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Therapeutic response to taxol of six human tumors xenografted into nude mice*

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Summary. To test the antineoplastic activity of taxol, a natural product isolated from yew (*Taxus baccata* L.), six human tumors transplanted into athymic mice were used (primary tumors of breast, endometrium, ovary, brain, lung and a recurrence of tongue tumor).

While the growth rates varied with the histopathological characteristics of different tumor types, all mice were treated at a mean tumor volume of 200 ± 8 mm^3.

Taxol was given SC at a dose level of 12.5 mg/kg per injection per day for 5 consecutive days out of 7 over a period of 3 weeks. With this schedule antitumor responses were obtained in all of the six neoplasms xenografted into nude mice. In the case of the ductal carcinoma of the breast total tumor regressions were observed in four of the five treated animals. In the five other experimental models taxol produced significant growth delays.

We believe that the results of these initial tests on the nude mouse – human tumor xenograft system are convincing and justify clinical assessment of this drug.

Introduction

Taxol, a diterpene of the taxan type, originally isolated from *Taxus brevifolia* L. [16] has also been extracted from several other species of the genus *Taxus* [12].

Studies have shown that this drug promotes and stabilizes microtubule assembly even in the absence of nucleoside triphosphate and microtubule-associated proteins (MAPs) [9, 10, 15] or at low temperatures [14]. The bundles observed with taxol in living cells often abrogate the organizing capacity of the centrosomes and kinetochores [3]. Thus, in vivo, taxol alters the associations between the microtubules and the other cell components [2, 3].

Taxol is also considered as a potent antitumor agent because of its efficacy against L 1210 and P 388 leukemias and several animal tumor models [4, 5]. The validity of such results, however, depends upon the reliability of the model used, and these animal tumors do not completely mirror the human situation. Experimental xenograft transplantation in athymic mice provides an appropriate model for predicting the antineoplastic effect of drugs in humans [1, 6, 7, 11, 13]. The different results obtained suggest a good correlation between the response to anticancer agents of human tumors in the patients and of xenografts growing in nude mice [6].

A preliminary report [8] described the antineoplastic activity of taxol in four transplanted human neoplasms: a primary tumor of the colon, a liver metastasis of a breast tumor, a primary tongue tumor, and a skin metastasis of a bronchial carcinoma. The administration of taxol gave statistically significant results except in the colon adenocarcinoma.

In the present study six other human tumors xenografted SC into nude mice have been tested for their therapeutic response to taxol.

Materials and methods

Drug. Taxol obtained from the Institut de Chimie des Substances Naturelles du C.N.R.S. was extracted from the

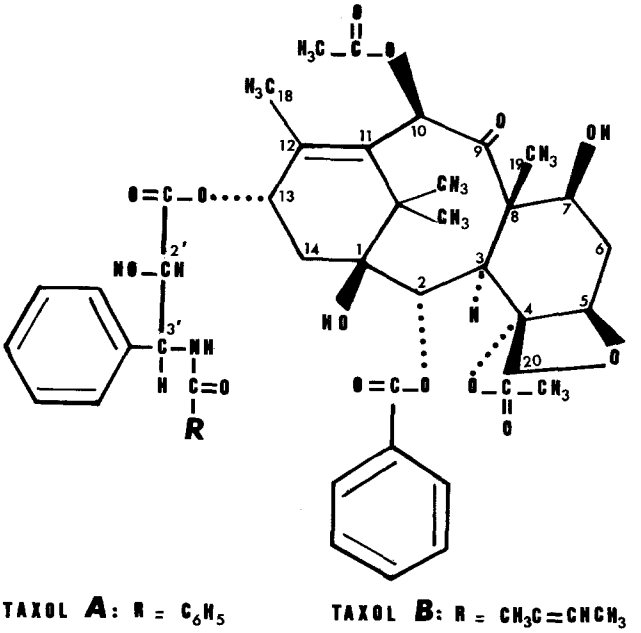

Fig. 1. Structural formula of taxol

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Abbreviations used: VT/VC % = (mean tumor volume in treated mice ÷ mean tumor volume in control mice) × 100; WT/WC % = (mean tumor weight in treated mice ÷ mean tumor weight in control mice) × 100

bark of *Taxus baccata* L. and prepared as previously described [12]. The product (Fig. 1) was a mixture of two derivatives (taxol A 70%, taxol B 30%).

