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# Treatment of an Nb-Pr-A.I.-3 (Autonomous) Tumor with Combination Chemotherapy

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***Summary.*** *The rat prostatic adenocarcinoma Nb-Pr-A.I.-3, an androgen-insensitive tumor, was evaluated with the following treatments: cyclophosphamide, cis-platinum, adriamycin, and the following combination therapies: cyclophosphamide and cis-platinum; adriamycin and cis-platinum; adriamycin, cis-platinum, and cyclophosphamide. Successful therapeutic combinations included cyclophosphamide alone ($P < 0.001$) and the triple-drug combination ($P < 0.001$). Significance was based on the final tumor volume at the termination of the experiment. Cyclophosphamide treatment alone was the only therapeutic regimen that resulted in complete tumor regression (16%) (2/12). Cyclophosphamide therapy also resulted in the lowest number of animals with metastasis (25%). On the basis of this study, the authors rate this animal model as a suitable one for trials of various chemotherapeutic combinations as well as for determining combination or single agents useful in producing tumor regression and a decreased number of metastasis.*

## Introduction

The Nb-Pr-A.I.-3 tumor model has been studied by this laboratory unit and was initially described by Dr. R. L. Noble of the Vancouver Cancer Institute [2, 9, 10]. Experience with single-agent chemotherapy in this system revealed that 5-fluorouracil, cyclophosphamide, and adriamycin were successful in reducing the final volume of tumors treated [3]. Cyclophosphamide, however, has been the most effective single agent used. Parameters considered in these evaluations include final tumor volume, complete regression of tumor, and decrease in the number of metastases.

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Single-agent chemotherapeutic trials in patients being treated for metastatic carcinoma have shown some response [1, 12]. However, chemotherapy has been employed in the treatment of human prostate cancer only since the beginning of the 1970s [6, 8].

## Materials and Methods

Eighty-four Nb male rats were used in this experiment. The animals were between 6 and 9 weeks of age and weighed between 80 and 100 g. They were housed in steel cages, three to a cage, and fed standard laboratory chow and water ad lib. The tumors were measured twice weekly. At the termination of the experiment representative histologic samples of the tumor were evaluated for each treatment group. Metastatic lesions were identified in each animal at the time of necropsy. Representative sections were also evaluated for histologic confirmation.

### *Tumor*

The androgen-insensitive tumor Nb-Pr-A.I.-3 has been investigated in the past and several tumor characteristics evaluated, including doubling time, acid phosphatase, and non-response to hormonal therapy [4, 5]. All tumor transplants in this experiment were from a single donor animal with a tumor of $1.5 \times 2\ cm^3$. The tumor was excised under sterile conditions and placed in a 125-mm petri dish; the tumor was then minced into $2 \times 2$ mm wedges. The method of tumor implantation has been previously described and it was followed in the standard manner [4, 5]. Tumor volume was calculated from the formula:

$$\frac{w^2 \times 1}{\Delta y^2} = mm^3 .$$

### *Chemotherapy*

In this experiment, seven groups were evaluated and all control animals were treated with IP saline. Experimental groups received the following: $1\ cm^3$ saline IP weekly for 3 weeks (controls); cyclophosphamide 60 mg/kg weekly for 3 weeks; *cis*-platinum

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1 mg/kg every 3 weeks (two courses); cyclophosphamide 60 mg/kg and *cis*-platinum 1 mg/kg every 3 weeks (once); adriamycin 1.5 mg/kg weekly for 3 weeks; adriamycin 1.5 mg/kg, *cis*-platinum 1 mg/kg, and cyclophosphamide 60 mg/kg every 3 weeks (two courses); adriamycin 0.5 mg/kg and *cis*-platinum 1 mg/kg every 3 weeks (two courses). Treatment was initiated at the time when tumor volume was 90 ± 10 $mm^3$ and was terminated 4 weeks after the last administration of chemotherapy. Statistical significance was evaluated according to a two-tailed Student's *t*-test, final tumor volume in the treatment groups and in the control group being compared (Table 1).

