bjh research paper

Perifosine plus lenalidomide and dexamethasone in relapsed and relapsed/refractory multiple myeloma: a Phase I Multiple Myeloma Research Consortium study†

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†Previous presentations are given in Appendix I.

Summary

The combination of lenalidomide–dexamethasone is active in multiple myeloma (MM). Preclinical data showed that the Akt inhibitor, perifosine, sensitized MM cells to lenalidomide and dexamethasone, providing the rationale for this Phase I, multicentre, single-arm study to assess the safety and determine the maximum-tolerated dose (MTD) of perifosine–lenalidomide–dexamethasone in relapsed and relapsed/refractory MM. Patients received escalating doses of perifosine 50–100 mg daily and lenalidomide 15–25 mg once daily on days 1–21 of each 28-d cycle, plus dexamethasone 20–40 mg weekly thereafter, as indicated. Thirty-two patients were enrolled across four dose cohorts. MTD was not reached, with 31 patients evaluable for safety/tolerability. The most common all-causality grade 1-2 adverse events were fatigue (48%) and diarrhoea (45%), and grade 3–4 neutropenia (26%), hypophosphataemia (23%), thrombocytopenia (16%), and leucopenia (13%). Among 30 evaluable patients, 73% (95% confidence interval, 57·5–89·2%) achieved a minimal response or better, including 50% with a partial response or better. Median progression-free survival was 10·8 months and median overall survival 30·6 months. Response was associated with phospho-Akt in pharmacodynamic studies. Perifosine–lenalidomide–dexamethasone was well tolerated and demonstrated encouraging clinical activity in relapsed and relapsed/refractory MM.

Keywords: perifosine, lenalidomide, dexamethasone, relapsed multiple myeloma, Akt.

Novel agents, such as thalidomide, bortezomib and lenalidomide, used as single agents or in combination, have improved the clinical outlook for patients with relapsed and/or refractory multiple myeloma (MM) (Richardson *et al*, 2005, 2007a; Dimopoulos *et al*, 2007; Kropff *et al*, 2007; Orlowski *et al*, 2007; Weber *et al*, 2007; Kumar *et al*, 2008; Palumbo *et al*, 2008; Knop *et al*, 2009; Laubach *et al*, 2009). However, patients who relapse following therapy with these agents tend to have a particularly poor prognosis; therefore, additional classes of novel agents are required to improve patient outcome and survival (Kumar *et al*, 2009).

There is a rationale for inhibiting the Akt signalling pathway in patients with MM, as it promotes cell survival and proliferation, and mediates MM cell resistance to conventional therapeutics (Hideshima *et al*, 2001, 2004). Perifosine (KRX-0401; Keryx Biopharmaceuticals, Inc., NY, USA) is a novel, oral signal transduction modulator with multiple pathway effects, including Akt inhibition and activation of c-Jun N-terminal kinase (Hideshima *et al*, 2006). Preclinical data have shown that perifosine inhibits phosphorylation of Akt, induces cytotoxicity, and increases dexamethasone-, doxorubicin-, melphalan-, and

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bortezomib-induced cytotoxicity in MM cells (Hideshima *et al*, 2006).

In Phase II studies of patients with relapsed/refractory MM, perifosine in combination with dexamethasone, or dexamethasone plus bortezomib, has demonstrated acceptable tolerability and promising clinical activity, suggesting that it may augment the efficacy of established treatment regimens (Richardson *et al*, 2007b, 2008, 2011). This possibility is currently being assessed in a Phase III trial comparing perifosine–bortezomib–dexamethasone versus bortezomib–dexamethasone in relapsed/refractory patients previously treated with bortezomib (www.clinicaltrials.gov NCT01002248).

Lenalidomide–dexamethasone is another treatment regimen in patients with relapsed/refractory MM that could potentially be augmented with the addition of perifosine. Preclinical studies have provided the rationale for combining lenalidomide with phosphatidylinositol 3-kinase/Akt pathway inhibitors (Raje *et al*, 2004; Shi *et al*, 2005). Furthermore, *in vitro* experiments in MM cell lines suggest that perifosine may increase the cytotoxicity of lenalidomide–dexamethasone (Jakubowiak *et al*, 2007).

In the preclinical portion of this study, we assessed the impact of perifosine–lenalidomide–dexamethasone on cell growth in MM cells (see Appendix S1, Table SI, and Fig S1). Based on these analyses, a Phase I clinical study of the 3-drug combination was conducted by the Multiple Myeloma Research Consortium (MMRC; NCT00415064) in patients with relapsed or relapsed/refractory MM (Jakubowiak *et al*, 2008). The primary objectives of the trial were to determine the safety, maximum tolerated dose (MTD), and response rate.

Methods

Patients

Eligible patients were $\geq$ 18 years old with relapsed or relapsed/refractory, measurable MM that required a second- or third-line of therapy. Patients with refractory disease were defined as progressing on treatment or within 60 d of last treatment; patients refractory to thalidomide or thalidomide–dexamethasone were eligible. Patients who had been treated previously with either lenalidomide or dexamethasone were also eligible, unless they were refractory to lenalidomide–dexamethasone. Additional eligibility criteria included an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, adequate liver function (defined as aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase $\leq$3 times upper limit of normal, and bilirubin $\leq$ upper limit of normal) and renal function (serum creatinine $\leq$51·3 μmol/l), an absolute neutrophil count $\geq$ 1·0 $\times$ 10^9/l, and a platelet count $\geq$75 $\times$ 10^9/l within 14 d prior to enrolment.

Study design and treatment

This Phase I, multicentre, single-arm, open-label study was conducted at six MMRC centres in the USA. Patients were enrolled between December 2006 and June 2008. The study was approved by local review boards, and was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice (World Medical Association, 2009). All patients provided written informed consent prior to participation.

Patients received oral perifosine once daily with food in the evening/at bedtime, oral lenalidomide on days 1–21, and oral dexamethasone in 28-d cycles. At the end of cycle 8, a full disease assessment was performed. Patients who responded or had stable disease were permitted to continue treatment until disease progression or unacceptable toxicity.

