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# Immunobiology and Therapeutic Manipulation of Heterotransplanted Nb Rat Prostatic Adenocarcinoma

## Chemotherapy of Autonomous Tumor, 102 Pr, Heterotransplanted into Congenitally Athymic (Nude) Mice and Syngeneic Nb Rats

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***Summary.*** *Nb rat prostatic adenocarcinomas, previously induced by the administration of testosterone and estrogen, have been serially studied as heterotransplants into congenitally athymic (nude) mice and into groups of Nb rats. This animal system has been used to evaluate the chemotherapeutic efficacy of 5-fluorouracil and Ftorafur. The use of both species was to determine if there would be any significant difference in relative tumor growth in nude mice which lack functional T cells as opposed to intact Nb rats. The autonomous tumor, 102 Pr, is the subject of the thesis presented herein. One donor Nb rat bearing 102 Pr prostatic adenocarcinoma served as the donor for this experiment. The nude mice and Nb rats received the transplant on the same date and were subjected to the chemotherapies outlined above and were treated after there was sufficient increase in tumor volume from the 2 $mm^3$ wedge to assure growth and neovascularity (> 60 $mm^3$). Statistically significant data was presented revealing 5-fluorouracil to be efficacious in the treatment of these tumors. Also presented is data revealing differences in growth versus time in the respective recipient animal hosts. It is suggested herein that this combination animal model system could be used for screening potential cytotoxic chemotherapeutic agents.*

### Introduction

The Nb rat prostatic adenocarcinoma is an inbred strain developed by Dr. R. L. Noble of the Cancer Research Center in Vancouver, British Columbia. Briefly, it has been induced via administration of varying concentrations of testosterone and/or estrone into Nb rats resultant in a 20% production rate of prostatic adenocarcinomas [6]. It has been used for a study because of its versatility and similartiy to human prostatic adenocarcinoma: histologic, biochemical, and development of metastatic disease [4].

There are three variants of this tumor based on its hormonal characteristics: androgen-dependent, estrogen-dependent, and autonomous. These have been the subject of serveral reports [6, 7]. Recently, this laboratory unit has investigated the hormone dependence of heterotransplanted Nb rat prostatic adenocarcinomas into congenitally athymic (nude) mice and found that the hormonal dependency pattern which exists in Nb rats also exists in the heterotransplants of congenitally athymic nude mice [2]. Additionally we have used this model to evaluate, in a preliminary fashion, chemotherapy of heterotransplants into nude mice as well as undertaking investigation of chemotherapeutic and hormonal manipulation in Nb rats [3, 5].

In this report, we will present the chemotherapeutic effects of 5-fluorouracil and Ftorafur on the autonomous prostate tumor, 102 Pr, both in Nb rats and nude mice receiving these transplants at the same point in time.

### Materials and Methods

*Animals.* The nude mouse colony has been maintained by mating of nu/nu males with nu/+ females on a N:NIH(S) background. These animals remain vigorous for periods of at least 12–18 months unmanipulated and for periods of 8–14 months following chemotherapy, surgery and/or endocrine manipulation. Nude mice are housed under specific-pathogen-free (SPF) conditions until 6 weeks of age. They are then transferred to standard laboratory cages which have been freshly cleaned and autoclaved, and covered with a filtered lid (Whatman qualitative filter paper, London, England). All mice are

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given acid water (pH 2.8) and are fed regular Purina Lab Chow. Handling is done with a sterile cap, mask and disposable plastic gloves. Nude mice are transferred using long forceps to clean, disposable paper bags during cage cleaning and always returned to their original cage.

*Nb rats:* These animals are housed in clear polycarbonate cages with 2–3 animals per cage and are fed water ad lib and standard Purina Lab Chow. They are kept under conditions in which air, humidity, temperature, light and noise are controlled, and maintained at uniform physiologic levels. Experimental animals are between seven and ten weeks of age at the initiation of tumor transplantation.

