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Enhancement of Intermittent Androgen Ablation Therapy by Finasteride Administration in Animal Models

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One critically important problem in prostate cancer research is to find new approaches to slow down the transition of prostate cancer from an androgen-dependent state to a lethal androgen-refractory state. Intermittent androgen ablation therapy may slow down the development of androgen refractory tumors because intermittent recovery of androgens can induce differentiation of prostatic epithelial cells. However, the advantage of inducing differentiation of prostate cancer cells by intermittent recovery of androgens is compromised by the disadvantage of androgenic induction of prostate cancer cell proliferation. The biologically most active androgen is dihydrotestosterone (DHT), which is converted from testosterone (T) by 5α-reductase. Our recent studies showed that T is more potent than DHT in enhancing differentiation but weaker in stimulating proliferation, which led to our hypothesis that intermittent androgen suppression (IAS) can be enhanced by finasteride, an inhibitor of T to DHT conversion. We have worked out conditions to deliver T and finasteride over a long time period in nude mice, which allowed us to test our hypothesis. Our experiments recently showed that finasteride administration during IAS significantly slowed the growth of LNCaP tumors.
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Introduction:

Conversion of T to DHT is essential for prostate development.

T and DHT are two major biologically active androgens (1). T is synthesized in testis and then transported to target organs, such as the prostate, via blood circulation. T can be converted to DHT in the prostate by 5a-reductase (2, 3). Both T and DHT bind to the same AR. DHT is more potent than T in activating promoters containing ARE, most likely due to the higher binding affinity of AR to DHT relative to that of T (4-7). The conversion of T to DHT is necessary for normal prostate development because 5α-reductase inactivation prevents normal prostate development (8, 9). It was thought that the conversion is merely an amplification step for androgen action (10). However, it cannot be ruled out that T and DHT have overlapping yet different biological functions in vivo. In fact, our recent studies suggest that T is more potent than DHT in inducing androgen-response genes during the regrowth of the rat ventral prostate (11).

Androgens regulate homeostasis of prostate.

Androgens are required for the structural and functional integrity of the prostate (12). Androgen ablation by castration leads to rapid prostate regression via massive apoptosis (13, 14). On the other hand, androgen replacement stimulates rapid proliferation and differentiation of a regressed prostate until it reaches the normal size (12, 15). Androgen action in a regressed prostate is different from that in the fully-grown prostate because androgens do not stimulate proliferation in a fully-grown prostate (Table 1) (12). During the regrowth of a regressed prostate, androgens induce and then nullify proliferation, establish apoptotic potential while inhibiting apoptosis, and induce and maintain differentiation.

<table>
<thead>
<tr>
<th>Androgen</th>
<th>Regressed Prostate</th>
<th>Fully-Grown Prostate</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>Proliferation &amp; Differentiation</td>
<td>No Significant Change</td>
</tr>
<tr>
<td>-</td>
<td>No Significant Change</td>
<td>Apoptosis &amp; Dedifferentiation</td>
</tr>
</tbody>
</table>

+ represents androgen replacement and - represents androgen ablation or administration of anti-androgens. Differentiation is defined as the expression of prostate-specific markers. Dedifferentiation is defined as loss of prostate-specific marker expression.

Androgen action is intimately associated with prostate cancer pathogenesis.

Androgens are thought to play important roles in prostate cancer pathogenesis (16-18). One of the risk factors for prostate cancer is the presence of the functional testis. Prostate cancer cells are derived from glandular epithelial cells and are initially androgen-dependent. Androgen ablation remains as the standard therapy for metastatic prostate cancer. Unfortunately, androgen ablation therapy is only palliative and eventually patients relapse with androgen-refractory prostate cancer that is currently incurable (18).
Development of androgen-refractory prostate cancer.

The mechanisms of prostate cancer progression from an androgen-dependent state to a lethal androgen-refractory state have been studied extensively. Mutations followed by clonal selection appears to be the mechanism of androgen-independent progression in several prostate cancer models, including the Dunning R3327 rat prostatic adenocarcinoma and LAPC9 human prostate cancer cells (19, 20). Another mechanism for androgen-independent progression involves adaptation. The androgen-independent progression of Shionogi mouse tumor and LNCaP human tumor involve the adaptation (21-24). It is possible that multiple mechanisms are involved in the development of androgen-refractory prostate cancer.

Intermittent androgen ablation therapy.