There were four dose levels in the dose-escalation Phase of the study. Dose level 1: perifosine 50 mg/d; lenalidomide 15 mg/d; dexamethasone 20 mg/d on days 1–4, 9–12, and 17–20 for the first four cycles, and 20 mg/d on days 1–4 of subsequent cycles. Dose level 2: as level 1, except the lenalidomide dose was 25 mg/d. Dose level 3: perifosine 100 mg/d; lenalidomide 15 mg/d; dexamethasone 40 mg once weekly for the first four cycles and 20 mg per week for subsequent cycles. Dose level 4: as level 3, except the lenalidomide dose was 25 mg/d.

Six patients were planned to be enrolled at each dose level and monitored for one cycle for dose-limiting toxicities (DLTs). Once the MTD had been determined, an additional six patients would be treated at that dose. In August 2007, after the first 12 patients were enrolled (at dose levels 1 and 2), the protocol was amended to limit dexamethasone-related adverse events (AEs) and followed a low-dose dexamethasone regimen (40 mg weekly for cycle 1–4 and 20 mg weekly for subsequent cycles), as described previously (Rajkumar *et al*, 2010a).

A DLT was defined as a grade $\geq$3 non-haematological toxicity, grade 4 haematological toxicity (thrombocytopenia with platelet count $<25 \times 10^9$/l on more than one occasion despite transfusion support, or grade 4 neutropenia lasting >5 d and/or resulting in neutropenic fever), or an inability to receive the day 1 dose in cycle 2 due to toxicity. The MTD was defined as the dose level prior to that resulting in a DLT, i.e. the dose level at which no more than one out of up to six patients experienced a DLT.

After completion of cycle 1, dose modifications were permitted based on investigator assessment of AEs. For persistent grade 2 AEs, perifosine could be reduced from 100 mg/d to 50 mg/d, or from 50 mg/d to 50 mg every other day. For patients on lenalidomide 25 mg, the dose could be reduced to 15 mg, with further 5 mg decrements to 5 mg/d, if required. Dose adjustments of dexamethasone were at the discretion of the treating investigator.

Study objectives

The primary objectives of this study were to determine the safety and the MTD of perifosine in combination with lenalidomide and dexamethasone, and also the response rate of perifosine in combination with lenalidomide and dexamethasone, in patients with relapsed and relapsed/refractory MM. The secondary objective was to observe the duration of response of perifosine in combination with lenalidomide and dexamethasone, in patients with relapsed and relapsed/refractory MM.

Safety, toxicity, and efficacy assessment

Toxicities were monitored by the investigators throughout the trial and for up to 30 d after the last administration of study medication. AEs were graded according to National Cancer Institute Common Terminology for Adverse Events, Version 3.0 (National Cancer Institute, 2006).

Efficacy was assessed by investigators according to the modified European Group for Blood and Marrow Transplant response criteria (Bladé *et al*, 1998). Response, defined as at least minimal response (MR), was assessed after two cycles and every cycle thereafter, and was classified according to the International Uniform Response Criteria (IURC) (Durie *et al*, 2006) with an addition of MR and near complete response (nCR). Assessments of response, relapse, and progression were based on measurement of serum/urine M-protein levels (at baseline and on day 1 of each cycle), bone marrow evaluation, skeletal survey, and, if applicable, assessment of plasmacytoma.

Determination of phospho-Akt

Bone marrow core biopsies or aspirates were collected before and after the first cycle from consenting patients and immunohistochemically stained with an anti-phospho-Akt antibody (Ser473; Cell Signaling Technologies, Danvers, MA). The percentage of neoplastic plasma cells [identified with an anti-CD138 antibody (Cell Marque Corporation, Rocklin, CA) and histopathological features] positive for phospho-Akt was assessed by a haematopathologist in a blinded fashion. High and low phospho-Akt-positive staining was defined as >40% and $\leq$40% of stained plasma cells, respectively.

Statistical analysis

Safety was analysed in all patients who received at least one dose of study medication. Observed response rates were reported as percentages with 95% confidence intervals (CIs). Progression-free survival (PFS) and overall survival (OS), which were measured from the time of treatment initiation to event, disease progression or death, were analysed using log-rank tests. The Kaplan-Meier method was used to estimate median times for PFS and OS, and the corresponding 95% CIs. For these survival analyses, data were censored as of the cut-off date on 10 July 2011. The minimal level of significance was $P \leq 0{\cdot}05$.

Results

Patient characteristics and disposition

Of 32 patients enrolled, 30 were evaluable for efficacy, and 31 for safety and tolerability. Baseline patient characteristics are listed in Table I. Of evaluable patients, eight were thalidomide-naïve and 22 had received prior thalidomide, of whom 13 (59%) were refractory to thalidomide. In addition, two patients had received prior lenalidomide, and 14 patients had received prior bortezomib, of whom 6 (43%) had progressed on bortezomib. Of these six patients, four had progressed on bortezomib as the last line of therapy prior to this study. Eight patients (27%) who otherwise met all other eligibility criteria had three or four prior lines of therapy, based on exceptions granted by the investigators.

Six patients received dose level 1, six received dose level 2, eight received dose level 3 (two of 8 patients did not complete cycle 1 due to non-compliance and a non-treatment-related splenic aneurysm, respectively, and were not included in the MTD assessment or the efficacy analysis), and six received dose level 4. The median number of cycles received was 5·5

Table I. Patient characteristics and disposition at baseline.

Characteristic	Patients (N = 32)
Male, *n* (%)	17 (53)
Median age, years (range)	64 (37–79)
Patient status, *n* (%)	
Relapsed	17 (53)
Relapsed/refractory	15 (47)
Multiple myeloma type, *n* (%)	
IgA	10 (31)
IgG	21 (66)
Kappa LC	1 (3)
Performance status, *n* (%)	
0	11 (34)
1	16 (50)
2	5 (16)
Median lines of therapy, *n* (range)	2 (1–4)
Received $\geq$ 3 lines of therapy, *n* (%)	8 (27)
Prior therapy, *n* (%)	
Thalidomide/dexamethasone*	24 (75)
Dexamethasone	30 (94)
Bortezomib	14 (44)
Autologous stem cell transplantation	23 (72)
Lenalidomide	2 (6)
VAD	8 (25)

Ig, immunoglobulin; VAD, vincristine, doxorubicin, dexamethasone.
*Of these patients, 15 (63%) were refractory to thalidomide/dexamethasone (47% of all patients); two of these patients were non-evaluable for efficacy.

