Liposomal daunorubicin (DaunoXome) in multiple myeloma: a modified VAD regimen using short-term infusion

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Liposomal daunorubicin replacing conventional anthracyclines may reduce toxicity and enhance efficacy of chemotherapy. In this phase I study, we evaluated liposomal daunorubicin (DaunoXome) in combination with vincristine and dexamethasone for toxicity, pharmacokinetics and potential efficacy in patients with multiple myeloma. The main objective was to determine the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of liposomal daunorubicin combined with vincristine and dexamethasone (VLDD). Additionally, pharmacokinetics were determined at higher dose levels. Seventeen multiple myeloma patients were enrolled in this trial; 76% of the patients had relapsed or refractory multiple myeloma. Successive cohorts received liposomal daunorubicin at doses of 40, 60, 80 and 100 mg/m^2 on day 1 in combination with vincristine 1.4 mg/m^2 (day 1) and oral dexamethasone (40 mg, days 1–4). DLT occurred at 100 mg/m^2. Liposomal daunorubicin at 80 mg/m^2 was well tolerated in this protocol and should be used for further phase II studies with the VLDD regimen. In this phase I trial, 64% of the patients achieved a partial remission or a minor response. ***Anti-Cancer Drugs*** **14:793–799 © 2003 Lippincott Williams & Wilkins.**

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Introduction

Multiple myeloma represents a rapidly expanding research field with important new insights obtained during recent years [1–5]. Nevertheless, this disease remains incurable using conventional treatment with a median survival of about 42 months [6]. There have been continuous attempts to improve the response rates, overall survival and quality of life in myeloma patients [7]. However, more effective treatment options are still urgently needed. The continuous infusion of vincristine and doxorubicin over 96 h in combination with oral dexamethasone (VAD) is one of the most effective treatment options for patients with relapsed or primary refractory multiple myeloma [8]. Furthermore, this regimen has advantages in the treatment of multiple myeloma patients with poor renal function or can be used as an induction therapy in patients eligible for high-dose chemotherapy with autologous stem cell transplantation because of its swift tumor cell kill without inflicting hematopoietic stem cell compromise [9–11]. On the other hand, application of anthracyclines may be limited by their toxicity, especially in elderly patients with pre-existing cardiac disease [12]. The VAD protocol requires a central venous catheter, and thus is associated with complications and considerable inconvenience. Given the efficacy of VAD, attempts should be made to make this treatment more convenient.

DaunoXome is a liposomal formulation of daunorubicin entrapped within the inner aqueous spaces of small unilamellar vesicles, ranging from 40 to 80 nm in diameter, composed of distearylphosphatidylcholine : cholesterol in a 2 : 1 molar ratio. Because of the high stability of the liposomal membrane in the circulation, daunorubicin is protected against enzymatic degradation. DaunoXome interacts with cells in several ways. After endocytosis, the bilayer can be degraded and the drug is released into the cytoplasm. Liposomes can adsorb to cells and slowly release their contents [13].

Tumor cells are preferentially targeted. Preclinical data of a murine solid tumor model confirmed a 10-fold increased *in vivo* delivery of entrapped daunorubicin to solid tumors compared to conventional daunorubicin. Subsequently, efficacy studies indicated improved tumor regression in this model [14]. With the same dosage, liposomal daunorubicin achieved a 35-fold greater area under the concentration curve in plasma than free daunorubicin

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[14]. Since liposomal daunorubicin provides sustained plasma and intracellular anthracycline levels, we performed a phase I/II trial with a modification of the VAD protocol by replacing the 96-h anthracycline infusion with a short-term infusion of liposomal-encapsulated daunorubicin. The current study was performed to determine the maximum tolerated dose (MTD) and the dose-limiting toxicity (DLT) of the VLDD regimen. Furthermore, we evaluated the pharmacokinetics of the liposomal daunorubicin in multiple myeloma patients.

Patients and methods

Seventeen patients with multiple myeloma having adequate cardiac, renal and hepatic function were enrolled. Diagnosis was made according to the criteria of the British Columbia Cancer Agency; staging was performed according to the criteria of Salmon and Durie [15].

Inclusion criteria

Diagnosis of multiple myeloma, stage II or III according to Durie and Salmon, previous treatment: relapsed or primary refractory disease or first-line treatment before high-dose chemotherapy, age 18–75, Karnofsky index > 60%.