One urgent challenge in prostate cancer research is to develop new approaches to inhibit or to slowdown the development of androgen-refractory prostate cancer. Intermittent androgen ablation therapy was developed, attempting to delay the emergence of androgen-refractory prostate tumors relative to the continuous androgen ablation therapy. The rationale is that intermittent recovery of androgens can promote prostate cancer cell differentiation and enhance their dependence on androgens (24, 25). However, androgens are also proliferative to prostate cancer cells, which is undesirable in the therapy. The goal of our proposal is to increase the efficacy of intermittent androgen suppression by enhancing the differentiation effects while inhibiting the proliferative effects via finasteride administration.

Finasteride enhances the expression of many androgen-response genes during T-stimulated regrowth of the regressed prostate.

One interesting question in androgen action is whether or not the expression of androgen-response genes is differentially regulated by T and DHT. Finasteride, a 5α-reductase inhibitor, had little or no effect on the expression of the surveyed androgen-response genes in testis-intact rats (11). However, the induction of half of the surveyed androgen-response genes, including prostatein C3, adrenomedullin and calreticulin, are further enhanced by finasteride during T-stimulated regrowth of a regressed rat ventral prostate (Fig. 3) (11). This unexpected observation suggests that T is more potent than DHT in inducing androgen-response genes in prostate regrowth.

Since finasteride only enhances androgen-response gene expression in a regressed prostate but not in a fully-grown prostate, finasteride is expected to enhance the expression of androgen-response genes in prostate tumor regrowth induced by intermittent recovery of androgens but not in prostate tumors untreated with androgen ablation therapy.

Body:

Task 1: Determine quantitatively the relative potency of T versus DHT in the induction of androgen-response genes during the prostate regrowth (Month 1-36).

a. Animal manipulation and collecting prostatic tissue and serum samples.

b. Measurement of serum and intraprostatic T and DHT.
c. Measurement of DNA contents in the rat ventral prostate in the presence and absence of finasteride.

d. Northern and Western blot analysis of androgen-response gene expressions in the rat ventral prostate in the presence or absence of finasteride.

We did not focus on Task 1 last year because Task 2 is the key to the project and we have already presented some data in our first annual report. Without the success of Task 2, the whole project may have very little clinical relevance. Also, the success of Task 2 would facilitate the accomplishment of Task 1 and Task 3. During the 2nd year of the funding period, we focused our effort on Task 2.

Task 2: Test the effect of finasteride on intermittent androgen ablation therapy of xenograft androgen-sensitive prostate tumors in nude mice (Months 1-36).

a. Establish Shionogi and LNCaP androgen-sensitive tumor models in nude mice.

b. Determine the impact of finasteride on intermittent androgen ablation in Shionogi model by collecting tumor specimens and serum samples for analysis.

c. Determine the impact of finasteride on the time required to establish androgen-independent PSA expression in LNCaP tumor model undergoing intermittent androgen suppression.

As indicated in our 1st annual report, we have worked out approaches to delivery of exogenous testosterone at physiologic levels and finasteride over a prolonged period in nude mice. This allowed us make the following progresses.

a. Establishment and hormone manipulation of LNCaP xenograft tumors in nude mice.

Approximately 1x10^6 low passage of commercially available LNCaP cells were inoculated subcutaneously with 0.25 ml of Matrigel (Becton Dickinson, Bedford, MA) in the flank region of 6-8 week old athymic male nude mice. Tumors were allowed to grow until they reached 5-10 mm in diameter. All mice were then castrated via a trans-scrotal approach under tribromoethanol anesthesia and considered ‘on-cycle’. Fourteen days following castration, the mice were assigned to one of four groups and considered ‘off-cycle’:

group 1 (testosterone [T] + finasteride [F] implants)
group 2 (T only)
group 3 (F only)
group 4 (continuous androgen ablation [CAA], no implants).

Silastic tubings were implanted subcutaneously in the flank region contralateral to the tumor. The mice were distributed so that mean tumor volume at time of randomization was equivalent among the four groups. Pellets were extracted after fourteen days and constituted the end of a full cycle. Cycles were repeated until mouse death, tumor overgrowth (> 2 cm), tumor ulceration, or other circumstances required euthanasia.
Tumor volume was calculated as \((\text{length} \times \text{width}^2)/2\). Tumor volume and serum PSA were measured at each intervention. Testosterone and finasteride pellets were prepared as described in previous annual report.

Tumor was obtained at time of euthanasia and will be used for measurement of hormonal concentrations and molecular analysis.

b. **Finasteride administration during the off-cycle of intermittent androgen ablation therapy significantly reduced the tumor growth rate.**

At the time of LNCaP tumor size reaching 5-10 mm in diameter, orchiectomy was performed. From the time of orchiectomy to initial treatment, all groups exhibited similar tumor volume growth (Fig. 1). This increase is consistent with findings of other investigators.