(range, 1–37). There were no DLTs at dose levels 1 and 2, one DLT at dose level 3 (grade 3 nausea), and no DLTs at dose level 4.The MTD was therefore not reached. However, based on drug tolerability and cumulative toxicity beyond cycle 1, plus emerging experience from other perifosine studies (Richardson *et al*, 2011), the maximum planned dose level 4 was used for an extension cohort of 12 patients in total.

Safety and tolerability

The most frequent all causality grade 1-2 AEs were fatigue (48%), diarrhoea (45%), hyperglycaemia and nausea (32% each). The most common grade 3–4 haematological AEs were neutropenia (25%), thrombocytopenia (16%) leucopenia (13%), and lymphopenia (10%). The most common grade 3–4 non-haematological AEs were hypophosphataemia (23%), arthralgia (10%), and hyperglycaemia (10%). No grade 3–4 peripheral neuropathy or deep vein thrombosis were reported (Table II).

AEs were manageable with supportive care, dose reductions, or interruptions. Perifosine dose was reduced in nine patients: one at dose level 3 due to rash; eight at dose level 4 due to diarrhoea (three patients), nausea (two patients), hyperglycaemia, upper respiratory infection, and anaemia (one patient each). Lenalidomide dose was reduced in 11 patients: one at dose level 1 due to thrombocytopenia; two at dose level 2 due to thrombocytopenia and hypophosphataemia, respectively; eight at dose level 4 due to thrombocytopenia (three patients), hypophosphataemia (three patients), neutropenia, and anaemia (one patient each). Dexamethasone dose was reduced in seven patients: one at dose level 1 due to psychological changes; one at dose level 2 due to difficulty sleeping; five at dose level 4 due to dizziness (two patients), muscle pain (two patients), and abdominal bloating (one patient). Two patients discontinued treatment after completion of cycle 1: one at dose level 3 due to grade 3 nausea; one at dose level 4 due to persistent lenalidomide-related cytopenia. There were no treatment-related deaths.

Efficacy

Overall, 22 (73%) evaluable patients achieved at least a MR; at least partial response (PR) rate [nCR/CR + very good partial response (VGPR) + PR] was 50% (Table III), including seven patients (23%) achieving a VGPR or better. The median time to first response was 1 cycle (range, 1–3 cycles); the median duration of response was 9·2 months (range, 2–35 months). In the subset of patients with relapsed but not refractory disease ($N = 17$), at least PR was 71% and at least MR was 82%; and in patients with refractory disease ($N = 13$) at least PR was 23% and at least MR was 62%. Among the thalidomide-exposed patients ($N = 22$), PR or better was 45%, 78% in the thalidomide-relapsed patients (7 of 9), and 23% in the thalidomide-refractory patients (3 of 13). In the small subset of bortezomib-refractory patients,

Table II. Summary of the most common all-causality adverse events*.

Adverse event ($n = 31$)	Grade 1 or 2, n (%) >20% of patients	Grade 3 or 4, n (%) $\geq$10% of patients
Haematological		
Neutropenia	–	8 (26)
Leucopenia	–	4 (13)
Lymphopenia	–	3 (10)
Thrombocytopenia	9 (29)	5 (16)
Anaemia	8 (26)	3 (10)
Non-haematological		
Hypophosphataemia	–	7 (23)
Arthralgia	–	3 (10)
Fatigue	15 (48)	–
Hyperglycaemia	10 (32)	3 (10)
Back pain	–	3 (10)
Peripheral oedema	7 (23)	–
Diarrhoea	14 (45)	–
Nausea	10 (32)	–
Vomiting	9 (29)	–
Constipation	9 (29)	–
Elevated ALT†	9 (29)	–
Elevated blood urea‡	8 (26)	–
Elevated AST†	8 (26)	–
Rash	7 (23)	–
Dyspnea	9 (29)	–
Muscle spasms	8 (26)	–
Cough	9 (29)	–
Pain	9 (29)	–
Upper respiratory tract infection	11 (35)	–

ALT, alanine aminotransferase; AST, aspartate aminotransferase.
*According to the National Cancer Institute Common Terminology for Adverse Events, Version 3.0. (National Cancer Institute 2006).
†>3× the upper limit of normal range.
‡Greater than the upper limit of normal range.

Table III. Summary of clinical response, according to the modified European Group for Blood and Bone Marrow Transplant criteria (Bladé *et al*, 1998).

Response ($n = 30$)	n (%)	Duration, weeks (range)
nCR	4 (13)	117+, 115+, 114, 24
VGPR	3 (10)	141, 34, 17
PR	8 (27)	34 (11–112)
$\geq$PR	15 (50)	43 (11–141)
MR	7 (23)	41 (9–114)
$\geq$ MR	22 (73)	45 (9–141)
SD*	6 (20)	14 (8–19)
PD	2 (7)	8, 4

nCR, near complete response; VGPR, very good partial response; PR, partial response; MR, minimal response; SD, stable disease; PD, progressive disease; ORR, overall response rate.
*SD defined as <25% reduction in M-protein.

two of six patients achieved PR or better, and four achieved MR or better.

Survival analysis

After a median follow up of 30 months (range, 3·4–51·3 months), median PFS was 10·8 months in all evaluable patients and 11·7 months in patients who achieved a MR or better (Fig 1A). Median OS was 30·6 months in all evaluable patients and was not reached in patients who achieved at least a MR or better (Fig 1B). As of 10 July 2011, 15 of the 30 evaluable patients were still alive, and seven patients had not progressed. For those patients who achieved a MR or better, nine patients had died and 17 patients had progressed. Patients with relapsed but not refractory disease had longer median PFS and OS than patients with refractory disease (27·7 vs. 3·9 months, $P = 0·0002$; not reached versus 16·7 months, $P = 0·0006$; Fig 2).