Athymic mice. Six- to eight-week-old female homozygous Swiss nude mice (purchased from IFFA-CREDO Animal Breeding Center) were used throughout this study. The animals were housed in a separate room maintained at 26 °C, with a 14-h light cycle. All cages, bedding and food were sterilized.

Tumors. Tumor tissues obtained from patients during surgery were immediately placed in sterile ice-cold medium (RPMI 1640, EUROBIO) and transplanted into nude mice within 1–2 h of resection. Selected pieces of human tissue were rinsed with sterile medium containing antibiotics and cut into small cubes (2 × 2 mm). Four to six such fragments were implanted SC through a skin incision on the back of each athymic mouse.

Paraffin-embedded tumor tissues were processed for histological determination and stained with hematoxylin eosin and saffron. For electron microscopy small tumor fragments underwent double fixation with glutaraldehyde and osmium tetroxide and embedded in Epon 812. Ultrathin sections were routinely stained with uranyl acetate and lead citrate.

The main characteristics of the six human tumors xenografted into nude mice are shown in Table 1. Breast, endometrial and ovarian neoplasms were relatively well differentiated but did not contain steroid receptors. The brain tumor was derived from a glioblastoma multiforme. It contained a mixture of small anaplastic cells and a population of spindle cells. The bronchogenic adenocarcinoma was poorly differentiated. The last human neoplasm chosen for this study was a recurrence of a tongue tumor: it was a well-differentiated epidermoid carcinoma with parakeratosis. A detailed histological study and chromosomal analysis of these tumors will be reported elsewhere.

Serial transplantations. For serial transfers donor mice were sacrificed by ether anesthesia and the tumors were dissected free from murine tissues and minced in sterile medium with antibiotics. About 100 mg tumor tissue was introduced SC in the subscapular region of 6- to 8-week-old female nude mice.

Table 1. Characteristics of human tumors grown in female nude mice[a]

Patients		Tissue of origin	Histopathological characteristics	No. of passages
Sex	Age			
♀	74 Years	Breast (primary tumor)	Infiltrating ductal carcinoma	9
♀	62 Years	Endometrium (primary tumor)	Adenocarcinoma	10
♀	39 Years	Ovary (primary tumor)	Cystoadeno-carcinoma	7
♂	55 Years	Brain (primary tumor)	Glioblastoma	3
♂	54 Years	Lung (primary tumor)	Adenocarcinoma	5
♂	34 Years	Tongue (recurrence)	Epidermoid carcinoma	19

[a] at the time of the experiment.

Preliminary toxicity studies. The acute toxicity studies (i.e., after a subcutaneous single-dose administration) were performed in groups of non-tumor-bearing Swiss athymic mice. The LD_{50} of taxol obtained in these experiments was over 200 mg/kg.

The dose used for the subacute cumulative toxicity studies (i.e., multiple-dose injections) was 1/20 of the evaluated LD_{50} value (12.5 mg/kg). The mice received SC injections on 6 days per week for 4 weeks. All the animals were observed for 3 months. At the initiation of the treatment a slight body weight loss was sometimes seen. Moreover, no mortality, no histological damage and no toxic effects were observed in the nude mice treated with taxol.

Evaluation of the therapeutic response. The dose used was 12.5 mg/kg per injection and per day for 5 consecutive days out of every 7 over a period of 3 weeks. Taxol was dissolved in ethanol followed by the addition of the same volume of EMULPHOR EL 760 and subsequently by 18 volumes of sterile distilled water. The resultant emulsion was immediately injected SC in a volume of 0.1 ml/10 g body weight. Control groups received 15 SC injections of vehicle alone. The tumors were measured weekly in three dimensions using a caliper. The tumor volume was calculated using the formula $V = \Pi/6 \times \text{length} \times \text{width} \times \text{thickness}$.

After sacrifice all tumors were removed and weighed immediately. Thus, the response to drug therapy was evaluated at the end of each experiment by comparing the mean volume and the mean weight of the treated and untreated tumors. Student's *t*-test was used to assess the statistical significance of the observed differences in average final tumor volumes and weights.

Results

Growth curves

In this study (Figs. 2–7) the treatment was started when the tumor transplants were well established (average tumor volume = 200 $mm^3 \pm 8$). Thus the time from transplantation to treatment (DTS) varied among the tumor types. The data are tabulated in Table 2.