## Results

### *Tumor Volumes*

Control groups had an average tumor volume of 92,000 ± 11,500 $mm^3$, those treated with cyclophosphamide 31,000 ± 3,600 $mm^3$; with *cis*-platinum 88,050 ± 7,800 $mm^3$; with cyclophosphamide and *cis*-platinum 79,000 ± 13,450 $mm^3$; with adriamycin

**Table 1.** Evaluation of therapy

| Group ($n$ = 12) | Final tumor volume ± SD | $P$ value[a] | METS | Complete tumor regression |
|---|---|---|---|---|
| Controls | 92,000 ± 11,500 | – | 6/12 | 0 |
| Cyclophosphamide | 31,000 ± 3,600 | 0.001 | 3/12 | 2 |
| *cis*-Platinum | 88,050 ± 78,000 | NS | 5/12 | 0 |
| Cyclophosphamide, *cis*-platinum | 79,000 ± 13,450 | NS | 7/12 | 0 |
| Adriamycin | 102,210 ± 14,300 | NS | 7/12 | 0 |
| Adriamycin, *cis*-platinum | 120,540 ± 28,660 | NS | 6/12 | 0 |
| Adriamycin, *cis*-platinum, cyclophosphamide | 18,052 ± 4,625 | < 0.001 | 4/12 | 0 |

