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An EORTC-ECSG phase I study of LU 79553 administered every 21 or 42 days in patients with solid tumours

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Abstract

A single-agent dose-escalating phase I and pharmacokinetic study on the naphthalamide agent, LU 79553, was performed to determine its safety profile, maximum tolerated dose (MTD) and recommended dose for phase II studies. LU 79553 was given intravenously (i.v.) every 3 weeks to patients with advanced solid cancers (an extended cohort of patients also received the drug every 6 weeks). 59 patients were enrolled into the study (50 patients in the 3-weekly schedule and 9 patients in the 6-weekly schedule). Dose levels studied ranged from 10 mg/m^2 to 160 mg/m^2. Neuro-muscular toxicity was identified as the dose-limiting toxicity (DLT). This muscular toxicity was observed after administrating total doses of 160–450 mg/m^2 (median 330 mg/m^2). Non-DLTs consisted of diarrhoea, nausea and vomiting, fatigue and local venous phlebitis. The major haematological toxicities observed were anaemia and neutropenia (and were mainly observed at the two highest dose levels). The proposed dose for phase II studies using the 3-weekly regimen is 100 mg/m^2/course (60 min infusion in 500 ml normal saline), but a close clinical follow-up of the patients for neuromuscular toxicity is mandatory. Prolongation of the treatment interval to 6 weeks, based upon the long half-life of the drug in the plasma and tissue, observed during this study, seemed not to be feasible in this heavily pretreated group of patients.

Keywords: LU 79553; Bis-intercalating naphthalamide; Phase I; Pharmacokinetics

1. Introduction

Naphthalamides are a new class of DNA intercalating agents, which have demonstrated significant antitumour activity [1–4]. LU 79553 (*N*,*N*-Bis[2-(1,8-naphthalamido)-ethyl]-1,3-diaminopropano-bismethanesulphonate), a bis-intercalating naphthalamide and a topoisomerase II inhibitor, has demonstrated a higher binding affinity for DNA and significant antitumour efficacy against a panel of established tumour cell lines, including several multidrug resistant-positive sublines *in vitro*. It has also shown potent activity in various human tumour xenografts grown in nude mice, including induction of long-term tumour-free survival in several of these models [3,5]. The main toxicity in preclinical studies consisted of myelosuppression and local vein irritation at the injection site, which appeared to be concentration-dependent. Changes in heart muscle were seen, but were completely reversible. Studies in rats and dogs show intermediate to high clearance and a terminal half-life of approximately 24 h. The purposes of this phase I study, conducted in patients with solid tumours were (1) to determine the maximum tolerated dose (MTD) of LU 79533 administered as a single intravenous (i.v.) infusion every 3 weeks; (2) to determine the dose-limiting toxicities (DLTs) of LU 79553; (3) to derive a recommended dose for phase II studies; (4) to characterise the pharmacokinetic variables of LU 79553; and, finally, (5) to document any antitumour activity.

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2. Patients and methods

2.1. Selection of study population

In addition to giving informed consent, patients had to fulfill all of the following criteria to be eligible: histologically- or cytologically-confirmed diagnosis of a solid tumour not amenable to established forms of treatment; age $\geqslant 18$ years; World Health Organization (WHO) performance status of $\leqslant 2$; estimated life expectancy of $\geqslant 3$ months; granulocyte count $\geqslant 2\times10^9$ cells/l; platelet count $\geqslant 100\times10^9$ cells/l; bilirubin $<1.25\times$ the upper normal limit (UNL); aspartate aminotransferase (ASAT) alanine aminotransferase, (ALAT) $<3\times$UNL; serum creatinine $\leqslant 120$ μmol/l (1.4 mg/dl); creatinine clearance 60 ml/min; normal left ventricular ejection fraction (LVEF); no prior chemotherapy, immuno- or extensive radiotherapy at least four weeks prior to entry into the study (6 weeks for nitrosoureas and mitomycin).

2.2. Treatment administration

LU 79553 (Knoll AG, Ludwigshafen, Germany) was provided in vials (50 mg/5 ml or 200 mg/20 ml) containing the following: sodium acetate/acetic acid for adjustment of pH (about 5) and isotonicity.

