c interval	2
Unstable bradycardia	1
Cardiac arrest	1
Presyncopal attack	1
T-wave inversions	1
Pulmonary hypertension	1
Bacteraemia	1
Renal failure	1
Neuralgia	1
Intracranial haemorrhage	1

*The number of patients is equal to the number of occurrences, since no adverse event listed occurred more than once in any given patient.

ATO-induced cell death in both cell lines and patient samples (Grad *et al*, 2001). ATO is not sensitive to drug efflux pump mechanisms and is capable of overcoming pro-survival cytokines, interleukin-6 and Bcl-xL in chemorefractory MM (Grad *et al*, 2001). ATO may also functionally alter diverse signalling pathways by inhibiting mitogen-activated protein kinase (MAPK) phosphatases (Cavigelli *et al*, 1996) and NF-κB activation (Kapahi *et al*, 2000).

The combination of ATO and AA with or without melphalan has shown synergistic effects on chemoresistant

MM both *in vitro* and *in vivo* (Dai *et al*, 1999; Grad *et al*, 2001; Campbell *et al*, 2004a). The effect of overcoming resistance may be due, at least in part, to the inhibition of NF-κB activation by ATO, because specific inhibition of NF-κB downregulated apoptosis inhibitors and sensitises MM cells to melphalan (Mitsiades *et al*, 2002; Campbell *et al*, 2004a). On the basis of the above preclinical observations, treatment of myeloma with ATO has also been evaluated in two clinical studies with other agents that inhibit NF-κB activation in MM cells, including bortezomib (Berenson *et al*, 2005) and dexamethasone (Wu *et al*, 2005). GSH depletion by AA, as a way of enhancing ATO (Grad *et al*, 2001) and melphalan (Campbell *et al*, 2004a) cytotoxicity, may also contribute to the efficacy of MAC, as a primary determinant of MM cell sensitivity to both melphalan and ATO (Gupta *et al*, 1989) seems to be intracellular GSH and elevated GSH levels are associated with chemoresistance (Bellamy *et al*, 1991; Grad *et al*, 2001).

In contrast, an *in vitro* study recently demonstrated that AA may protect leukaemia and MM cell lines from ATO toxicity by reducing intracellular ROS (Karasavvas *et al*, 2005). Moreover, AA suppressed bortezomib-mediated inhibition of proteasome activity and abrogated the cell killing by this proteasome inhibitor *in vitro* (Zou *et al*, 2006). These paradoxical effects have been associated with intracellular AA concentration and the timing of when these drugs are given in relation to each other.

The activity of ATO as single agent for patients with relapsed and/or refractory MM has been demonstrated in two separate studies (Munshi *et al*, 2002; Hussein *et al*, 2004). In a small phase II study of ATO alone in heavily pretreated refractory myeloma, ATO alone (0·15 mg/kg/d for 60 d) produced MRs in three of 14 patients (21%) (Munshi *et al*, 2002). A similar response rate was observed when ATO was administered to 24 patients with MM at 0·25 mg/kg on days 1–5 and 8–12 of a 28-d cycle (Hussein *et al*, 2004). The dose of ATO in the MAC regimen, 0·25 mg/kg/d, given in a more convenient twice-weekly dosing schedule, except during the first week of cycle 1, produced a higher response (48%) than in the two single-agent trials (Munshi *et al*, 2002; Hussein *et al*, 2004). The dose of melphalan at 0·1 mg/kg is approximately one-third of the amount that is generally used in conventional treatment regimens for patients with MM. In this study, lower doses of oral melphalan, ATO and AA not only produced a high response rate but were associated with predictable and manageable toxicities in a previously treated MM patient population. The observation that many patients who previously were treated with melphalan responded (44%) to MAC supports clinically the *in vitro* observation that ATO can overcome chemotherapy resistance (Campbell *et al*, 2004b).