*Tumor Transplantation.* The donor animals carrying the 102 Pr tumor had been implanted with a 2 $mm^3$ wedge 38 days earlier, and at the time of sacrifice, the tumor was 1.5 $cm^3$. The tumor was minced using fine iris scissors and a scalpel over a sizing grid to obtain 2 $mm^3$ tumor wedges in a bath of 1640 RPMI media containing 100 units of Pen-Strep per ml. Immediately following this procedure, which takes about ten min, these wedges were implanted into 24 nude mice and 24 Nb rat recipients. The animals were observed and tumor volume measured twice weekly by an unbiased observer. When tumor volume had reached a size of > 60 $mm^3$, chemotherapy was initiated. At this time, animals were divided randomly into groups of eight, the chemotherapeutic regime consisted of administration of 80 mg/kg IP q. week × 3 of 5-fluorouracil, 30 mg/kg IP q. week × 3 of Ftorafur and the saline control groups received intraperitoneal saline at the same time the treatment groups received chemotherapy. The experiment was terminated at least 30 days following the last chemotherapeutic administration. Necropsy was performed at the termination of the experiment. Normal tissues were sent for histologic evaluation as well as samples of the tumors.

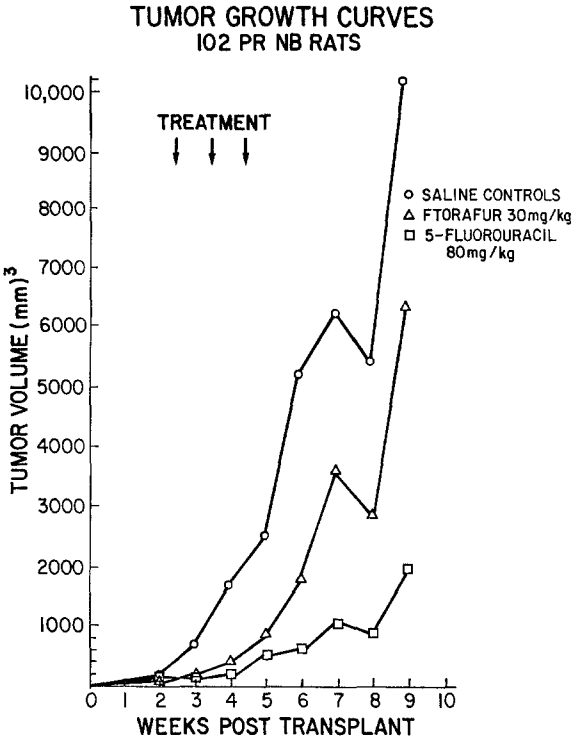


**Fig. 1.** Chemotherapy of 102 Pr autonomous tumor in Nb rats. Note the shift of the growth curve to the right associated with both chemotherapies. 5-fluorouracil yields a more marked response when compared to Ftorafur

## Results

Chemotherapy with heterotransplanted 102 Pr revealed similar results in both species. 5-fluorouracil treatment was associated with a smaller tumor volume increase in the nude mice. The average increase was 700 $mm^3$. In the Nb rats, it was 2,100 $mm^3$. Ftorafur in mice resulted in a 2,816 $mm^3$ increase and, in rats, a 4,266 $mm^3$ increase compared to the saline control group in mice of 7,000 $mm^3$ increase and, in rats, of 10,000 $mm^3$ increase. Additionally, 5-fluorouracil was more efficacious than Ftorafur in both species. The dose of Ftorafur employed was chosen as one that is well below the $LD_{10}$ for attempts at determining the effect of this low dose ($P < 0.0085$ in mice and $P < 0.008$ in rats). In the control animals, there is a constant linear progression in tumor-growth size throughout the course of the experiment whereas, in chemotherapeutic treated animals, there is a rapid fall off in terms of tumor volume, especially seen in 5-fluorouracil treatment, that persists. However, at the completion of this experiment, there appears to be a tendency towards regrowth and further progression in tumor volume. Ftorafur treatment in either species revealed a similar retardation of tumor-growth volume (Fig. 1 and 2; Table 1 and 2). In the Nb rat experiment, 5-fluorouracil treatment was significant with $P < 0.008$ whereas, in the Ftorafur group, it was significant with $P < 0.032$. Similarly, in rats as in mice, the saline control group yielded a steady progression in tumor volume increase whereas a distinct retardation in tumor-growth rate was observed in the 5-fluorouracil treated groups of animals. However, Ftorafur, which did show a decrease in total tumor volume, was not as significant as 5-fluorouracil. There is apparent increase in tumor volume after the completion of therapy. This suggests that either another course of cytotoxic chemotherapy is indicated or a larger dose of the agents should be used. However, this was two times the average tumor increase as opposed to the 5-fluorouracil treated group.