Morphological changes. The histological characteristics of xenografts were similar to those of original human tumors. These findings were confirmed by electron microscopy. The histopathological features of the treated tumors were almost the same as those of the control groups, except that there was more frequent intratumoral hemorrhage and a slight increase in necrosis.

Toxicity

No deaths from drug toxicity were observed in the 40 treated mice throughout these experiments.

Breast tumor

Figure 2 shows the growth curves of one breast neoplasm treated with taxol (5 mice) or vehicle alone (6 mice) from

Table 2. Effect of taxol on human tumors transplanted into athymic mice[a]

	Breast tumor		Endometrial tumor		Ovarian tumor		Brain tumor		Lung tumor		Tongue tumor	
	Controls (6)[b]	Treated (5)	Controls (6)	Treated (5)	Controls (7)	Treated (6)	Controls (17)[b]	Treated (11)	Controls (7)	Treated (7)	Controls (7)	Treated (6)
DTS	30	30	14	14	28	28	21	21	32	32	7	7
Average tumor volume at the start of treatment (cm^3) ± SEM	0.20 ± 0.02	0.23 ± 0.04	0.23 ± 0.01	0.26 ± 0.06	0.20 ± 0.03	0.20 ± 0.02	0.19 ± 0.007	0.19 ± 0.01	0.19 ± 0.02	0.22 ± 0.02	0.17 ± 0.003	0.17 ± 0.01
Average tumor volume at sacrifice (cm^3) ± SEM	4.35 ± 0.6	0.4	5.81 ± 0.9	1.67 ± 0.7	5.67 ± 0.9	2.02 ± 0.4	7.87 ± 0.7	2.27 ± 0.4	4.37 ± 0.9	1.36 ± 0.6	4.17 ± 0.7	1.13 ± 0.4
P			0.01 > *P* > 0.001		0.02 > *P* > 0.01		*P* > 0.001		0.05 > *P* > 0.02		0.01 > *P* > 0.001	
Average tumor weight at sacrifice (g) ± SEM	3.4 ± 0.34	0.4	4.55 ± 0.44	1.8 ± 0.72	4.8 ± 0.8	1.75 ± 0.4	7 ± 0.6	2.06 ± 0.5	4.73 ± 1.1	1.6 ± 0.55	4.9 ± 0.3	0.84 ± 0.25
p[d]			0.02 > *P* > 0.01		0.02 > *P* > 0.01		*P* > 0.001		0.05 > *P* > 0.02		*P* > 0.001	
No. of tumor regressions/ No. of treated mice	–	4/5	–	0/5	–	0/6	–	1/11	–	1/7	–	1/6

[a] Schedule of treatment: The dose schedule used was 12.5 mg/kg per SC injection per day for 5 consecutive days out of 7 over a period of 3 weeks. The control group received vehicle alone

[b] Figures in parentheses show numbers of animals in the groups

[c] DTS, days from tumor transplantation to start of treatment

[d] Statistical analysis of difference between control versus treated groups was performed using Student's *t*-test

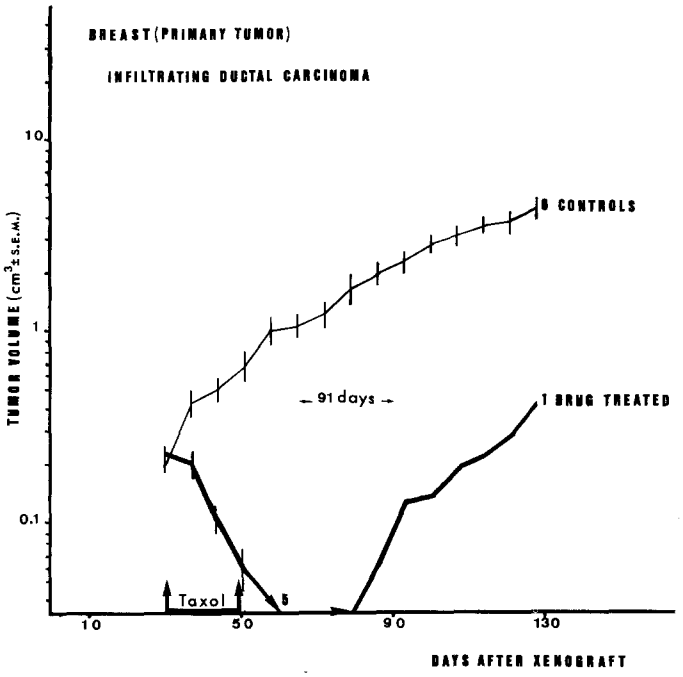

Fig. 2. Response to taxol of human breast tumor in its 9th passage, transplanted into nude mice. Treatment started 30 days after xenograft