Exclusion criteria

Neutropenia < 500/μl or thrombocytopenia < 100 000/μl, active infection, heart failure (NYHA III or IV) or myocardial infarction in the last 3 months, impaired liver function with bilirubin > 2.0 mg/dl or ASAT or ALAT > 3 × normal range, impaired renal function with creatinine > 3.0 mg/dl, uncontrolled diabetes mellitus. Patient characteristics are described in Table 1. The study was initiated after ethical approval in accordance with the Declaration of Helsinki. Informed consent was obtained from all patients.

Treatment schedule

The VLDD protocol consisted of vincristine 1.4 mg/m$^{2.}$ (maximum 2 mg) i.v. bolus, day 1, liposomal daunorubicin (DaunoXome) i.v., day 1 (in 250 ml 5% dextrose, 60-min infusion), and dexamethasone 40 mg/day p.o., days 1–4 and 9–12. The initial dose of liposomal daunorubicin was 40 mg/m^2 and was escalated in 20 mg/m^2 steps until DLT and MTD had been reached. The doses of dexamethasone and vincristine were not changed. The treatment courses were repeated every 3 weeks (day 22). At least three patients were treated on each dose level. If no DLT occurred, chemotherapy cycles were repeated until disease progression or until maximal response to treatment.

Table 1 Patient characteristics

Age [median (range)]	60 (39–70)
Sex	
male	13
female	4
Disease stage (Salmon and Durie)	
II	1
III	16
No. of previous treatments	
0	4
1	8
2–3	4
>3	1

DLT was defined as either ANC < 500/μl, platelet count < 25 000/μl or bleeding, each non-hematological toxicity WHO grade ≥ 3, persistence of ANC < 1500/μl or platelet count < 100 000/μl until day 22, as well as persistence of each toxicity WHO grade ≥ 2 until day 22 of the first cycle (excluding alopecia and vomiting). The criteria of MTD was fulfilled if three patients treated on the same dose level experienced DLT or if any patient experienced a life-threatening toxicity.

Response was defined according to Chronic Leukemia–Myeloma Task Force—Criteria. For minor response, a reduction of the M-component by 25–50% was required.

Chemicals and reagents for pharmacokinetic studies

DaunoXome was obtained from Nexstar Pharmaceuticals (presently Gilead Science, Germany) in a ready-to-use vial with 50 mg liposomal-encapsulated daunorubicin at a concentration of 2 mg/ml. Caelix was obtained from Essex Pharma (Germany) in a ready-to-use vial with 50 mg encapsulated doxorubicin at a concentration of 2 mg/ml. Doxorubicin as analytical reagent was obtained as a HCl salt from Farmitalia (presently Pharmacia & Upjohn, Italy), daunorubicin as a pure HCl salt from Rhône-Poulenc-Rohrer (presently Aventis Pharma, France). Doxorubicin and Caelix were used as internal standards for the free and encapsulated Daunorubicin in the entire extraction procedure and quantitative analysis. All anthracyclines were gratefully accepted as a gift from the named industries.

All chemicals used were of analytical grade. Triton X-100, acetonitrile and methanol were purchased from Merck (Germany). Solid-phase extraction was performed using 100 mg Discovery DSC-18 1-ml cartridges from Supelco (Germany) in combination with a 24 SPE solid-phase extraction manifold from Baker (Germany).

Biological materials

Blood samples taken from healthy volunteers were stored at 4°C overnight, centrifuged for 10 min at 8000 r.p.m. and used as reference plasma samples for spiking. Patient blood samples were taken before and immediately after the application of liposomal daunorubicin (DaunoXome) as a 1-h infusion. Sample time points were 1, 2, 4, 12 and 24 h after the beginning of the infusion. Blood samples

were centrifuged for 10 min at 8000 r.p.m. and stored at −78°C.

Extraction procedure

Samples were thawed to room temperature and centrifuged at 8000 r.p.m. for 10 min. Then 50 µl internal standard solutions (2 or 10 µg/ml in 50 mmol/l citrate solution, pH 2.5) of both free doxorubicin and Caelix were mixed with 200–900 µl plasma sample, and the necessary amount of a 50 mmol/l citrate solution (pH 2.5) to result in a total sample volume of 1 ml in a 1.5-ml reaction vial.