LU 79553 was administered as an i.v. infusion every 3 weeks or after full recovery from the toxicities experienced during the previous course. The starting dose was 10 mg/m^2 and was based on the LD10 (lethal dose in 10%) in mice (162 mg/m^2). Dose escalation took place according to a modified Fibonacci scheme. Proposed dose levels were 10, 20, 33, 50, 70, 90, 120 and 160 mg/m^2. Changes in dose escalation were allowed depending upon the observed clinical and pharmacokinetic data.

LU 79553 was administered as a single 15-min i.v. infusion every 3 weeks, using a peripheral vein. The appropriate dose of LU79553 was initially dissolved in 100 ml of normal saline. In later stages of treatment, infusion durations of 60 and 180 min (dissolved in 500–1000 ml normal saline) were applied as well, because of local vein irritation.

A minimum of 3 evaluable patients receiving a total of four evaluable courses was to be entered at the non-toxic dose levels. If significant ($\geqslant$grade 2 with the exception of adequately treated nausea, vomiting, constipation and grade 2 alopecia and fatigue) toxicity was observed at a given dose level, at least 3 more patients were to be treated at that level. If delayed cumulative toxicities were observed, more prolonged intervals between treatment courses were allowed. At a given dose level, at least 2 weeks were to pass between entry of the first and the next 2 patients. The study was completed once the MTD and the DLTs had been established.

2.3. Maximum tolerated dose and proposed dose for phase II

Using the common toxicity criteria (CTC) of the National Cancer Institute (NCI), DLT was defined as any of the following events occurring during treatment with LU 79553: $\geqslant$grade 3 non-haematological toxicity, unless easily preventable or treatable; grade 4 vomiting despite therapy with serotonin receptor antagonists; febrile neutropenia; and grade 4 thrombocytopenia.

The MTD was defined as the highest dose level at which at least 2 of 6 patients experienced a DLT. The recommended dose for phase II studies was defined as the dose level below the MTD.

2.4. Safety and efficacy variables

All patients were evaluable for toxicity from the time of their first dose of LU 79553. Patients were evaluated after each course for toxicities, which were recorded and graded according to the NCI-CTC criteria. If no CTC grade was available, toxicity was graded as: 1 = mild; 2 = moderate; 3 = severe; 4 = life-threatening.

Toxicity was evaluated by measuring the following variables: haemoglobin, white blood count, granulocytes, platelets twice per week; sodium, potassium, calcium, phosphate, serum creatinine, bilirubin, total protein, albumin, glucose, alkaline phosphatase, ASAT, ALAT, gamma-glutamyl transferase (GT), lactate dehydrogenase (LDH) weekly; urinalysis and physical exam weekly; LVEF every other course.

All patients who received at least two courses of LU 79553 were considered evaluable for response. Standard tumour measurement procedures to assess response (WHO criteria) were used every other course and/or if the patient went off study for toxicity or significant clinical changes. Positron Emission Tomography (PET) scanning has also been included into the response assessment.

The duration of a complete remission was calculated from the date on which complete remission was documented until relapse. The duration of a partial response was dated from the first day of treatment until relapse.

2.5. Pharmacokinetics

Pharmacokinetic studies of LU 79553 were performed in all patients during the first course of treatment whenever possible, and in at least 2 patients per dose level. Blood samples were obtained at the following time points:

- 15-min administration schedule: prior to infusion; 10 min after the start of infusion; immediately before the end of infusion; 5 min after the end of infusion and then 15, 30, 60, 180 min; 6, 10, 24, 48 h after the end of infusion.

- 60-min administration schedule: prior to infusion; 30 min after the start of infusion; immediately before the end of infusion; 5 min after the end of infusion and then 15, 30, 60, 180 min, 6, 10, 24 and 48 h after the end of infusion.
- 180-min administration schedule: prior to infusion; 90 min after the start of infusion; immediately before the end of infusion; 5 min after the end of infusion and then 15, 30, 60, 180 min, 6, 10, 24 and 48 h after the end of infusion.

Blood samples (4.5 ml) were collected in heparin-containing tubes, centrifuged at 3000*g*, and 2–3 ml of the plasma had to be immediately frozen at −18 °C. All samples were shipped on dry ice.

2.5.1. Urine

After taking a pretreatment sample, the total urine output was to be collected and kept in a refrigerator. The urine collected during each time interval was thoroughly mixed, the total volume recorded on the collection form and two duplicate samples of 10 ml were transferred to a polypropylene tube, tagged and stored at −20 °C.