Among the 22 thalidomide-exposed patients, of which 59% were refractory to both thalidomide and dexamethasone, median PFS and OS were 5·2 and 17·2 months, respectively. Median PFS was significantly higher in thalidomide-relapsed patients than in thalidomide-refractory patients (12·9 vs. 3·9 months; $P = 0·0145$; Fig 3A). Median OS was not reached in thalidomide-relapsed patients and was 16·7 months in thalidomide-refractory patients ($P = 0·0568$; Fig 3B). As of July 10, 2011, 15 patients who had received prior thalidomide had died, and 19 had progressed.

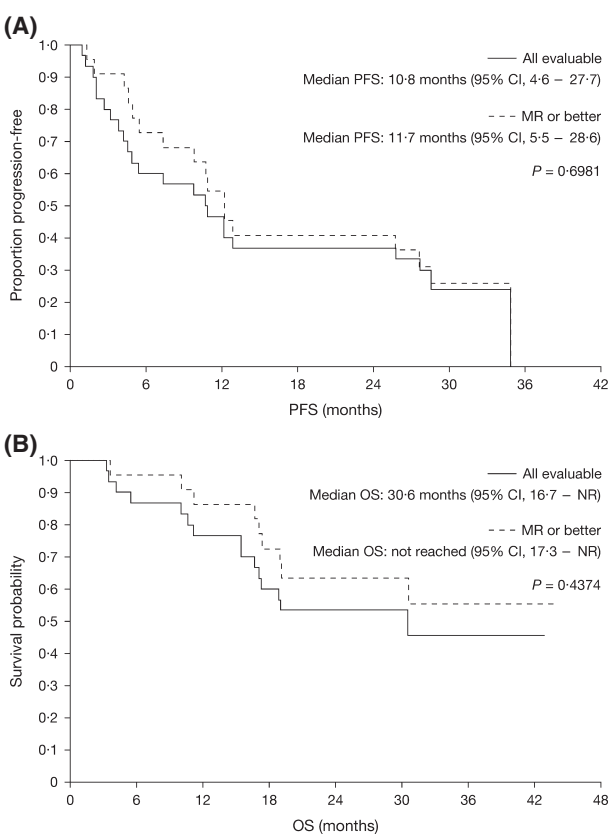

Fig 1. Kaplan–Meier plot of PFS (A) and OS (B) in all evaluable patients ($n = 30$) and patients who achieved $\geq$MR ($n = 22$). Abbreviations: CI, confidence interval; MR, minimal response; NR, not reached; OS, overall survival; PFS, progression-free survival.

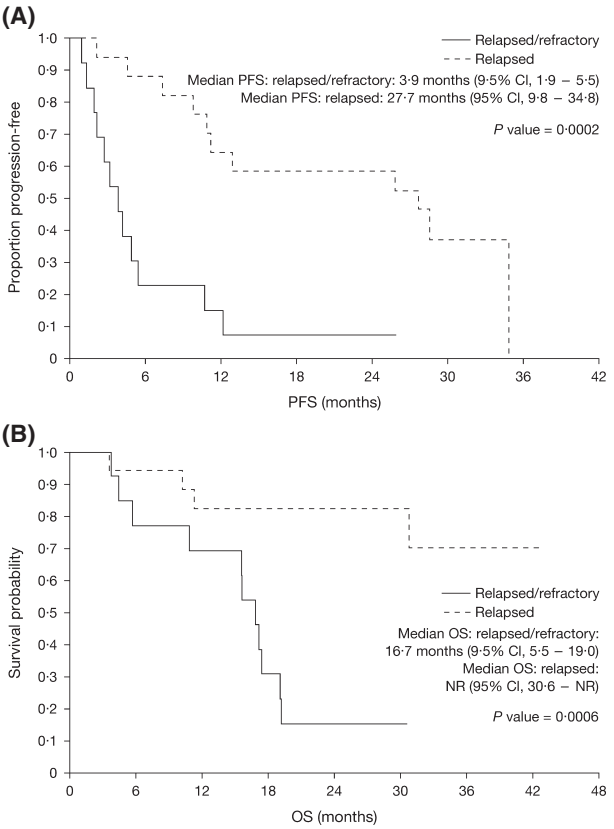

Fig 2. Kaplan–Meier plot of PFS (A) and OS (B) in relapsed but non-refractory patients ($n = 17$) and relapsed/refractory patients ($n = 13$). Abbreviations: CI, confidence interval; NR, not reached; OS, overall survival; PFS, progression-free survival.

Assessment of phospho-Akt

In exploratory analysis, baseline bone marrow phospho-Akt immunostaining was assessed in 13 patients, 11 were positive for phospho-Akt (range of 10–90% plasma cells; Fig S2A–D). PFS was longer in patients with high immunostaining of phospho-Akt than those with low staining (25 vs. 5 months, respectively, $P = 0·17$; Fig S2E).

Phospho-Akt immunostaining postcycle 1 was also assessed in seven of the 13 patients. Three of these seven patients achieved at least PR, which was associated with a change from baseline positive phospho-Akt to either not detectable or decreased immunostaining. In the four other patients, there was no detectable change in phospho-Akt staining; none achieved a PR or better (data not shown).

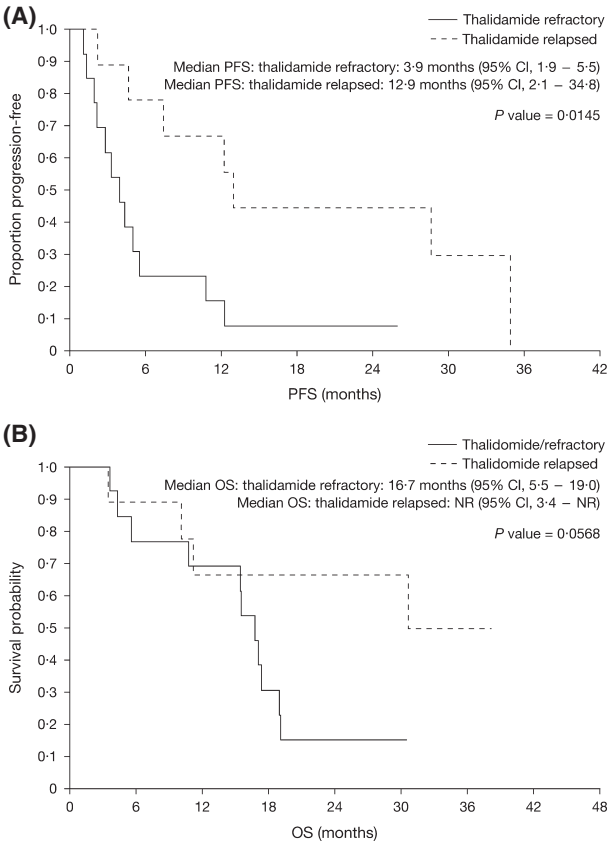

Fig 3. Kaplan–Meier plot of PFS (A) and OS (B) in thalidomide-relapsed ($n = 9$) and thalidomide-refractory patients ($n = 13$). Abbreviations: CI, confidence interval; NR, not reached; OS, overall survival; PFS, progression-free survival.