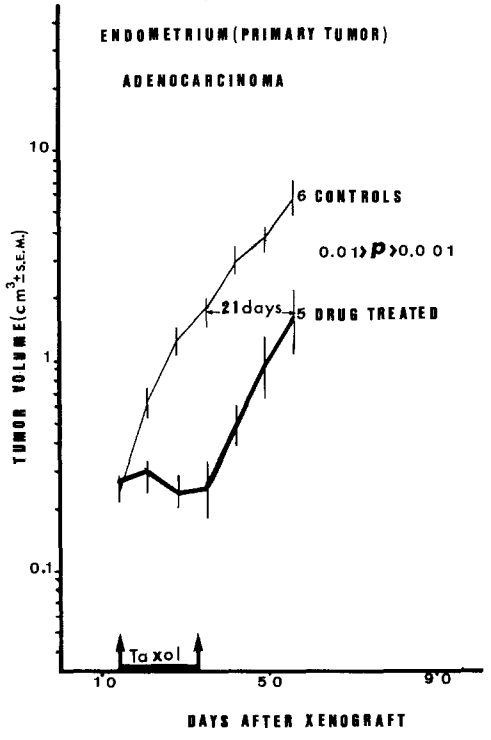

Fig. 3. Response to taxol of human endometrial tumor in its 10th passage transplanted into nude mice. Treatment started 14 days after xenograft

the 30th day after xenograft to the 49th day. Total tumor regression was observed for 20 days (from 60th to 80th day) in all animals. At day 80 one tumor reappeared and its growth rate was slightly faster than that observed in the control group. At the end of the experiment (128 days after transplantation) tumor-bearing animals were killed (6 controls and 1 drug-treated). A significant growth delay (91 days) and a drastic reduction of subcutaneous tumor volume and weight were observed in the taxol-treated mouse. In the four other treated animals no palpable tumor was observed 6 months after treatment.

Endometrial and ovarian tumors

The models for endometrial and ovarian tumors showed similar patterns of drug sensitivity (Fig. 3, 4 and Table 2). There were no apparent tumor regressions in the treated groups. Slight growth delays were obtained (21 and 23 days), although statistically significant reductions in the tumor volumes and weights were observed (VT/VC % = 29, $P < 0.01$, and 36, $P < 0.02$; WT/WC % = 39.5, $P < 0.02$, and 36, $P < 0.02$, respectively).

Brain tumor

The treatment for the brain tumor began 3 weeks after the transplantation (Fig. 5 and Table 2). Of the 28 mice in this study, 27 were killed 86 days after xenograft. Only 1 of 11 treated mice showed tumor regression. The histological examination of the implant (128 days after transplantation) revealed no tumor cells in the scar tissues. A growth delay of 34 days was obtained and a statistically significant inhibition was observed in the treated group (VT/VC % = 29, $P < 0.001$; WT/WC % = 29, $P < 0.001$).

Lung tumor

The taxol treatment against the bronchogenic adenocarcinoma transplanted into nude mice began 32 days after xenograft (Fig. 6 and Table 2). Total disappearance of the tumor was observed in 1 of 7 treated animals. At the end of the experiment (102 days after xenograft) a growth de-

