

# Prophylactic Noscapine Therapy Inhibits Human Prostate Cancer Progression and Metastasis in a Mouse Model

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**Abstract.** *Background:* Noscapine has demonstrated potent antitumour activity and minimum toxicity in cancer models. Recently, noscapine has been shown to limit tumour growth and lymphatic metastasis of PC3 human prostate cancer mice. The prophylactic effects of noscapine are not known. *Materials and Methods:* Nude mice received oral noscapine (300 mg/kg per day; 'treatment'; n=10) or diluent ('control'; n=10) for 56 days, beginning 7 days after inoculation with PC3 human prostate cancer cells; or noscapine for 70 days, beginning 7 days before inoculation ('pretreatment'; n=10). *Results:* Mean total tumour volumes were 1731.6±602.0 mm<sup>3</sup> in the control group, 644.3±545.1 mm<sup>3</sup> in the noscapine pretreatment group and 910.9±501.1 mm<sup>3</sup> in the noscapine treatment group (p<0.001 pretreatment vs. control, p<0.05 pretreatment vs. control, p<0.001 pretreatment vs. treatment group), with no evidence of toxicity. Noscapine pretreatment and treatment also reduced tumour weight, the incidence of metastasis and primary tumour inhibition rate. *Conclusion:* Pretreatment with oral noscapine limited tumour growth and lymphatic metastasis of PC3 human prostate cancer in this mouse model and conferred a significant additional benefit over noscapine treatment in final tumour volume.

Noscapine, an opium-derived alkaloid commonly used as an antitussive agent in cough medicine (1, 2), is currently under investigation as a potential chemotherapeutic agent for use in human cancer. Noscapine is known to bind to tubulin, arresting mitosis in many mammalian cells (3, 4), and has displayed potent antitumour activity in a number of cancer models, including tumours of the breast (4) and bladder (3), as well as ovarian cancer (5), malignant melanoma (6) and glioblastoma

(7). Furthermore, noscapine is readily absorbed after oral administration and shows little or no toxicity in humans (8).

Despite recent advances, metastatic prostate cancer remains largely untreatable. Moreover, the treatments that currently exist tend to be associated with significant side-effects and reduced quality of life (9). A previous study (10) demonstrated that oral administration of noscapine was effective in reducing primary tumour growth and lymphatic metastasis of PC3 human prostate cancer cells transplanted into immunodeficient nude mice, without visible signs of toxicity to other tissues or induction of additional morbidity. Here, we investigate whether noscapine may also demonstrate a chemopreventative benefit when administered to mice in advance of inoculation with PC3 human prostate cancer cells.

## Materials and Methods

*Cell culture.* PC3 human prostate cells (ATCC, Rockville, MD, USA) were incubated with RPMI-1640 (Gibco-BRL, Life Technologies, Inc., Grand Island, NY, USA) supplemented with 10% foetal bovine serum. Cells were incubated at 37°C in 5% CO<sub>2</sub>/95% air. Cell viability was determined by Trypan blue exclusion.

*Animals.* Male, athymic, Sim (NCr) nude mice, 5-6 weeks of age, were obtained from Nxgen Bio Sciences (Sorrento Valley, San Diego, CA, USA). The animals were bred and maintained in a HEPA-filtered environment with cages; food and bedding were sterilized by irradiation and autoclaving. The animal diets were obtained from Newco Distributions, Inc (Rancho Cucamonga, CA, USA). Drinking water was autoclaved and chlorinated, but no antibiotic was added. A total of 30 animals were used for the study. All protocols involving these mice were subject to guidelines established by the institutional Animal Care Committee in accordance with national regulations concerning the use of animals in scientific experimentation.

*Establishment of subcutaneous tumour model.* Thirty mice were randomly divided into three groups of ten animals and each mouse was marked by ear-cut for identification. Suspensions of 1×10<sup>6</sup> PC3 human prostate cancer cells in 0.2 ml of phosphate-buffered saline (PBS) were injected into the subcutaneous space of dorsal skin within 40 min of harvesting the cells from culture. Two groups ('control' and 'treatment') were inoculated before treatment and one group ('pretreatment') began treatment before inoculation.