Discussion

This is the first clinical trial to assess perifosine–lenalidomide–dexamethasone in patients with relapsed or relapsed/refractory MM. The combination was well tolerated with manageable AEs, and demonstrated encouraging and durable antitumour activity in this patient population, which included 47% patients with relapsed/refractory disease.

Perifosine, the first in class Akt inhibitor, has emerged as a promising new drug in the search for new anti-myeloma therapies, beyond proteasome inhibitors and immunomodulatory drugs. Encouraging results achieved with perifosine plus bortezomib–dexamethasone (Richardson *et al*, 2011) in relapsed/refractory MM, has led to the development of an ongoing Phase III trial with this 3-drug combination (www.clinicaltrials.gov NCT01002248). The current study was based on a rationale that perifosine may enhance the activity of lenalidomide and dexamethasone. This 3-drug combination may also provide a more patient-friendly all-oral drug regimen in MM. In this context, establishing that the regimen is well tolerated, with no overlapping toxicities was important. DLTs were limited and the MTD was not reached at maximum planned doses. Extended use was associated with gastrointestinal toxicities and fatigue, which did lead to dose attenuations in later treatment cycles. However, these toxicities were easily manageable and patients who responded tolerated the treatment well for extended periods of time, meeting the objectives of a patient-friendly all-oral regimen.

Is this 3-drug regimen also fulfilling a promise of being more efficacious than the 2-drug combination of lenalidomide–dexamethasone? Because of the design of this Phase I study, the evaluation of efficacy was limited to providing preliminary evidence and was not powered to evaluate the impact of adding perifosine to lenalidomide–dexamethasone in terms of efficacy and duration of response. In addition, comparison with historical data on lenalidomide–dexamethasone in relapsed MM (MM-009 and MM-010), is limited (Dimopoulos *et al*, 2007; Weber *et al*, 2007). The patient population in these studies included mainly relapsed rather than refractory disease, and dexamethasone was used at higher doses, which may be associated with greater efficacy (Rajkumar *et al*, 2010b), whereas the patient population enrolled into the current study was more pretreated and included a significant proportion (47%) of refractory patients. Therefore, an observed response rate of at least PR or better of 50% plus time-to-event data, PFS (10·8 months) and OS (30·6 months) are encouraging. A subset analysis of non-refractory patients enrolled into our study, more comparable to the patient population enrolled into MM-009 and MM-010 studies, indicated a PR or better rate of 70%, and PFS was 27·7 months, which appears improved when compared to historical data with lenalidomide–dexamethasone (Dimopoulos *et al*, 2007; Weber *et al*, 2007). Efficacy and survival data, in the current study, for those patients previously treated with thalidomide are also encouraging, even though a high proportion (59%) of patients were refractory to thalidomide and dexamethasone, making comparison to historical data difficult (Dimopoulos *et al*, 2007; Weber *et al*, 2007; Kumar *et al*, 2008).

Our exploratory pharmacodynamic study data (see Appendix S1, Table SI, and Fig S1) suggest that the clinical efficacy of perifosine–lenalidomide–dexamethasone is positively associated with phospho-Akt; the activity of the 3-drug combination appeared to be more likely in patients with higher baseline phospho-Akt. Although this observation is based on just a few patients, the correlative data could represent the first steps towards the rational selection of individualized therapy with Akt inhibitors. The data also suggest that perifosine may be particularly effective in patients with Akt-dependent MM, a sub-group of MM (Zollinger *et al*, 2008). Additional studies are ongoing to investigate the potential relationship between perifosine activity and phospho-Akt. Findings may show whether patients with an activated Akt genotype would benefit in particular from the addition of perifosine, therefore raising the possibility of individualized therapy according to a patient's phospho-Akt status.

Other Phase I/II studies have investigated whether the addition of a third agent, such as doxorubicin (Knop *et al*, 2009), vorinostat (Siegel *et al*, 2009), or cyclophosphamide (Schey *et al*, 2010), can enhance the activity of lenalidomide–dexamethasone in patients with relapsed and/or refractory MM. As with the current study, it has been difficult to demonstrate improved efficacy and survival of the 3-drug combinations over the lenalidomide–dexamethasone regimen, due to a lack of comparable patient populations. Therefore, trials will be required to directly compare emerging 3-drug combinations in patients with relapsed and relapsed/refractory disease.

In conclusion, perifosine–lenalidomide–dexamethasone shows acceptable tolerability and encouraging clinical activity in patients with relapsed or relapsed/refractory MM, considering that close to 50% of the enrolled patients were refractory to prior treatments. While the study was neither designed nor powered to assess an impact of an addition of perifosine on efficacy data, the combined clinical and correlative data appear to suggest that adding perifosine to lenalidomide–dexamethasone may provide potential for added clinical benefit in this setting (Jakubowiak *et al*, 2007). Based on these findings, further clinical evaluation, including a head-to-head study of perifosine–lenalidomide–dexamethasone versus lenalidomide–dexamethasone, and the evaluation of an impact of pretreatment Akt expression and number of prior therapies is warranted and may complement the ongoing randomized Phase lll study evaluating perifosine with bortezomib and dexamethasone (www.clinicaltrials.gov NCT01002248).