3. Results

3.1. Patient characteristics and LU 79553 administration

59 patients were entered into this study from 14 November 1995 until 10 February 1998. Of the 59 patients, 50 were treated on a 3-weekly schedule (q3 weeks) and 9 were treated on a 6-weekly schedule (q6 weeks). Table 1 summarises the patient characteristics. A total of nine cohorts of patients were treated with LU 79553 in the 3-weekly schedule at dose levels ranging from 10 to 160 mg/m^2 (Table 2). The 59 patients received a total of 132 cycles of treatment with LU 79553, the median number of cycles per patient being 2 (1–8).

The first cohort of patients treated with 10 mg/m^2 was expanded to 6 patients because of a grade 2 skin changes (exanthema) observed in 1 patient. Moreover, at the first dose level, 1 patient died due to a non-treatment-related haemorrhage at the tumour site and was replaced. Thus, 7 patients were treated at the 10 mg/m^2 dose level. These patients and the ones treated at 20 and 33 mg/m^2 did not develop clinically relevant toxicities.

At 50 mg/m^2, 3 patients were treated. Since 1 patient developed a local phlebitis at the infusion site, the cohort was expanded to 6. As none of the additional patients at 50 mg/m^2 developed signs of phlebitis, further patients were treated with 70 mg/m^2. At this dose level, 1 patient developed a grade 2 local phlebitis; hence, this cohort of patients was also expanded to a total of 6 patients without any further significant toxicities. None of the patients treated at the dose level of 90 mg/m^2 experienced relevant clinical toxicities. However, the first 2 patients at the 120 mg/m^2 dose level developed phlebitis at the infusion site. Consequently, it was decided that all treatments at this dose of LU 79553 or higher were to be administered in 1000 ml of 0.9% NaCl over 180 min. Despite this measure, 1 patient at 120 mg/m^2 developed grade 1 phlebitis in course 1 and phlebitis grade 2 in course 2.

The first patient in the 160 mg/m^2 cohort developed thrombophlebitis. After 4 patients had been treated in this cohort, it became apparent that neuro-muscular toxicity, which had developed in 2 patients still on the study at 120 mg/m^2, was dose-limiting. Hence, further accrual at 160 mg/m^2 was suspended.

Table 1
Patient characteristics

	q3 weeks	q6 weeks	All patients
Number of patients	50	9	59
Median age (range)	57 (32–77)	58 (28–67)	57 (28–77)
Male/Female	25/25	6/3	31/28
Median performance status	1 (0–2)	1 (0–2)	1 (0–2)
Prior therapy			
Radiation only (RT)	5	–	5
Chemotherapy only	17	3	20
RT + chemotherapy	15	2	17
No prior therapy	13	2	15
Not available	0	2	2
Tumour type			
Lung	8	2	10
Colorectal	6	0	6
Breast	5	1	6
Pancreas	5	1	6
Pleural mesothelioma	5	0	5
Renal	4	0	4
Head & neck	4	1	5
Other	13	4	17

q, every.

Table 2
LU 79553 administration

Dose level (mg/m^2)	No. of patients	No. of courses
q3 weeks		
10	7	15
20	3	9
33	4	11
50	6	16
70	6	22
90	3	8
120	6	14
160	4	7
100	11	20
q6 weeks		
100	9	10

q, every.

Following these observations, a total of 6 patients were treated at 100 mg/m^2 using an infusion volume of 500 ml normal saline via the peripheral route over 60 min. Because the first 4 patients had gone off-study after one or two courses, we were unable to document potential cumulative and delayed muscular toxicity. 5 additional patients were treated at this dose level and schedule.

Table 3 summarises the sites (peripheral versus central) and duration of infusions of LU 79553 throughout the study, according to the occurrence of local phlebitis.

3.2. Toxicities

In this phase I study the severity of most adverse events was graded using the NCI-CTC toxicity scale. 35 (59%) patients experienced serious adverse events. 6 (10%) patients died during the study. Only one fatality was considered related to LU 79553, as the patient had fever and neutropenia. Another 6 patients discontinued due to toxicity: 4 patients with neuro-muscular toxicity grade 3, 1 patient with grade 3 thrombophlebitis and 1 patient with grade 3 fatigue.

