

**EUROPEAN PATENT SPECIFICATION**

**EPI 1 00 589 B1**

20 **Increase in the anticancer activity of taxol in the presence of propionyl L-carnitine (PLC) *in vivo*.**

25 [0167] Cell cultures and tumour inoculation conditions

[0168] Cell cultures of L-MM3 murine breast cancer cells were used, cultured at 37°C in plastic flasks in a humidified atmosphere with 5% CO<sub>2</sub>. The cells were grown in DMEM supplemented with 10% FCS and in the presence of 2 mM L-glutamine and 80 µg/ml of gentamicin. The subconfluent cells were collected during the exponential growth phase using trypsin-EDTA and re-suspended in DMEM. They were then injected subcutaneously in female Balb/c mice weighing 20 g at a density of 4 x 10<sup>5</sup>.

Tumour measurement method

30 [0169] The tumour was measured with a calliper three times a week as soon as it became palpable. The tumour mass is calculated on the basis of the measurements of the two dimensions (length and width), expressed in mm, according to the following formula:

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$$\frac{(\text{length} \times \text{width}^2)}{2} = \text{tumour volume (mm}^3\text{)}.$$

[0170] If we consider conventionally a tumour density equal to 1, the result is that the tumour volume is equal to (mm<sup>3</sup> = mg).

40 Taxol preparation method

[0171] Agent used: taxol (paclitaxel INDENA). The agent is weighed, solubilised in the specific vehicle (12 mg/ml), and stored at +4°C, sheltered from the light. At the time of use, it is diluted 1:4 with saline solution in phosphate buffer (PBS, SIGMA) and injected.

45 Agent vehicle: Cremophor EL (BASF).

[0172] Cremophor is diluted 1:1 with ethanol and stored sheltered from the light. On the day of treatment it is diluted 1: 4 with PBS.

[0173] The animals were selected and treated as described in the previous examples.

50 Treatment conditions

[0174] Schedule A). The mice were treated i.p. with 30 mg/kg of taxol and s.c. with 100 mg/kg of PLC according to the following schedule.

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A)	Day	Treatment
	0	Inoculation of 400,000 cells/mouse

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A)	Day	Treatment
	12	Administration of 100 mg/kg s.c. of PLC
	13	PLC
	14	PLC
	15	PLC + Taxol (30 mg/kg)
	16	PLC
	17	PLC + Taxol
	18	PLC
	19	PLC + Taxol
	20	PLC
	21	PLC + Taxol
	22	PLC
	23	PLC
	24	PLC
B)	Day	Treatment
	0	Inoculation of 400,000 cells/mouse
	4	Administration of 100 mg/kg s.c. of PLC
	5	PLC
	6	PLC
	7	PLC
	8	PLC + Taxol 30 mg/kg i.p.
	9	PLC
	10	PLC + Taxol
	11	PLC
	12	PLC + Taxol
	13	PLC
	14	PLC + Taxol
	15	PLC
	↓	
	59	PLC

[0175] In both treatment schedules, the control, taxol and PLC groups were inoculated with the same number of cells.

[0176] In addition, the taxol treatment was given according to the same procedures and at the same times both in the group treated with taxol alone and in the one treated with taxol and PLC.

[0177] Treatment with PLC, whether alone or in combination with taxol, in treatment schedule A) starts on day 12 /after inoculation of the tumour) and ends on day 24; in treatment schedule B), the treatment starts on day 5 after inoculation of the tumour and ends at the end of the experiment, i.e. on day 59.

**RESULTS**

Experiment A)

[0178]

Animals with tumours/total number of animals				
Day	Control	Taxol	Taxol + PLC	PLC
19	8/13	6/13	4/12	4/12
22	10/13	6/13	6/12	6/12
25	11/13	7/13	6/12	9/12
28	12/13	9/13	6/12	11/12

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Animals with tumours/total number of animals				
Day	Control	Taxol	Taxol + PLC	PLC
36	13/13	11/13	7/12	12/12
46	13/13	11/13	8/12	12/12
Tumour size				
Day	Control SE	Taxol + PLC	Taxol + PLC	PLC
0	0	0	0	0
22	2.30 ± 0.23	0.8 ± 0.4	0.4 ± 0.3	0.6 ± 0.3
25	3 ± 0.6	0.96 ± 0.4	0.5 ± 0.32	1.2 ± 0.38
28	3.9 ± 0.6	1.4 ± 0.4	0.8 ± 0.52	3 ± 0.4
36	9.5 ± 0.6	5.5 ± 0.5	3.4 ± 1.25	9.5 ± 0.8
46	14.3 ± 0.86	11 ± 1.5	7.6 ± 2.15	15 ± 1.1

[0179] On applying the non-parametric Mann-Whitney test for unpaired data, significant differences were found at all observation times in the taxol + PLC group versus the control group, with  $p < 0.003$ , and only at the last observation time (day 46) did the significance level drop to  $p < 0.034$ . It should be noted that the values for the taxol group on day 46 were not significantly different from the control group values.

Experiment B)

[0180]

Animals with tumours / total animals				
Day	Control	Taxol	Taxol + PLC	PLC
31	4/11	2/10	1/10	3/10
45	8/11	5/10	4/10	8/10
59	10/11	6/10	6/10	8/10
Tumour size				
Day	Control	Taxol	Taxol + PLC	PLC
0	0	0	0	0
26	0.4 ± 0.2	0.1 ± 0.6	0.00	0.4 ± 0.3
31	0.6 ± 0.3	0.2 ± 0.2	0.00	0.5 ± 0.3
37	1.9 ± 0.8	0.45 ± 0.2	0.050 ± 0.050	1.3 ± 0.6
41	3.1 ± 1.3	1.750 ± 1.1	0.1 ± 0.060	2.2 ± 1.0
45	3.59 ± 1.3	2.250 ± 1.130	0.4 ± 0.2	3.9 ± 1.2
53	7.2 ± 2	5.000 ± 2.2	2 ± 0.8	5.6 ± 1.4
59	9.6 ± 1.9	5.6 ± 2.2	3.950 ± 1.5	8 ± 2.2

[0181] The Wilcoxon statistical test was applied in this experiment, which revealed that only the control group was significantly different from the Taxol + PLC group, with  $p < 0.05$ .