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Authorship contribution

This study was designed as a collaborative effort within the MMRC by the primary investigator (Andrzej Jakubowiak, AJJ), co-investigators and the sponsor, Keryx Biopharmaceuticals, Inc. Statistical analysis was provided by the sponsor. All authors had full access to the primary data, and the final analysis. AJJ, PGR, PS, and KCA designed the research; AJJ, PGR, TZ, MA, JK, MK, SK, CWR, and TH performed the research; AJJ, PGR, TZ, MA, and JK enrolled patients; AJJ, CH, PS, and LG collected patient data; MK, SK, CWR, and TH performed the laboratory experiments; AJJ, PGR, MK, TH, PS, and KCA analysed and interpreted the data; MK and Michael Chen performed the statistical analysis; and AJJ, PGR, MK, PS, and KCA wrote the manuscript.

Conflicts of interest

Employment: PS (Keryx), EP (Keryx), LG (Keryx)

Consultant or advisory role: AJJ (Millennium, Onyx, Bristol-Myers Squibb), PGR (Millennium, Celgene, Bristol-Myers Squibb, Johnson & Johnson, Novartis), TZ (Celgene), MA (Millennium), JLK (Millennium, Celgene, Keryx, Novartis, Onyx), TH (Acetylon), KCA (Millennium, Celgene, Merck, Novartis, Onyx, Bristol-Myers Squibb)

Stock ownership: PS (Keryx), EP (Keryx), LG (Keryx), KCA (Acetylon)

Honoraria: AJJ (Ortho-Biotech, Celgene, Millennium, Bristol-Myers Squibb, Exelixis), TZ (Celgene), MA (Millennium)

Research funding: MA (Celgene, Millennium, Ortho-Biotech), JLK (Merck, Celgene), KG No conflicts of interest declared

Patents: EP (Keryx)

Other renumeration: AJJ (speakers' bureau Celgene, Millennium, Ortho-Biotech), TZ (speakers' bureau Millennium)

The remaining authors declare no competing financial interests.

Appendix I

Previous presentations: This work was presented in part at the following meetings: American Society of Hematology Annual Meeting, Atlanta, GA, December 8–11, 2007; Jakubowiak A, Zimmerman T, Alsina M, *et al* A Multiple Myeloma Research Consortium (MMRC) multicentre Phase I trial of perifosine (KRX-0401) in combination with lenalidomide and dexamethasone in patients with relapsed/refractory multiple myeloma (MM): updated results. *Blood (ASH Annual Meeting Abstracts)* 110; 2007 (abstract 1169).

American Society of Hematology Annual Meeting, San Francisco, CA, December 6–9, 2008; Jakubowiak A, Richardson P, Zimmerman TM, *et al* Phase I results of perifosine (KRX-0401) in combination with lenalidomide and dexamethasone in patients with relapsed or refractory multiple myeloma (MM). *Blood (ASH Annual Meeting Abstracts)* 112; 2008 (abstract 3691). American Society of Hematology annual meeting, Orlando, FL, December 4–7, 2010; Jakubowiak A, Richardson PG, Zimmerman T, *et al* Final Phase I results of perifosine in combination with lenalidomide and dexamethasone in patients with relapsed or refractory multiple myeloma (MM). *Blood (ASH Annual Meeting Abstracts)*116: 2010 (abstract 3064).

XIth International Myeloma Workshop, Kos, Greece, June 25–30, 2007; Jakubowiak A, Richardson P, Zimmerman T, *et al* A Phase I trial of perifosine (KRX-0401) in combination with lenalidomide and dexamethasone in patients with relapsed or refractory multiple myeloma: preliminary results. Multiple Myeloma Research Consortium (MMRC) trial. *Hematologica* 92(Suppl 2): 154-155, 2007 (abstract PO-606). XIIth International Myeloma Workshop, Washington, DC, February 26–March 1, 2009; Jakubowiak A, Richardson PG, Zimmerman T, *et al* Phase I trial of perifosine, lenalidomide and dexamethasone in relapsed or refractory myeloma. *Clin Lymphoma Myeloma* 9(Suppl 1):S56-57, 2009 (abstract A347).

Supporting Information

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

Fig S1. Cultured NCI-H929 cell survival in the MTT assay following incubation for 72 h with perifosine–lenalidomide–dexamethasone (at concentrations of 2.5 μmol/l, 500 nmol/l, and 20 nmol/l, respectively), lenalidomide–dexamethasone, or perifosine alone.

Fig S2. Phospho-Akt immunostaining in bone marrow samples. Representative samples of immunohistochemical staining for CD138 and phospho-Akt in bone marrow core biopsies of patients with high or low phospho-Akt are shown (A–D).

Table S1. Combination indices (according to CalcuSyn analysis) derived from MTT cell survival assays with cultured NCI-H929 MM cells following incubation for 72 hours with increasing concentrations of perifosine–lenalidomide–dexamethasone.

Appendix S1. Growth-inhibition assay.

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References

Bladé, J., Samson, D., Reece, D., Apperley, J., Bjorkstrand, B., Gahrton, G., Gertz, M., Giralt, S., Jagannath, S. & Vesole, D. (1998) Criteria for evaluating disease response and progression in patients with multiple myeloma treated by high-dose therapy and haemopoietic stem cell transplantation. Myeloma Subcommittee of the EBMT. European Group for Blood and Marrow Transplant. *Br J Haematol*, **102**, 1115–1123.

Dimopoulos, M., Spencer, A., Attal, M., Prince, H. M., Harousseau, J.L., Dmoszynska, A., San, M.J., Hellmann, A., Facon, T., Foa, R., Corso, A., Masliak, Z., Olesnyckyj, M., Yu, Z., Patin, J., Zeldis, J.B. & Knight, R.D. (2007) Lenalidomide plus dexamethasone for relapsed or refractory multiple myeloma. *New England Journal of Medicine*, **357**, 2123–2132.

Durie, B.G., Harousseau, J.L., Miguel, J.S., Blade, J., Barlogie, B., Anderson, K., Gertz, M., Dimopoulos, M., Westin, J., Sonneveld, P., Ludwig, H., Gahrton, G., Beksac, M., Crowley, J., Belch, A., Boccadaro, M., Turesson, I., Joshua, D., Vesole, D., Kyle, R., Alexanian, R., Tricot, G., Attal, M., Merlini, G., Powles, R., Richardson, P., Shimizu, K., Tosi, P., Morgan, G. & Rajkumar, S.V. (2006) International uniform response criteria for multiple myeloma. *Leukemia*, **20**, 1467–1473.