This mixture was first extracted on the solid-phase cartridge preconditioned with 1 ml methanol and 1 ml 50 mmol/l citrate solution (pH 2.5) under light vacuum. The cartridge was washed with 1 ml purified water, and the eluted plasma and washing water were retained in a 2-ml reaction vial in the sample extraction manifold for the second extraction process. The retained analyte and internal standard (free daunorubicin and free doxorubicin) on the SPE cartridge were eluted with a 500 µl solution of methanol citrate (50 mmol/l pH 2.5, 80 vol%) into a 1.5-ml reaction vial and used for injection into the HPLC system without further processing.

The retained sample containing the still liposomal-encapsulated daunorubicin and the internal standard Caelix (liposomal-encapsulated doxorubicin) was mixed for 1 min with 100 µl Triton X-100 solution (10 vol%) in order to breakdown the liposomes. After that, the extraction procedure was performed again as described above. A similar approach for differentiated measurement was published by Bellott *et al.* [16]; however, in this paper the internal standard doxorubicin was given to the samples as free substance only after breaking down the liposomes.

Chromatographic separation and quantification

The chromatographic separation and detection of analyte and internal standard was measured by HPLC with fluorescence detection. The system consisted of a Waters 510 solvent pump, a Rheodyne injection valve with a 20-µl PEEK loop, GL-Interscience column 3.0 × 250 mm Inertsil Ph3 and a Shimadzu Fluorescence Monitor RF 535. Separation was performed using an isocratic run with an eluent of acetonitrile citrate (250 mmol/l pH 2.5) 80 : 20 v/v and an excitation wavelength of 480 nm; emission detection was performed at 590 nm.

Calibration curve and recovery of the analytes

After spiking of methanol citrate (50 mmol/l pH 2.5). 80:20 v/v solutions with doxorubicin and daunorubicin two calibration curves in the range of 5–900 and 900–10 000 ng/ml were obtained with 10 concentration points each and a determination in triplicate. The limit of detection was 15 ng/ml, the limit of quantification was 30 ng/ml, and correlation coefficient r was 0.9992 for 5–900 ng/ml (coefficient of variation 4.01%) and 0.9991 for 900–10 000 ng/ml (coefficient of variation 2.25%).

Recovery was measured as in-day repeatability with $n = 10$ spiked plasma samples for three concentrations (50, 900 and 10 000 ng/ml) for both free and liposomal-encapsulated daunorubicin. A recovery of 100 ± 16% was reached for the free and the encapsulated daunorubicin with an intra-day precision of ± 8% for the whole concentration range. These values are in good agreement with accepted guidelines for bioanalytical determination methods. Valoo software from Analytical Software (Germany) was used for determination of calibration parameters.

Pharmacokinetic data analysis

Plasma concentrations per time point were measured in duplicate for nearly all patient samples. For further data analysis the mean of these two determinations was used. Pharmacokinetic data were processed using the TopFit 2.0 software package from Heinzel *et al.* The data were measured for each individual patient and chemotherapy cycle with equally weighted data points. Kinetic values were determined using a non-compartment model; the area under the curve (AUC) was measured with the linear trapezoidal rule.

Results

Toxicity

Seventeen patients received a total of 54 courses and all were evaluable for toxicity. Patients received at least 1 and maximal 6 cycles of VLDD chemotherapy. After the first cycles six patients experienced WHO grade 3 neutropenia and two patients WHO grade 4 neutropenia. Concerning non-hematological toxicity after the first cycle, in one patient grade 3 diarrhea occurred, one patient experienced an infection grade 3 and in two patients alopecia grade 2 occurred (Table 2). No patient received granulocyte colony stimulating factor during the first cycle of treatment. Referring to all cycles, neutropenia was the most common toxicity. WHO toxicity grade 3 and 4 neutropenia occurred in 13 (24%) and 6 (11%) of 54 cycles, respectively (Table 3). Other hematological toxicities were observed less frequently and were less severe. Anemia of WHO grade 3 occurred in only 4 cycles (7%), no grade 4 anemia, and no grade 3 or 4 thrombocytopenia occurred. Non-hematological toxicity was mild. No relevant cardiotoxicity was observed. Eight patients developed an infection, three infections of WHO grade 1, four infections of grade 2 and one infection of grade 3. Alopecia of WHO grade 2 and 3 occurred each in two patients. One patient who had received one cycle of VLDD with 100 mg/m^2 of liposomal daunorubicin experienced an acute diverticulitis with subsequent colon