[a] Student's *t*-test

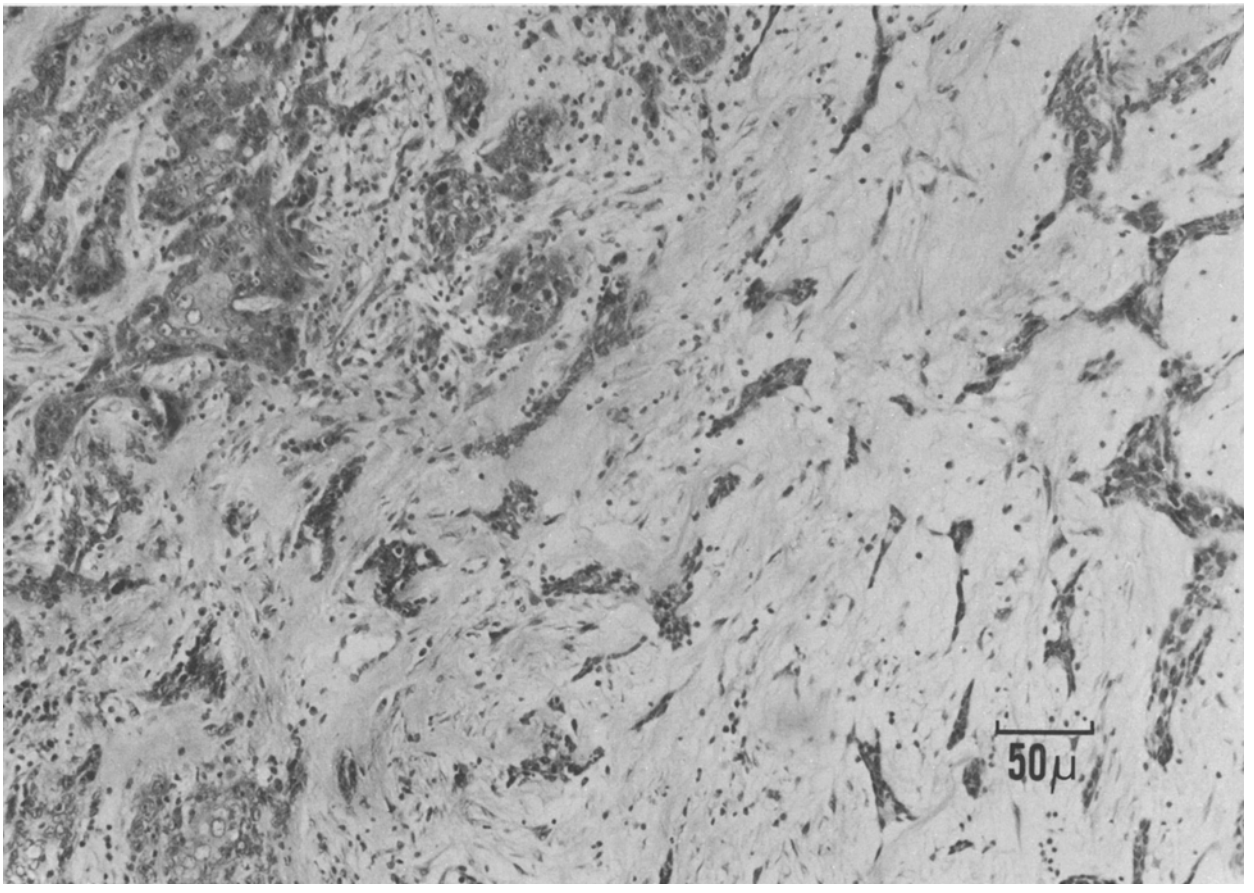


**Fig. 1.** This section is post triple-drug chemotherapy (*cis*-platinum, adriamycin, and cyclophosphamide) at 263 power. Note loss of cellular architecture with marked increase in fibrous stroma

alone 102,210 ± 14,300 $mm^3$; with adriamycin and *cis*-platinum 120,540 ± 28,660 $mm^3$; and with adriamycin, *cis*-platinum, and cyclophosphamide 18,052 ± 4,256 $mm^3$. It can be seen from these final tumor volumes that cyclophosphamide alone and the triple-drug therapy reduced the tumor volume significantly compared with controls.

*Toxicity*

Mortality resulting from the chemotherapeutic trials outlined in this report was minimal, with one animal dying after the last chemotherapeutic administration in each of the combination chemotherapeutic groups. The final weights of the animals were 228 ± 30 g, and they did not vary in a statistically significant manner from one group to another. The effect of chemotherapy on the cellular architecture can be appreciated from Fig. 1, which is a representative photomicrograph of tumors treated with adriamycin, cyclophosphamide, and *cis*-platinum, showing cellular destruction and loss of cellular architecture as well as an increase in fibrous stroma.

## Discussion

Until recently, therapy for disseminated prostatic carcinoma lagged behind therapy for other solid tumors. However, effective response rates have been reported for treatment of prostatic carcinoma with both single-agent and combination therapy [1, 6, 8, 12]. The main reason for the late appearance of chemotherapy on the scene for treatment of human prostatic carcinoma has been the initial good response of hormonal manipulation, and also perhaps the variable course of the disease [14, 15]. More recent reports concerning combination chemotherapy in a limited clinical series give objective response rates of over 40% [7, 11, 13]. This Nb-Pr-A.I.-3 tumor was evaluated with combination treatment because it is not affected by hormonal manipulation and may represent a similar situation to that seen in the clinical setting in which the patient no longer responds to hormonal therapy. In the present experiment triple-drug therapy with cyclophosphamide, adriamycin, and *cis*-platinum resulted in the lowest final tumor volume, even lower than was obtained with cyclophosphamide treatment alone, which is the single most active agent in treatment of prostatic tumors in this animal model. One can note from Table 1 that treatment with cyclophosphamide alone was the only treatment that resulted in any animals having complete tumor regression, and this was obtained in only 16%. This result differs from experience with cyclophosphamide treatment, either single or in combination, of the other androgen-insensitive (autonomous) tumors, where cyclophosphamide has resulted in tumor regression in as many as 50% of animals treated. Also, in the treatment of other androgen-insensitive tumors, the metastatic rate in cyclophosphamide treatment groups has been consistently below 20% [3]. The discrepancy in terms of treatment results in this tumor as against other androgen-insensitive tumors is of importance. The advantages of this animal model are: first, several tumors that have been classified and characterized at this laboratory unit: second, multiple treatment modalities can be used in these different tumors to determine the most efficacious treatment. The criteria currently being evaluated include reduction in final tumor volume, decreased number of metastases, and number of animals with complete tumor regression. Use of this model system will be continued in future, to evaluate combination and single-agent chemotherapy and new modalities of treatment as they become available.

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