*Histology.* The histologic picture at the outset of the experiment from the donor and at the conclusion of the experiment from both nude mice and Nb rats has remained stable. The donor tumor consists of compact cords and nests of cells with prominent nuclei and evidence of mitotic activity. 5-fluorouracil treated tumors revealed a similar histology. However, there may appear to be a slight increase in fibrosis in areas and also some evidence of necrosis (Figs. 3 and 4).

*Toxicity.* Nb rats treated with 5-fluorouracil at the dosage of 80 mg/kg had a mortality of two of eight animals.

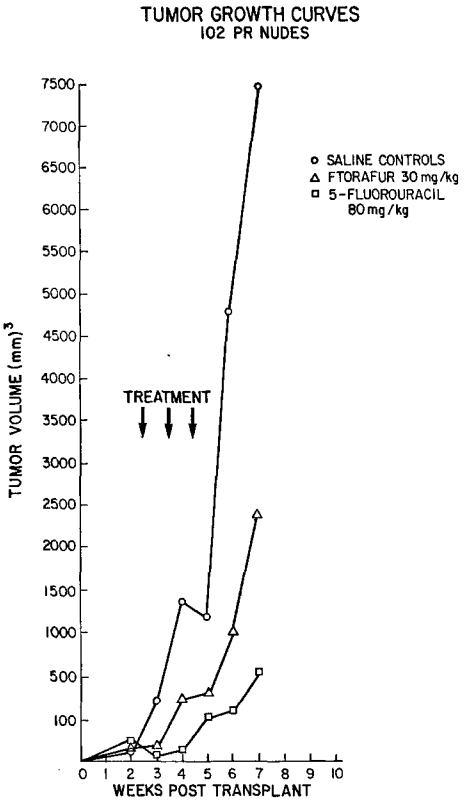


**Fig. 2.** 5-fluorouracil and Ftorafur chemotherapy of heterotransplanted Nb rat 102 Pr prostate adenocarcinoma in congenitally athymic nude mice. Note similarity in response to these agents in nude mice as compared to treatment in Nb rats (Fig. 1)

**Table 1.** Chemotherapy

| Group | $\bar{X}$ Tumor increase ($mm^3$) | *P* Value[a] |
|---|---|---|
| Nb Rats | | |
| Saline | 11,000 ± 2,030 | – |
| 5-flourouracil | 2,100 ± 795 | < 0.008 |
| Ftorafur | 4,266 ± 1,100 | < 0.032 |
| Nude Mice | | |
| Saline | 7,000 ± 1,406 | – |
| 5-flourouracil | 700 ± 300 | < 0.0085 |
| Ftorafur | 2,816 ± 752 | < 0.036 |

[a] Student *t*-test

**Table 2.** Percent decrease of tumor volume

| | $\left(\frac{\text{Treatment}}{\text{Controls}}\right)$ | | |
|---|---|---|---|
| | Saline ($mm^3$) | 5-fluorouracil | Ftorafur |
| Rats | 10,00 | 21% | 42.6% |
| Mice | 7,000 | 10% | 40.2% |

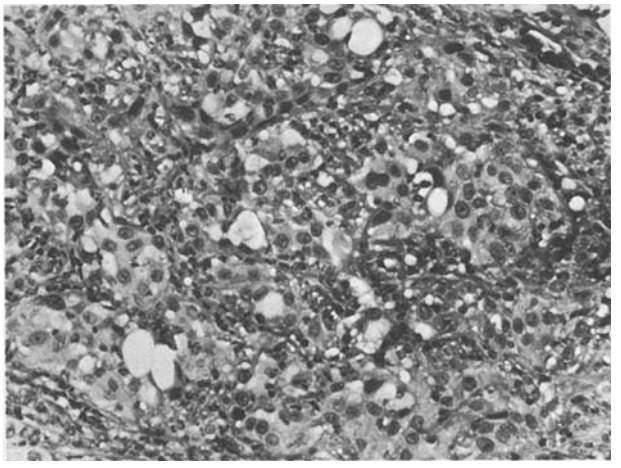

**Fig. 3.** Donor tumor, 102 Pr: Note sheets and cords of malignant cells with minimal gland formation. At this magnification, hyperchromatic nuclei are seen. H & E × 275