Table 2 Toxicity during the first cycle ($n=17$)

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Hemoglobin	3	6	7	1	0
Leukocytes	2	3	4	6	2
Granulocytes	4	4	1	6	2
Platelets	12	2	3	0	0
Bilirubin	17	0	0	0	0
ASAT/ ALAT	16	1	0	0	0
AP	15	2	0	0	0
Mucositis	15	1	1	0	0
Nausea	14	2	1	0	0
Diarrhea	16	0	0	1	0
Renal	16	1	0	0	0
Allergic	17	0	0	0	0
Cutaneous	17	0	0	0	0
Infections	13	1	2	1	0
Alopecia	14	0	1	2	0
Cardiac	16	1	0	0	0
Neurotoxicity	14	2	1	0	0
Pain	17	0	0	0	0

Table 3 Toxicity referring to the number of cycles ($n=54$)

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Hemoglobin	13	24	13	4	0
Leukocytes	4	18	13	15	4
Granulocytes	12	12	11	13	6
Platelets	45	5	4	0	0
Bilirubin	54	0	0	0	0
ASAT/ ALAT	52	2	0	0	0
AP	49	3	1	1	0
Mucositis	50	2	2	0	0
Nausea	50	3	1	0	0
Diarrhea	53	0	0	1	0
Renal	53	3	0	0	0
Allergic	54	0	0	0	0
Cutaneous	54	0	0	0	0
Alopecia	50	0	2	2	0
Infection	45	4	4	1	0
Cardiac	47	6	1	0	0
Neurotoxicity	33	16	5	0	0
Pain	54	0	0	0	0

Table 4 Toxicity during the treatment referring to the number of patients ($n=17$)

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Hemoglobin	2	7	4	4	0
Leukocytes	1	1	3	8	4
Granulocytes	1	1	0	10	5
Platelets	11	2	4	0	0
Bilirubin	17	0	0	0	0
ASAT/ALAT	16	1	0	0	0
AP	15	1	0	1	0
Mucositis	14	1	2	0	0
Nausea	14	2	1	0	0
Diarrhea	16	0	0	1	0
Renal	15	2	0	0	0
Allergic	17	0	0	0	0
Cutaneous	17	0	0	0	0
Alopecia	13	0	2	2	0
Infections	9	3	4	1	0
Cardiac	13	3	1	0	0
Neurotoxicity	6	7	4	0	0
Pain	17	0	0	0	0

Table 5 Plasma concentrations of liposomal daunorubicin and free daunorubicin in patients receiving 100 mg/m^2 liposomal daunorubicin

Time after infusion (h)	Mean	SEM	No. of cycles
Concentration of liposomal daunorubicin (ng/ml)			
0	0	0	7
1	18558	4082	7
2	9466	2714	7
4	6217	2088	6
24	89	46	7
48	0		1
Concentration of free daunorubicin (ng/ml)			
0	0	0	7
1	6961	702	7
2	8306	991	7
4	5998	616	6
24	398	97	7
48	32		1

perforation, which made surgery necessary. This was evaluated as DLT and determined the end of this study. No deaths occurred during the study. Eleven patients experienced neurotoxicity grade 1 or 2 due to vincristine, but no grade 3 or 4 neurotoxicity was observed. Table 3 shows the rate of side-effects referring to the number of administered cycles. The cumulative toxicity experienced by the patients is given in Table 4.

Response

All patients were evaluable for response to chemotherapy. Five patients (29%) achieved a partial response of multiple myeloma, six (35%) patients had minor response, one patient (6%) had no change and in five patients (29%) disease progression occurred during treatment.

Pharmacokinetics

The pharmacokinetics of liposomal daunorubicin and free daunorubicin were determined in three patients who received 100 mg/m^2 and two patients who received 80 mg/m^2. Overall, the pharmacokinetics were determined during 8 cycles of chemotherapy.