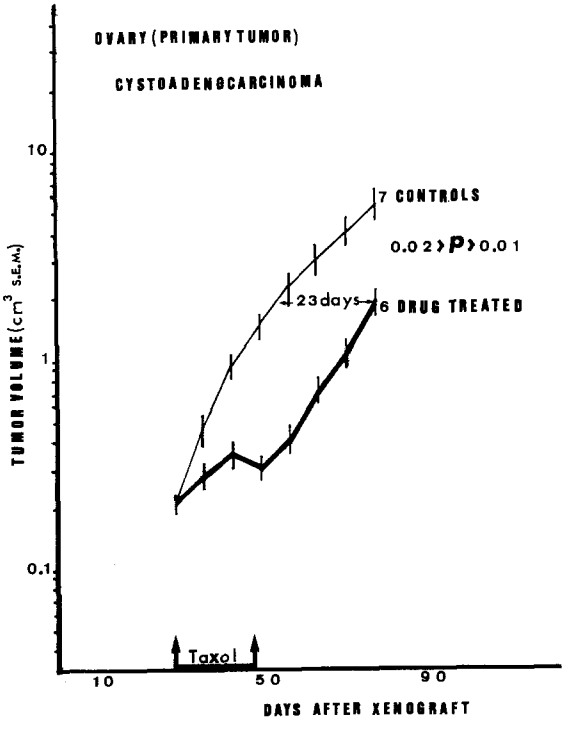

Fig. 4. Response to taxol of human ovarian tumor in its 7th passage transplanted into nude mice. Treatment started 28 days after xenograft

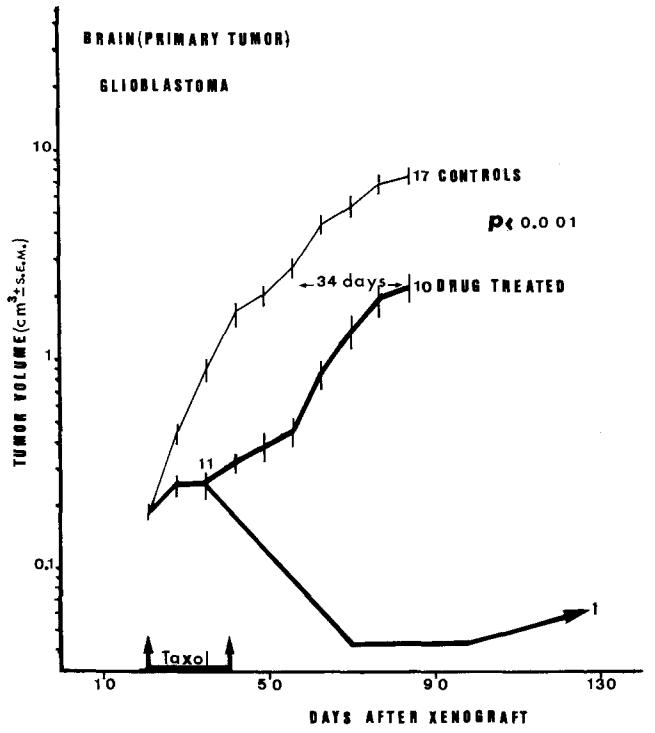

Fig. 5. Response to taxol of human brain tumor in its 3rd passage transplanted into nude mice. Treatment started 21 days after xenograft

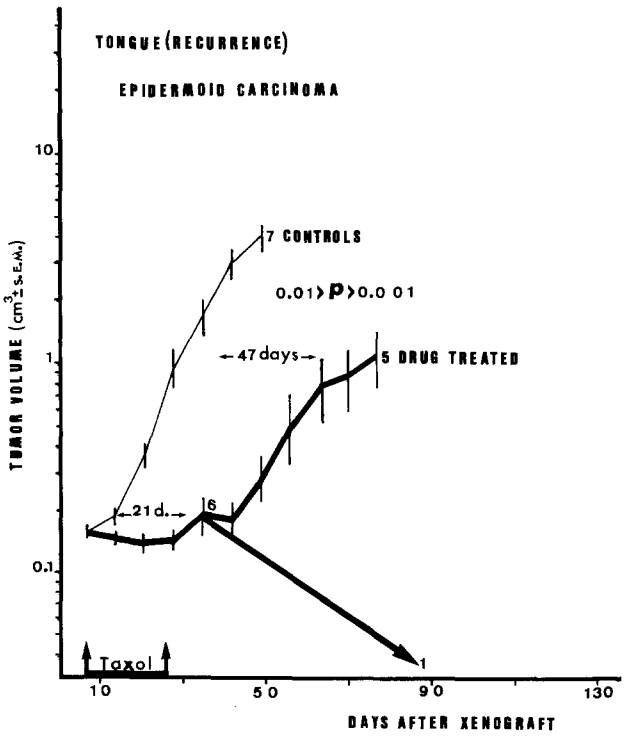

Fig. 7. Response to taxol of human tongue tumor in its 19th passage transplanted into nude mice. Treatment started 7 days after xenograft