The major non-haematological toxicities observed in the 3-weekly schedule were neuro-muscular presenting clinically as a mixed syndrome of severe weakness (sometimes with pain in both legs), myalgia and arthralgia, asthenia/fatigue/malaise. Non-DLTs consisted of diarrhoea, nausea and vomiting, and thrombophlebitis. The major haematological toxicities observed were anaemia and neutropenia. Neutropenia was mainly seen at the two highest dose levels (120 and 160 mg/m^2). Details on muscular and haematological toxicities are summarised in Table 4.

MTD was reached at 120 mg/m^2 with 3 out of 6 patients developing neuro-muscular toxicity grade 3 (DLT). This muscular toxicity appeared after a total dose of 160–450 mg/m^2 (median 330 mg/m^2). At the highest dose level of 160 mg/m^2, 1 out of 4 patients developed muscular toxicity grade 3 after one course. Consequently, the muscular toxicity was identified as the DLT at the dose level of 120 mg/m^2 of LU 79553 given as an i.v. infusion every 3 weeks and appeared to be related to the total dose administered. Furthermore, clinical pharmacokinetic studies have shown the terminal half-life of LU 79553 to be much longer than expected from animal studies. These findings indicated that the current interval of 3 weeks between drug administrations seemed to be too short and could give rise to drug accumulation. Therefore, the 3-weekly administration schedule was replaced by a 6-weekly schedule. 9 patients entered this schedule. 8 patients received only a total of eight courses due to the development of progressive disease and one further patient recieved 2 courses. 2 patients developed muscular toxicity (grades 2 and 3) in the 6-weekly schedule after one and two courses, respectively (cumulative doses of 100 and 200 mg/m^2) (Table 4).

Although EMG's became normal in most cases, complete recovery from the clinical symptoms was not reached in all patients. In some patients flu-like symptoms and slight, transient CPK elevations were observed at the onset of muscular weakness.

At the 100 mg/m^2 dose level (60 min infusion in 500 ml normal saline, between the MTD and the previous 70 mg/m^2 level) 11 patients were entered. Only 2 of them reached the required three courses; the other patients dropped out due to progressive disease. One of these two patients developed muscular toxicity after a cumulative dose of 300 mg/m^2. No creatine kinase (CPK) elevation was observed.

In 1 out of 6 patients treated at the 70 mg/m^2 dose level, a reduction in LVEF was observed (from 65 to 43%) occurring 20 days after the second course, without any clinical symptoms. The LVEF graduately improved to 52% over the following 7 weeks.

Myalgia and/or arthralgia was seen in 7 patients, mostly grade 2 and occurred at the higher dose levels only. One patient (90 mg/m^2) had myalgia and arthralgia grade 3 after course 5 (duration 7 days). 2 patients (120 mg/m^2) had myalgia and arthralgia grade 2 after three

Table 3
Site (peripheral versus central) and duration of LU 79553 infusion (every 3 weeks schedule)

Dose level (mg/m^2)	Total no. of courses	No. of courses with an infusion duration of		
		15 min[a]	180 min[b]	60 min[c]
10–90	81	80	1	0
120	14	8	6	0
160	7	0	7	0
100	20	0	0	20

[a] 100 ml (peripheral vein).
[b] 1000 ml peripheral vein at least for the first course (central access was used in case of local toxicity at the infusion site).
[c] 500 ml (peripheral vein, central access in 4 patients from the first cycle).

Table 4
LU 79553-related neuro-muscular and hematological toxicities

Toxicity	No. of pts	No. of patients with ⩾3 courses	Neutropenia/ thrombocytopenia	Neuromuscular toxicity	
Dose levels (mg/m^2)			No of patients with Grades 3 & 4	Grade 2	Grade 3
q3 weeks:					
70	6	3	0/0	1	1
90	3	1	1/0	0	1
120	6	3	3/1	0	3
160	4	0	3/1	0	1
100	11	2	1/0	0	1
q6 weeks:					
100	9	0	1/0	1	1

pts, patients.

courses (duration 14 days). One patient (160 mg/m^2) had myalgia grade 2 after course 1 and, finally, 3 patients (100 mg/m^2) had arthralgia grade 1/2 (duration 1–17 days).

A total of 26 patients experienced asthenia, fatigue and/or malaise in one or more courses. However, in the majority of cases, the severity was no more than grades 1 or 2, and approximately 50% of the events were considered to be unrelated or unlikely to be related to LU 79553.