At a dose level of 100 mg/m^2 of liposomal daunorubicin, patients had a mean high peak plasma concentration of 18 558 ng/ml (Table 5). Liposomal daunorubicin exhibited a mean terminal half-life of 2.6 h in patients receiving 100 mg/m^2 and 2.7 h in patients receiving 80 mg/m^2 (Table 6). The mean AUC was determined in patients treated with 100 mg/m^2 as 112 367.8 ng/ml h. The mean total plasma clearance was 27.6 ml/min/m^2 and the mean volume of distribution at steady state was 4.2 l/m^2. The mean residence time (MRT) at dose levels of 100 and 80 mg/m^2 was 3.1 and 3.7 h, respectively. As expected, the peak plasma concentration of liposomal daunorubicin was reached directly after the end of infusion.

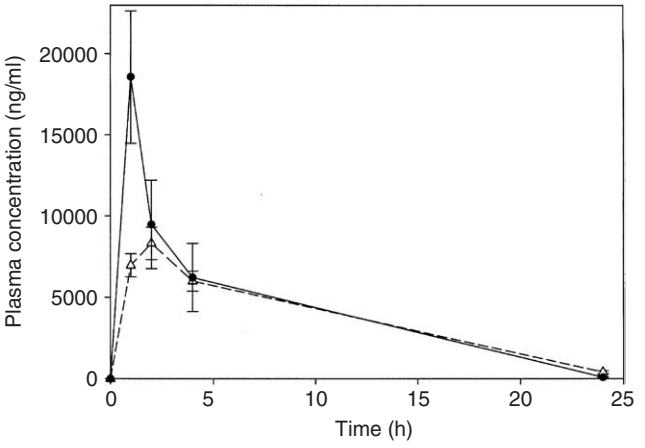
Measuring plasma levels of free daunorubicin revealed a peak plasma level of free daunorubicin 2 h after

Table 6 Pharmacokinetic parameters of liposomal daunorubicin and free daunorubicin calculated in a non-compartment analysis

	Dose level (mg/m^2)	$t_{1/2}$ (h)	$AUC_{(total)}$ (ng/ml·h)	$MRT_{(total)}$ (h)	Cl ($ml/min \cdot m^2$)	V_z (l)	V_{ss} (l)
Liposomal daunorubicin							
mean	100	2.6	112367.8	3.1	27.6	4.8	4.2
SEM ($n=6$)		0.3	31886.5	0.2	10.9	1.1	1.5
Mean	80	2.7	99050.7	3.7	42.6	7.8	8.0
SEM ($n=2$)		0.4	39842.2	0.3	16.0	2.9	3.2
Free daunorubicin							
mean	100	5.7	86068.8	5.7	17.3	8.9	5.8
SEM ($n=6$)		0.9	15149.4	1.3	1.3	2.2	1.9
mean	80	6.8	82997.7	8.2	15.3	9.4	7.3
SEM ($n=2$)		1.1	18577.4	1.6	2.1	2.7	2.3

$t_{1/2}$ = plasma half-life, AUC = area under the plasma concentration curve, MRT = mean residence time, Cl = clearance, V_z = volume of distribution in pseudo distribution equilibrium, V_{ss} = volume of distribution at steady state.

Fig. 1



Mean plasma concentrations (±SEM) of liposomal daunorubicin (circles) and free daunorubicin (triangles) at a dose level of 100 mg/m^2.

administration of 100 mg/m^2 DaunoXome and 4 h after administration of 80 mg/m^2, probably due to the delayed release from the liposomes. Data are shown only for a dose level of 100 mg/m^2 (Fig. 1).

Discussion

The results of this study suggest that the VLDD protocol is well tolerated even in heavily pretreated patients. In this phase I study, neutropenia was the most common toxicity (Tables 2 and 3). One severe infection occurred as DLT and determined the end of this trial at the dose level of 100 mg/m^2 liposomal daunorubicin (DaunoXome). A dose of 80 mg/m^2 liposomal daunorubicin resulted in the maximum acceptable toxicity in combination with vincristine and dexamethasone in this protocol. No acute or chronic cardiotoxicity was documented in the current study, confirming the data of Mohrbacher *et al.* in multiple myeloma patients [17]. Some authors described that patients were able to tolerate cumulative doses of liposomal daunorubicin above 1000 mg/m^2 [18,19].