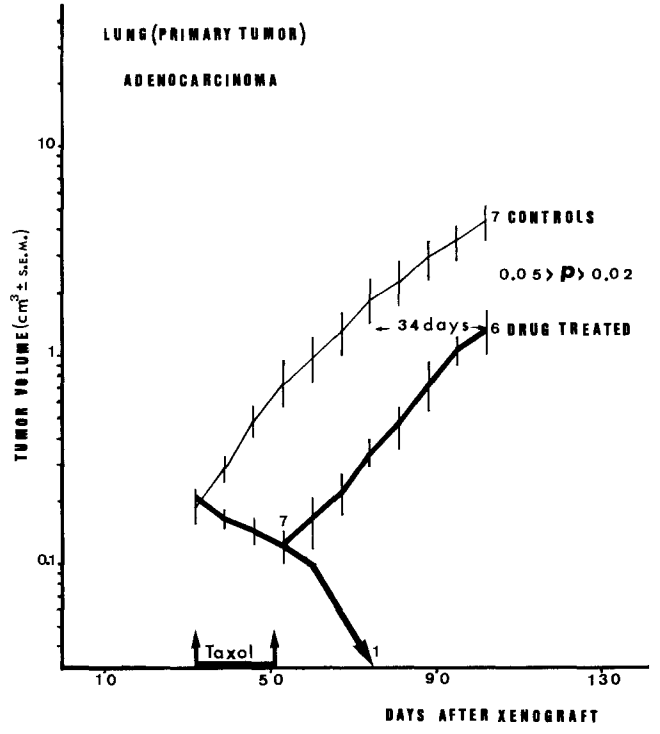

Fig. 6. Response to taxol of human lung tumor in its 5th passage transplanted into nude mice. Treatment started 32 days after xenograft

lay of 34 days was obtained. A statistically significant reduction in the tumor volumes (VT/VC % = 31, $P < 0.05$) and weights (WT/WC % = 34, $P < 0.05$) was produced by 15 taxol injections given SC.

Tongue tumor

The antitumor activity of taxol on the recurrence of an epidermoid carcinoma treated 7 days after xenograft was high and statistically significant (Fig. 7 and Table 2). In the control group the tumor growth was fast. Since animals were always killed before a lethal tumor burden with significant necrosis was reached, control and treated groups were sacrificed after different periods (7 weeks for controls and 11 weeks for the treated group). In the treated group 1 out of 6 mice had total tumor regression. At the end of the experiment taxol had produced a growth delay of 47 days. The tumor volume and weight ratios (mean volume or weight of treated tumors at 7 weeks versus that of control tumors at 11 weeks) $\times 100$ were 27 ($P < 0.01$ and 17 ($P < 0.001$), respectively.

Discussion

Human neoplasms heterotransplated into athymic mouse have now become one of the most valuable tumor models for the assessment of antineoplastic therapy [1, 6, 7, 11, 13]. This is an important advance over the previous situation, in which the tumor specimens could only be grown as monolayers or single-cell suspensions in an artificial medium outside any living environment or as xenografts that were quickly rejected by the hosts. But the limitations of the nude mouse system are evident: human neoplasms have an animal stroma, and the metabolism of the organism in which they are growing is not the metabolism of a human being.

In this preliminary investigation we have used the same experimental protocol for all tumors whatever their

histopathological characteristics. Moreover, for the tests on each neoplasm the drug was always given after an average growth delay observed in previous passages to give a common well-defined tumor volume. In each group the animals with tumors growing more slowly or more quickly than average were discarded. Thus a good uniformity of each batch was obtained. To verify the data based on estimations of the tumor volumes, at the end of the experiment all the neoplasms were weighed. There was always an excellent correlation between the estimated volumes and the measured weights.

We believe we have established that taxol has antitumor activity against six different human neoplasms xenografted into athymic mice. The effect on breast tumor was very marked and for four out of five mice we observed total disappearance of the subcutaneous tumor. In contrast, for endometrial and ovarian xenografts the effect on the growth rate was poor and only occurred at all during the period of drug injection. For the three other models (lung, brain, and tongue) the effects were intermediate between these extremes, with a definite action on the growth rate and a total remission for a small fraction of the tumors.

We are now extending those experiments, still using human xenografts in nude mice. In particular, we are comparing the therapeutic response to taxol and to vinblastine (another well-known mitotic inhibitor). To do this we are testing the response of several human tumors with the same histogenesis but provided by different patients. Preliminary results suggest that taxol shows an antitumor activity at least as good as that of vinblastine.

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