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**Study design.** The noscapine pretreatment group (n=10) received oral noscapine (300 mg/kg, provided by Netzah Israel Pharmacy, Tel Aviv, Israel) diluted in acidified deionised water (pH 4.0) and delivered by gavage. Treatment began 7 days before inoculation of tumour cells and continued daily for 70 days. For the noscapine treatment (n=10) and control (n=10) groups, oral noscapine (300 mg/kg) or acidified deionised water, respectively, was administered daily by gavage, beginning on day 7 after inoculation of tumour cells and continuing for 56 days (Figure 1). Body weight was determined and tumour volume measured twice a week for each animal. All animals were checked daily for mortality or signs of morbidity during the treatment. The final tumour weight and presence of metastases were determined after animals were sacrificed by CO<sub>2</sub> inhalation at the termination of the study.

**Data collection.** An electronic balance (Ohaus Adventure Pro Scale) was used to measure body weight. Tumour volumes were determined by measuring two perpendicular diameters using callipers; the volume was then calculated using the standard formula  $V=(L \times W^2)/2$ . The tumour growth inhibition rate (IR) was calculated according to the following formula:  $IR (\%) = (1 - TW_t / TW_c) \times 100$ , in which TW<sub>t</sub> and TW<sub>c</sub> are the mean tumour weight of a treated group and control, respectively. Tumour images were obtained at the end of the study with a Canon digital camera. Primary tumours and macro-metastasis imaging were acquired by whole-body imaging.

**Histological assessment of primary tumours and confirmation of metastasis.** Tissue samples of the primary tumour, metastatic lymph nodes and lungs were removed and processed with 10% formalin for haematoxylin and eosin staining and subsequent microscopic examination. Lung metastases were assessed by histological examination under microscopy.

**Statistical analysis.** Comparison of body weights and tumour sizes between each group were analyzed using Dunnett's post hoc procedure (based on the one-tailed *t*-test).

## Results

**Body weight.** None of the mice died during the study. Although the control group experienced a decline in mean body weight over the course of the study, body weight was maintained or slightly increased in the two noscapine-treated groups ( $p < 0.001$  pretreatment vs. control and  $p < 0.01$  treatment vs. control at day 63; Figure 2). The lack of cachexia among the experimental animals demonstrates that noscapine causes little or no toxicity and is well tolerated at high doses.

**Primary tumour growth.** Primary tumour growth by volume for the three treatment groups is shown in Figure 3. The final primary tumour volumes were  $1731.6 \pm 602.0 \text{ mm}^3$  in the control group,  $644.3 \pm 545.1 \text{ mm}^3$  in the noscapine pretreatment group and  $910.9 \pm 501.1 \text{ mm}^3$  in the noscapine treatment group ( $p < 0.001$  pretreatment vs. control,  $p < 0.05$  pretreatment vs. control,  $p < 0.001$  pretreatment vs. treatment group). At sacrifice, the mean total tumour weight was  $2.03 \pm 0.75 \text{ g}$  in the

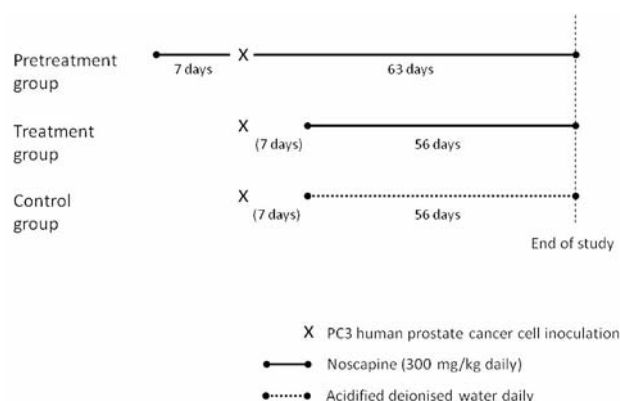


Figure 1. Study design. Mice in the noscapine pretreatment group received oral noscapine (300 mg/kg) 7 days before inoculation of tumour cells, continuing daily by gavage for a total of 70 days. Mice in the noscapine treatment or control groups received oral noscapine (300 mg/kg) or vehicle, respectively, daily by gavage for 56 days beginning 7 days after inoculation.

control group,  $0.56 \pm 0.68 \text{ g}$  in the noscapine pretreatment group and  $0.91 \pm 0.46 \text{ g}$  in the noscapine treatment group ( $p < 0.01$  pretreatment and treatment groups vs. control;  $p > 0.05$  pretreatment vs. treatment group).