Some diarrhoea (grades 1 or 2) occurred at the higher dose levels, while nausea (mostly grade 1) and vomiting (grades 1 or 2) were also seen at the lower dose levels.

3.3. Antitumour activity

Of the 59 patients treated in the two schedules (q3 and q6 weeks), 47 were evaluable for response. The reasons for exclusion from response evaluation were mainly the absence of measurable lesions or the administration of only one course. One patient with pleural mesothelioma who had received three courses of LU 79553 at 120 mg/m^2 developed a partial response that lasted 55 weeks. 11 patients with different tumour types had stable disease, including 2 patients with minor responses, vulva (cumulative dose: 450 mg/m^2) and renal cancer (cumulative dose: 360 mg/m^2) treated at the dose levels 90 and 120 mg/m^2, respectively, and one pancreatic cancer patient (cumulative dose: 300 mg/m^2) with an unconfirmed partial response treated at 100 mg/m^2. No responses or stable disease were seen for patients given the 6-weekly schedule.

3.4. Pharmacokinetic results

Following infusions of 10 to 120 mg/m^2 of LU 79553 over 15 min, linearity of plasma concentrations and AUC with dose were observed. In addition, the clearance and volume of distribution did not show any consistent changes with dose, i.e. these parameters were dose-independent. Peak plasma concentrations of LU 79553 occurred at, before or soon after the end of the infusion and were dose-dependent (Fig. 1). The pharmacokinetics of LU 79553 were best described by a multiphasic decline with an initial rapid decrease in plasma levels to approximately 5% of peak plasma levels in 1 h, and two further slower disposition phases (overall mean $t1/2_\beta$ and $t1/2_\gamma$ of approximately 1.5 and 29 h, respectively). However, there was also a very rapid distribution into very deep tissue compartments, and the plasma concentrations were sustained at a low level by drug returning from the tissue to the systemic circulation.

The model indicated that a mean 38% of the area under the plasma concentration curve was attributable to this slow disposition phase (γ-phase) associated with the return of the drug from the tissue. Therefore, there was substantial exposure of the target tissue to LU 79553, despite the initial rapid decrease in the plasma level.

Mean terminal half-life of LU 79553 for those patients where a 72-h sample was taken was approximately 40 h, indicating that the contribution of the deep compartment described above may even be underestimated.

4. Discussion

A total of 59 patients were entered into the study, 50 patients in the 3-weekly schedule and nine patients in the 6-weekly schedule. Neuro-muscular toxicity was identified as the DLT at the dose level of 120 mg/m^2 of LU 79553 given as an i.v. infusion every 3 weeks. This toxicity appeared dose-dependent and was seen in patients receiving doses equal to or greater than 70 mg/m^2. The onset of muscle toxicity generally occurred after 2–5 courses of therapy, but was seen after one cycle in a patient treated at the highest dose level (160 mg/m^2) and 1 patient (100 mg/m^2) treated in the 6-weekly schedule. This muscular toxicity appeared generally after total doses of 160–450 mg/m^2 (median 330 mg/m^2). From the data obtained in patients receiving multiple cycles of LU 79553 in this study, it was not possible to define a clear threshold level of administered drug above which muscle toxicity could be expected. The muscle toxicity was reversible in some patients, but since follow-up was limited, this reversibility could not be demonstrated for all of these patients.

In an effort to circumvent the observed muscle toxicity, the above study was amended to increase the interval between cycles by administering 100 mg/m^2 (60 min infusion) of LU 79553 every 6 weeks. 9 patients were treated for a total of 10 infusions, as the 6-weekly schedule was found to be impractical because the majority of patients discontinued treatment for progressive disease before receiving their second cycle of therapy. Alterations in the dosing interval on this regimen did not alter the DLT because 2 patients who received this schedule of LU 79553 developed muscle weakness. It is of note that no 'therapeutic window' between the toxicity and the promising antitumour activity was seen at the higher dose levels, as both the confirmed and unconfirmed partial responses, as well as the minor responses, occurred in patients who had developed muscular toxicity at approximately the same total cumulative dose.