Hideshima, T., Nakamura, N., Chauhan, D. & Anderson, K.C. (2001) Biologic sequelae of interleukin-6 induced PI3-K/Akt signaling in multiple myeloma. *Oncogene*, **20**, 5991–6000.

Hideshima, T., Bergsagel, P.L., Kuehl, W.M. & Anderson, K.C. (2004) Advances in biology of multiple myeloma: clinical applications. *Blood*, **104**, 607–618.

Hideshima, T., Catley, L., Yasui, H., Ishitsuka, K., Raje, N., Mitsiades, C., Podar, K., Munshi, N.C., Chauhan, D., Richardson, P.G. & Anderson, K. C. (2006) Perifosine, an oral bioactive novel alkylphospholipid, inhibits Akt and induces in vitro and in vivo cytotoxicity in human multiple myeloma cells. *Blood*, **107**, 4053–4062.

Jakubowiak, A., Zimmerman, T., Alsina, M., Richardson, P., Kaufman, J., Kendall, T., Brozo, C., McAllister, A., Leister, C., Hideshima, T., Sportelli, P., Gardner, L., Birch, R., Henderson, I.C., Giusti, K. & Anderson, K. (2007) A Multiple Myeloma Research Consortium (MMRC) multicenter Phase I trial of perifosine (KRX-0401) in combination with lenalidomide and dexamethasone in patients with relapsed/refractory multiple myeloma (MM): updated results. *Blood (ASH Annual Meeting Abstracts)*, **110**, Abstract 1169.

Jakubowiak, A., Richardson, P.G., Zimmerman, T., Alsina, M., Kaufman, J., Harvey, C., Brozo, C., Kendall, T., McAllister, A., Hideshima, T., Sportelli, P., Gardner, L., Anderson, K. & Giusti, K. (2008) Phase I results of perifosine (KRX-0401) in combination with lenalidomide and dexamethasone in patients with relapsed or refractory multiple myeloma (MM). *Blood (ASH Annual Meeting Abstracts)*, **112**, Abstract 3691.

Knop, S., Gerecke, C., Liebisch, P., Topp, M.S., Platzbecker, U., Sezer, O., Vollmuth, C., Falk, K., Glasmacher, A., Maeder, U., Einsele, H. & Bargou, R.C. (2009) Lenalidomide, adriamycin, and dexamethasone (RAD) in patients with relapsed and refractory multiple myeloma: a report from the German Myeloma Study Group DSMM (Deutsche Studiengruppe Multiples Myelom). *Blood*, **113**, 4137–4143.

Kropff, M., Bisping, G., Schuck, E., Liebisch, P., Lang, N., Hentrich, M., Dechow, T., Kroger, N., Salwender, H., Metzner, B., Sezer, O., Engelhardt, M., Wolf, H.H., Einsele, H., Volpert, S., Heinecke, A., Berdel, W.E. & Kienast, J. (2007) Bortezomib in combination with intermediate-dose dexamethasone and continuous low-dose oral cyclophosphamide for relapsed multiple myeloma. *British Journal of Haematology*, **138**, 330–337.

Kumar, S.K., Rajkumar, S.V., Dispenzieri, A., Lacy, M.Q., Hayman, S.R., Buadi, F.K., Zeldenrust, S. R., Dingli, D., Russell, S.J., Lust, J.A., Greipp, P. R., Kyle, R.A. & Gertz, M.A. (2008) Improved survival in multiple myeloma and the impact of novel therapies. *Blood*, **111**, 2516–2520.

Kumar, S., Blade, J., Crowley, J., Goldschmidt, H., Hoering, A., Jagannath, S., Lahuerta, J.J., Laubach, J., Moreau, P., Morgan, G., Orlowski, R. Z., Palumbo, A., Richardson, P.G., San Miguel, J., Sezer, O., Siegel, D., Sonneveld, P., Szymonifka, J., Rajkumar, S.V. & Durie, B.G. (2009) Natural history of multiple myeloma relapsing after therapy with IMiDs and bortezomib: a multicenter international myeloma working group study. *Blood (ASH Annual Meeting Abstracts)*, **114**, Abstract 2878.

Laubach, J.P., Mahindra, A., Mitsiades, C.S., Schlossman, R.L., Munshi, N.C., Ghobrial, I.M., Carreau, N., Hideshima, T., Anderson, K.C. & Richardson, P.G. (2009) The use of novel agents in the treatment of relapsed and refractory multiple myeloma. *Leukemia*, **23**, 2222–2232.

National Cancer Institute. (2006) National Cancer Institute Cancer Therapy Evaluation Program Common Terminology Criteria for Adverse Events, Version 3.0. http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf,

Orlowski, R.Z., Nagler, A., Sonneveld, P., Blade, J., Hajek, R., Spencer, A., San, M.J., Robak, T., Dmoszynska, A., Horvath, N., Spicka, I., Sutherland, H.J., Suvorov, A.N., Zhuang, S.H., Parekh, T., Xiu, L., Yuan, Z., Rackoff, W. & Harousseau, J.L. (2007) Randomized Phase III study of pegylated liposomal doxorubicin plus bortezomib compared with bortezomib alone in relapsed or refractory multiple myeloma: combination ther-

apy improves time to progression. *Journal of Clinical Oncology*, **25**, 3892–3901.

Palumbo, A., Gay, F., Bringhen, S., Falcone, A., Pescosta, N., Callea, V., Caravita, T., Morabito, F., Magarotto, V., Ruggeri, M., Avonto, I., Musto, P., Cascavilla, N., Bruno, B. & Boccadoro, M. (2008) Bortezomib, doxorubicin and dexamethasone in advanced multiple myeloma. *Annals of Oncology*, **19**, 1160–1165.

Raje, N., Kumar, S., Hideshima, T., Ishitsuka, K., Chauhan, D., Mitsiades, C., Podar, K., Le, G.S., Richardson, P., Munshi, N.C., Stirling, D.I., Antin, J.H. & Anderson, K.C. (2004) Combination of the mTOR inhibitor rapamycin and CC-5013 has synergistic activity in multiple myeloma. *Blood*, **104**, 4188–4193.