Until now, only a few trials determined the toxicity and even less the efficacy of liposomal daunorubicin in patients with hematological malignancies [17,18,20–23]. There has been only one other phase I study performed in patients with multiple myeloma with liposomal daunorubicin [17]. The MTD was determined as 100 mg/m^2 in this study. This slight increase of MTD in this study in comparison to ours may be due to the higher percentage of untreated patients in the previous study. In a phase I study in patients with indolent non-Hodgkin's lymphoma, liposomal daunorubicin was combined with cyclophosphamide 750 mg/m^2 i.v., vincristine 1.4 mg/m^2 i.v. and prednisone 100 mg p.o. (CVP) [21]. The determined MTD in combination with CVP was similar to that in our study (70–80 mg/m^2).

Even though a phase I study is not designed to evaluate efficacy, it should be mentioned that the VLDD regimen induced an acceptable response rate. In this study, 76% of the patients had relapsed or refractory multiple myeloma and 64% of the patients achieved a partial remission or a minor response. It has been shown that the primary goal of conventional chemotherapy for multiple myeloma is stabilization of disease and the level of response is not associated with a survival benefit [24].

The VAD protocol requires a central venous catheter, and thus it is associated with some central venous catheter-related complications and considerable inconvenience. Several attempts to avoid the necessity of a central venous catheter by reducing the infusion time of doxorubicin in modified VAD regimens revealed contradictory results [25,26]. Given the higher dose and increased uptake of liposomal daunorubicin by tumor cells, the VLDD regimen may provide an acceptable alternative to the VAD regimen.

In a recently performed phase II study the aspect of the efficacy of liposomal anthracyclines was further illuminated [27]. In this study in previously untreated myeloma patients, pegylated liposomal doxorubicin in combination

with vincristine and dexamethasone (DVD) showed a response rate comparable to that of the VAD regimen.

Nevertheless, there was an increased incidence of palmar–plantar erythrodysesthesia with pegylated liposomal doxorubicin that had already been observed in the underlying phase I study [28]. This adverse event is associated with pegylation of liposomal doxorubicin and was not described with the liposomal formulation of daunorubicin. In a comparison of the liposomal anthracyclines, *in vitro* data showed a superior efficacy of liposomal daunorubicin compared to pegylated liposomal doxorubicin [23,29]. On the other hand, the prolonged plasma half-life of pegylated liposomal doxorubicin compared with liposomal daunorubicin may translate into a higher efficacy. Nevertheless, on the basis of the existing clinical data, no clear overall advantage of either liposomal daunorubicin or pegylated liposomal doxorubicin can be deduced concerning their efficacy or toxicity profile in patients with multiple myeloma.

Forssen *et al.* investigated the pharmacokinetics of liposomal daunorubicin for an 80 mg/m^2 dose in four patients [14]. For a dose of 80 mg/m^2, the peak levels for liposomal daunorubicin were 43.7 μg/ml and the initial half-life was 5.2 h. Most patients demonstrated monoexponential declines in plasma levels as observed in our study, although some patients exhibited biexponential kinetics. Conventional daunorubicin exhibited biexponential plasma clearance with a short initial half-life and a long terminal half-life probably caused by the high tissue uptake and high affinity to plasma proteins. Terminal half-life ranged from 2.4 to 10 h. Peak levels were nearly 100-fold higher for liposomal daunorubicin [14]. Another group determined the mean half-life with 4.8 h in seven patients receiving 50 mg liposomal daunorubicin (DaunoXome) [30]. We applied a new method for differentiated determination of liposomal and free doxorubicin in patient blood samples similar to a method published by Bellott *et al.* [16]. A peak plasma level of free daunorubicin was measured 2–4 h after administration of liposomal daunorubicin due to the release of daunorubicin from liposomes. The mean half-life in patients receiving 80 or 100 mg/m^2 determined in our study (2.7 or 2.6 h, respectively) was somewhat lower than previously described (Table 6) [14,30].

Conclusion

The VLDD regimen may represent an alternative to VAD in patients with multiple myeloma. Without the need of a central venous line this protocol was easier to administer in an outpatient setting and showed an acceptable toxicity profile. In this study the MTD of liposomal daunorubicin was 80 mg/m^2 in combination with vincristine and dexamethasone. This dose level should be used for further phase II studies. In this phase I study, 64% of the patients achieved a partial remission or a minor response.

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