**Incidence of metastasis.** Twenty percent of animals in the control group developed lymphatic metastasis, compared with 10% in the pretreatment group ( $p > 0.05$  vs. control) and no animals in the treatment group ( $p > 0.05$  vs. control). Lung metastatic rate was 60% in the control group, 10% in the noscapine pretreatment group ( $p > 0.05$  vs. control) and 20% in the noscapine treatment group ( $p > 0.05$  vs. control). There were no significant differences in the incidence of either lymphatic or lung metastasis between the two noscapine groups.

**Primary tumour inhibition rate.** The primary tumour inhibition rate as determined by volume and total tumour weight was 62.8% ( $p = 0.0005$  vs. control) and 29.9% ( $p = 0.004$  vs. control), respectively, for the pretreatment group and 47.4% ( $p = 0.004$ ) and 18.4% ( $p = 0.222$ ), respectively, for the treatment group. There were no significant differences in tumour inhibition between the two noscapine groups in primary tumour inhibition rate as determined by either volume or total weight.

## Discussion

Earlier studies have demonstrated that noscapine, a common antitussive in over-the-counter cough medicines, also has properties that limit the growth of certain types of cancer both *in vitro* and *in vivo*. In particular, we have previously demonstrated that noscapine is effective in reducing primary tumour growth and lymphatic metastasis of PC3 human prostate cancer cells in immunodeficient nude mice (9).

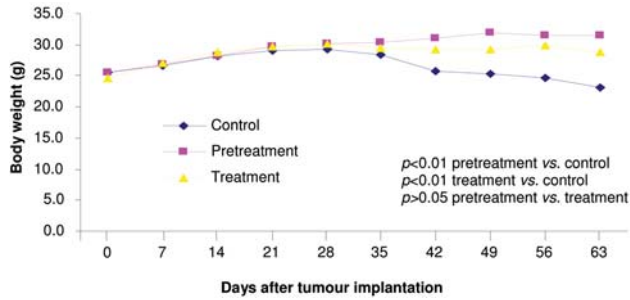


Figure 2. Mean body weights of the three treatment groups during the course of treatment. Animals were weighed before treatment and twice weekly after the initiation of treatment [oral noscapine, 300 mg/kg, or acidified deionised water (control)] as described in the Materials and Methods. Results shown are the mean of 10 animals in each group over the course of treatment.

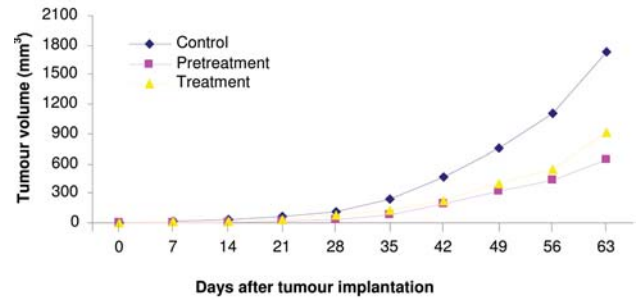


Figure 3. Tumour volume for noscapine pretreatment, treatment and control groups during the course of treatment. Tumours were measured with callipers and volumes calculated at the intervals shown in the Figure and as described in Materials and Methods. Results shown are the mean of 10 mice in each treatment group.

The high prevalence of prostate cancer as well as the significant morbidity and mortality associated with the disease make prostate cancer a suitable target for a risk-reduction approach. Furthermore, prostate cancer has a long latency period, providing ample opportunity to intervene prophylactically with chemopreventive agents at various stages of disease progression (11). Several agents are under investigation for reducing the risk of prostate cancer; however, research into prophylactic prostate cancer therapy is still in its infancy (12).

We report here that pretreatment with noscapine confers a significant benefit compared with control in both primary tumour growth and primary tumour growth inhibition rate, and exhibits an extremely favourable tolerability profile. Interestingly, pretreatment with noscapine also afforded an additional benefit over noscapine treatment in terms of final tumour volume. Our findings suggest that noscapine administered either as treatment or prophylaxis offers significant benefits in the management of prostate cancer, and that prophylaxis may offer some additional benefit over treatment.

Continuing research promises to expand our knowledge regarding the possibility of chemoprevention for prostate cancer. However, chemoprevention is not yet a reality in the clinic. In the future, it would be particularly interesting to examine the effects of noscapine as a prophylactic agent administered following prostate surgery.

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