Dose-dependent myelosuppression was noted in the 3-weekly schedule with grades 3 or 4 neutropenia seen in 16% of patients, thrombocytopenia in 4% and anaemia in 4%. Other grade 3 or 4 toxicities were seen in individual patients only and included malaise, myalgia and thrombophlebitis. Local venous toxicity, i.e. phlebitis, was seen in a dose-dependent manner, necessitating a prolongation of the infusion duration: 15 min for dose levels 10–90 mg/m^2, 60 min for 100 mg/m^2 and 180 min for the 120 and 160 mg/m^2 dose levels. LU 79553 was

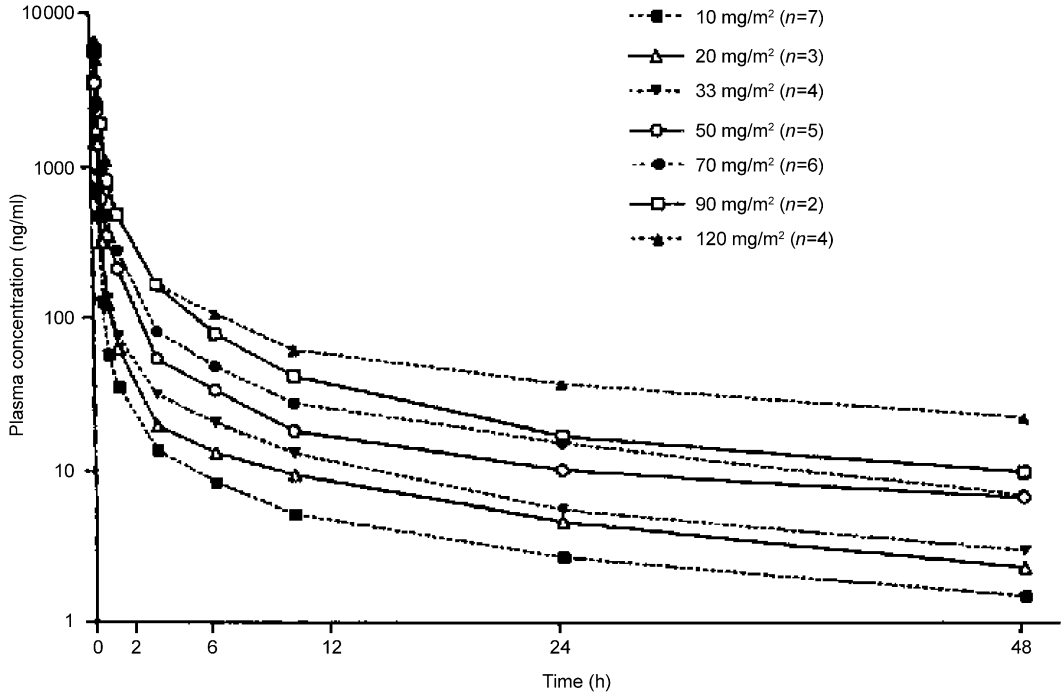

Fig. 1. Mean plasma concentrations of LU79553 following single 15 minute i.v. infusion.

known from the preclinical programme to cause irritation of the blood vessels. This characteristic of the compound was confirmed in this clinical trial. The administration of LU 79553 via a central catheter proved to be safer.

One patient at the 70 mg/m^2 dose level experienced an asymptomatic and reversible decrease in LVEF. Although there was a possible relationship to the drug administration, the patient was also a heavy alcohol user. None of the other patients in this study showed changes in LVEF over the course of the study.

Both model-independent and computer-modelled results indicated that plasma concentrations and AUC increased linearly with dose. Rate constants of disappearance and clearance were both observed to be dose-independent, with a half-life of disappearance of LU 79553 in humans of at least 40 h (considerably longer than the mean calculated value of 24 h for the 48-h data). The true value of this parameter may be even greater than 40 h; later blood sampling would be required to enable a definitive value to be determined. Since a large proportion of the plasma AUC was contributed by the return of the drug from tissues, which also controlled the rate of disappearance, it is reasonable to assume that dose-proportionality is also true for the tissue concentrations.

The recommended phase II dose was pre-defined as one dose level below the MTD in the phase I trial. Based on this definition, the proposed phase II dose for LU 79553 for the tested dosing regimen (q3 weeks) is: 100 mg/m^2/course (60 min infusion). However, even at this dose level and schedule, muscular toxicity was oberved and close clinical follow-up of the patients for neuro-muscular toxicity is mandatory. Prolongation of the treatment interval to 6 weeks, based upon the long half-life of the drug in plasma and tissue, seemed not to be feasible in the heavily pretreated group of patients tested in this study. Therefore, this dose and schedule cannot be recommended for future phase II testing.

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