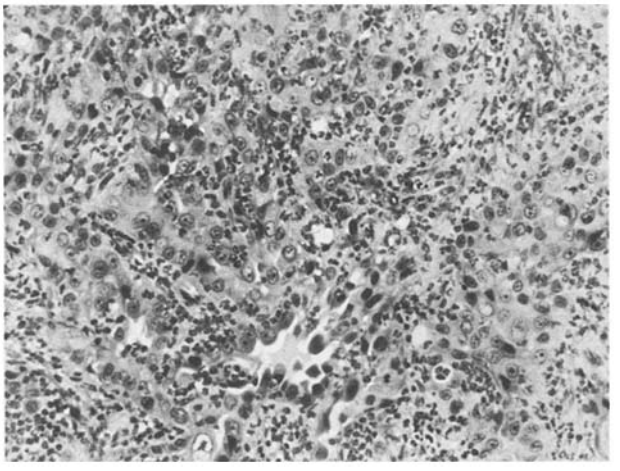

**Fig. 4.** Postchemotherapy (5-fluorouracil): Increased stroma and fibrosis can be appreciated as well as areas of necrosis. Also a suggestion of giant cell formation appears near the areas of necrosis. This may represent a response to chemotherapy and tumor regression

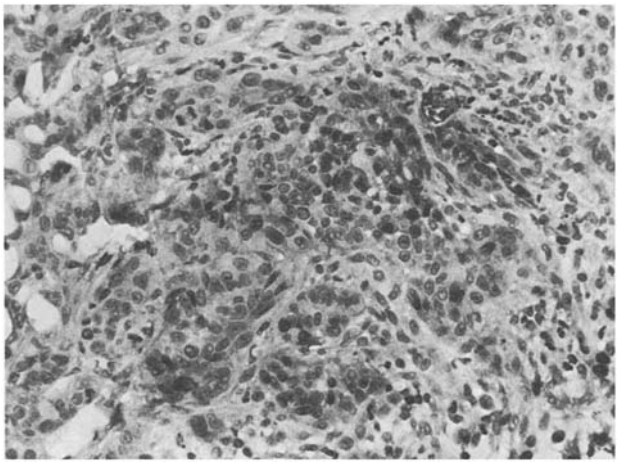

**Fig. 5.** Mediastinal metastasis taken from saline control group of nude mice, H & E × 275. Note nests of malignant cells with hyperchromatic nuclei in center of the photomicrograph

There were no Ftorafur deaths attributable to chemotherapy as would be anticipated at this dose. In congenitally athymic (nude) mice, one animal of the saline group appeared ill at the fourth week of the experiment and was sacrificed. One animal treated with Ftorafur died the day following the last chemotherapy and two animals in the 5-fluorouracil treated group also succumbed to chemotherapy. At the time of necropsy, one of the saline-treated animals was found to have a mediastinal mass that was grayish in color. A suitable section was taken for histology. This revealed a similar histologic pattern as seen in the donor tumor. It is believed to represent a metastatic deposit from the subcutaneous reimplanted tumor wedge (Fig. 5).

## Discussion

Chemotherapy of Nb rat prostatic adenocarcinoma, autonomous tumor, 102 Pr, with 5-fluorouracil and Ftorafur has shown similar results in tumor progression in both nude mice and Nb rats. 5-fluorouracil is one of the few chemotherapeutic agents that has been used in clinical trials and that has been found to be effective [1]. Ftorafur, fluorinated pyrimadine analogue, in preliminary studies, has been shown to have activity against human tumors [8]. It has been used in comparison with 5-fluorouracil because it is an analogue compound of 5-fluorouracil and proposed to have less toxic side-effects, however, maintaining its efficacy. The growth pattern of this autonomous tumor is similar in both species, with the relative tumor size not being affected differently by chemotherapy in either group, T-less nude mice or intact Nb rats. It is noted, however, that the effect of chemotherapy in either species is similar and this perhaps adds efficacy in combining both groups to assess the effects of chemotherapy alone in an immunocompetent host and chemotherapy in the immunosuppressed host. The finding of a metastatic lesion secondary to a subcutaneous implant has occurred in several of the Nb rat tumors heterotransplanted into nude mice. This may also be a helpful way of evaluating chemotherapeutic efficacy. Our unit has seen this phenomenon quite commonly up to 30% in Nb rats that received transplants in the subscapular area. The metastatic lesions have been predominantly to the lung, mediastinum and axillary lymph node [3].

The use of these combined animal model systems to screen potentially lethal agents with untoward side-effects may prove beneficial in discovering potentially valuable chemotherapeutic agents and regimes for use in clinical trials.

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