Melphalan plays an important role in enhancing activity against resistant MM. As single-agent ATO at 0·15 mg/kg/d was effective in less than one-quarter of patients (Munshi *et al*, 2002), its efficacy was likely to be improved when low-dose melphalan was added, as demonstrated in this study. A recently published phase I study showed a similar effect when low-dose melphalan was added to low doses of bortezomib in patients with relapsed or refractory MM (Berenson *et al*, 2006). Furthermore, preliminary results of a phase II trial of melphalan (9 mg/m^2) and prednisone (60 mg/m^2) plus bortezomib (1·3 mg/m^2) in elderly, previously untreated patients with myeloma also seem promising (Mateos *et al*, 2005). A combination of a lower dose of melphalan (4 mg/m^2) with prednisone (40 mg/m^2 daily) and thalidomide (100 mg) (MPT) produced a higher response rate than melphalan and prednisone (MP) alone in a study involving newly diagnosed elderly patients with MM (Palumbo *et al*, 2006). However, MPT was reported to have a high rate of adverse events, especially deep venous thrombosis, which necessitated discontinuation of thalidomide in more than one-third of the patients. Following prophylactic anticoagulation with low-molecular weight heparin, the risk of thromboembolic events was reduced significantly. Thus, the addition of low-dose melphalan to other newer anti-MM agents has been generally well tolerated and more effective in these early studies than single-agent treatment.

Overall, the anti-MM activity of MAC combination was encouraging, given that all patients had relapsed or were refractory to previous treatments and had PD at enrolment. Previous studies evaluating higher doses of oral melphalan-based therapy in a similar clinical setting have produced lower response rates (Palumbo *et al*, 2004). In our study, the combination of low-dose melphalan with ATO and AA achieved a higher response rate (48%) even among patients who had failed melphalan, thalidomide/lenalidomide, bortezomib and/or peripheral stem cell transplantation. The median time to response to MAC treatment was only 1·5 months, and the median duration of response was 12 months. By comparison, for patients treated with ATO alone in a phase II study, the median time to response and the median duration of response were 2·5 and 5 months respectively (Hussein *et al*, 2004). In addition, the median progression-free survival for all patients treated with MAC therapy was 7 months, with some patients remaining without PD for more than 2 years. Importantly, the median overall survival was 19 months, which is very encouraging given that this was a heavily treated population with an otherwise extremely poor prognosis. These results suggest that MAC therapy is more effective than ATO alone for the treatment of patients with relapsed or refractory MM. Notably, patients with objective responses to MAC were continued on maintenance therapy. The impact of the maintenance phase thus far is not clear and further study is needed to assess the value of ATO and AA as maintenance therapy.

At least one-third of the intent-to-treat population had elevated SCr levels at baseline, suggesting some degree of renal insufficiency in these patients. Most of these patients showed a reduction in SCr levels while on MAC therapy, suggesting that MAC therapy may potentially improve renal function in

patients with elevated baseline SCr. Importantly, patients with elevated baseline SCr levels treated with MAC not only experienced response rates and tolerability similar to patients with normal baseline SCr levels, but also showed frequent improvement in their SCr levels. These observations warrant further evaluation of the impact of MAC therapy on renal function.

The MAC regimen was well tolerated in the intent-to-treat population, with few serious haematological and non-haematological adverse events reported. Serious myelosuppression was observed in a few patients but was not associated with significant clinical toxicity, such as febrile neutropenia or sepsis. Notably, only one patient required a reduction in the dose of melphalan.

In earlier studies of patients with APL, 69–100% were found to have some prolongation of the QT_c interval (Ohnishi *et al*, 2000; Barbey *et al*, 2003; Unnikrishnan *et al*, 2004). Other groups have reported complete atrioventricular block and torsade de pointes, and sudden death (Huang *et al*, 1999; Westervelt *et al*, 2001). In this MAC study, only two (3%) patients had single occurrences of prolonged QT_c interval that necessitated a brief delay in their ATO administration. These results suggest that the addition of melphalan and AA to ATO in the MAC regimen does not increase the risk of cardiac events. A similar low incidence of cardiac events was reported in another study (Choueiri *et al*, 2005) in which ATO was given at 0·1–0·25 mg/kg/d twice weekly for 11 weeks, when electrolytes and concomitant medications were closely monitored.

The absence of glucocorticoids in the MAC regimen is another advantage. Many patients with relapsed or refractory disease are resistant to glucocorticoid-containing regimens and tolerate them poorly at the time of disease progression. In addition, this steroid-free regimen is suitable for many elderly patients with multiple co-morbid conditions that preclude the use of steroids. The high response rate and low toxicity profile with a prolonged progression-free survival produced by MAC combination therapy gives patients who experienced treatment failure after a wide variety of prior treatments another effective therapeutic option that allows them to be treated without negatively impacting their quality of life.

The results of this phase II study showed that the MAC regimen is an effective and well-tolerated treatment for heavily pretreated patients with relapsed or refractory MM. Further studies are warranted to assess efficacy and overall survival with the MAC combination regimen in comparison with other regimens that are currently considered as standard care for this patient population.

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