Rajkumar, S.V., Fonseca, R., Vesole, D., Jacobus, S., Greipp, P.R., Weiss, M. & Katz, M. (2010a) A Randomized Phase III Study of CC-5013 Plus Dexamethasone Versus CC-5013 Plus low Dose Dexamethasone in Multiple Myeloma With Thalidomide Plus Dexamethasone Salvage Therapy for non-Responders. http://www.ecog.org/cite/E4A03DOC20080806.PDF

Rajkumar, S.V., Jacobus, S., Callander, N.S., Fonseca, R., Vesole, D.H., Williams, M.E., Abonour, R., Siegel, D.S., Katz, M. & Greipp, P.R. (2010b) Lenalidomide plus high-dose dexamethasone versus lenalidomide plus low-dose dexamethasone as initial therapy for newly diagnosed multiple myeloma: an open-label randomised controlled trial. *Lancet Oncology*, **11**, 29–37.

Richardson, P.G., Sonneveld, P., Schuster, M.W., Irwin, D., Stadtmauer, E.A., Facon, T., Harousseau, J.L., Ben-Yehuda, D., Lonial, S., Goldschmidt, H., Reece, D., San-Miguel, J.F., Blade, J., Boccadoro, M., Cavenagh, J., Dalton, W.S., Boral, A.L., Esseltine, D.L., Porter, J.B., Schenkein, D. & Anderson, K.C. (2005) Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. *New England Journal of Medicine*, **352**, 2487–2498.

Richardson, P.G., Mitsiades, C., Schlossman, R., Munshi, N. & Anderson, K. (2007a) New drugs for myeloma. *Oncologist*, **12**, 664–689.

Richardson, P.G., Lonial, S., Jakubowiak, A., Krishnan, A., Wolf, J., Densmore, J., Singhal, S., Ghobrial, I., Stephenson, J., Mehta, J., Colson, K., Francis, D., Kendall, T., Obadike, N., Sullivan, K., Martin, J., Hideshima, T., Lai, L., Sportelli, P., Gardner, L., Birch, R., Henderson, I.C. & Anderson, K. (2007b) Multi-center Phase ll study of perifosine (KRX-0401) alone and in combination with dexamethasone (dex) for patients with relapsed or relapsed/refractory multiple myeloma: promising activity as combination therapy with manageable toxicity. *Blood (ASH Annual Meeting Abstracts)*, **110**, Abstract 1164.

Richardson, P., Wolf, J., Jakubowiak, A., Zonder, J., Lonial, S., Irwin, D.H., Densmore, J., Krishnan, A., Raje, N., Bar, M., Allerton, J., Schlossman, R., Ghobrial, I., Munshi, N., Martin, T., Laubach, J., Colson, K., Dean, S., Tocco, D., Steinfield, E., Kendall, T., O'Riley, K., Hideshima, T., Sportelli, P., Gardner, L. & Anderson, K. (2008) Phase I/II results of a multicenter trial of perifosine (KRX-0401) + bortezomib in patients with relapsed or relapsed/refractory multiple myeloma who were previously relapsed from or refractory to bortezomib. *Blood (ASH Annual Meeting Abstracts)*, **112**, Abstract 870.

Richardson, P.G., Wolf, J., Jakubowiak, A., Zonder, J., Lonial, S., Irwin, D., Densmore, J., Krishnan, A., Raje, N., Bar, M., Martin, T., Schlossman, R., Ghobrial, I.M., Munshi, N., Laubach, J., Allerton, J., Hideshima, T., Colson, K., Poradosu, E., Gardner, L., Sportelli, P. & Anderson, K.C. (2011) Perifosine plus bortezomib and dexamethasone in patients with relapsed/refractory multiple myeloma previously treated with bortezomib: results of a multicenter Phase I/II trial. *Journal of Clinical Oncology*, **29**, 4243–4249.

Schey, S.A., Morgan, G.J., Ramasamy, K., Hazel, B., Ladon, D., Corderoy, S., Jenner, M., Phekoo, K., Boyd, K. & Davies, F.E. (2010) The addition of cyclophosphamide to lenalidomide and dexamethasone in multiply relapsed/refractory myeloma patients; a Phase I/II study. *British Journal of Haematology*, **150**, 326–333.

Shi, Y., Yan, H., Frost, P., Gera, J. & Lichtenstein, A. (2005) Mammalian target of rapamycin inhibitors activate the AKT kinase in multiple myeloma cells by up-regulating the insulin-like growth factor receptor/insulin receptor substrate-1/phosphatidylinositol 3-kinase cascade. *Molecular Cancer Therapeutics*, **4**, 1533–1540.

Siegel, D., Weber, D.M., Mitsiades, C.S., Dimopoulos, M.A., Harousseau, J.-L., Rizvi, S., Howe, J., Reiser, D., Byrne, C., Anderson, K.C. & Richardson, P. (2009) Combined vorinostat, lenalidomide and dexamethasone therapy in patients with relapsed or refractory multiple myeloma: a Phase I study. *Blood (ASH Annual Meeting Abstracts)*, **114**, Abstract 305.

Weber, D.M., Chen, C., Niesvizky, R., Wang, M., Belch, A., Stadtmauer, E.A., Siegel, D., Borrello, I., Rajkumar, S.V., Chanan-Khan, A.A., Lonial, S., Yu, Z., Patin, J., Olesnyckyj, M., Zeldis, J.B. & Knight, R.D. (2007) Lenalidomide plus dexamethasone for relapsed multiple myeloma in North America. *New England Journal of Medicine*, **357**, 2133–2142.

World Medical Association (2009) World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. World Medical Association, Ferney-Voltaire, France.

Zollinger, A., Stuhmer, T., Chatterjee, M., Gattenlohner, S., Haralambieva, E., Muller-Hermelink, H.K., Andrulis, M., Greiner, A., Wesemeier, C., Rath, J.C., Einsele, H. & Bargou, R.C. (2008) Combined functional and molecular analysis of tumor cell signaling defines 2 distinct myeloma subgroups: Akt-dependent and Akt-independent multiple myeloma. *Blood*, **112**, 3403–3411.