![Change in tumor volume from orchiectomy to treatment randomization](image)

**Figure 1.** Tumor volume 2 weeks after castration, at the time of randomization for implantation of T- and/or F-pellets. Error bars indicate S.E.M.

Following one cycle of intermittent androgen ablation (orchiectomy followed by pellet administration), the change in mean tumor volume (+/- SEM) during the ‘off-cycle’ was similar in the T, F, and CAA groups (152% +/- 21%, 136% +/- 69%, 138% +/- 32%, figure 2). Mice treated with T+F during the ‘off-cycle’ had significantly less tumor growth (36% +/- 20%, \(p=0.002\)).
Figure 2: Percent change in LNCaP tumor volume following one cycle of intermittent androgen ablation with various treatment groups during ‘off-cycle’; bars are percent change in tumor volume (+/- SEM).

Figure 3: Percentage of mice by treatment group with tumor volume stabilization or decrease following one cycle of intermittent androgen ablation.
We then stratified the results by mice that experienced no change or a decrease in tumor growth during the 'off-cycle' (figure 5). The results were similar in the T, F, and CAA groups (8%, 0%, 18%, respectively). Mice treated with T+F during the 'off-cycle' had a higher proportion of tumors with an unchanged or decrease in tumor volume (45%).

For the mice completing the second cycle of intermittent androgen ablation, the change in mean tumor volume after two complete cycles in the T, F, and CAA, and T+F groups was 476% +/- 198%, 820% +/- 342%, 1426% +/- 482%, 223% +/- 104%, p=0.049, figure 4. The mice treated with T implants had decreased tumor growth compared to CAA. Mice treated with T+F had the least tumor growth of any group.

The growth suppression of intermittent androgen suppression (IAS) plus finasteride (T+F) appears to be less dramatic following two cycles relative to the first cycle. This may reflect that mice with more aggressive tumors were preferentially eliminated in the CAA, F, and T groups prior to completion of the second cycle.

Percent change in tumor volume following two cycles

![Bar chart showing percent change in tumor volume for different treatment groups.

Figure 4: Percent change in mean tumor volume by treatment group following two complete cycles of intermittent androgen ablation.

C. Finasteride plus intermittent androgen ablation appears to be more effective on xenograft tumors with smaller sizes.

To test whether tumor size at time of treatment randomization would have an effect on the efficacy of intermittent androgen suppression (IAS) plus finasteride, we analyzed the data based on tumor volume at the start of treatment (<0.33 cm³, 0.33-1.0 cm³, and >1.0 cm³, figure 5). The mice treated with T+F experienced significantly less tumor growth than those treated with T alone (<0.33 cm³: 17% vs 191%, p=0.006; 0.33-1.0 cm³: 41% vs 217%, p=0.017; and >1.0 cm³: 47% vs 134%, p=0.032). The advantages of IAS plus finasteride (T+F) diminish as initial tumor size increases.
size increases, suggesting that earlier treatment of tumors provides a more substantial growth suppressant.

Figure 5: Percent change in tumor volume stratified by size of tumor (<0.33 cm³, 0.33-1.0 cm³, >1.0 cm³) at time of treatment randomization (T versus T+F)

d. No significant difference in serum PSA levels was detected among the four groups of nude mice.

Serum was obtained by retro-orbital venipuncture. Serum PSA levels were determined by a commercial kit (IMx PSA, Abbott Laboratories, Abbott Park, IL). Serum PSA did not significantly differ between the four groups (data not shown). This finding indicates that LNCaP xenograft tumors in our experiment are already on the way to become independent of androgens, which is consistent with their growth in castrated hosts (Figure 1). This finding also means that it will virtually be impossible for us to use androgen-independent PSA expression as an endpoint in our proposed studies (Task 2c).

e. I would like to request a modification of Task 2.

Our experience in the past year with nude mice indicate that multiple surgeries, required for multiple cycles of intermittent androgen ablation therapy, caused a high death rate unrelated to tumor burden. Thus, we were not able to obtain statistically significant data regarding the advantages of finasteride plus IAS versus other types of hormonal manipulation on survival of animal hosts bearing LNCaP tumors. However, the data on the effect of finasteride plus IAS on the survival of nude mice bearing LNCaP tumors will be critically important to argue that finasteride indeed enhances the efficacy of intermittent androgen suppression (IAS).
I would like to conduct an experiment to determine the effect of finasteride plus testosterone replacement on the survival of nude mice with LNCaP xenograft tumors. In the revised experiment, one cycle of intermittent androgen ablation will be performed instead of multiple cycles of IAS. According to Dr. Nick Bruchovsky who invented the concept of IAS, the first cycle in IAS is the most important (personal communication). Furthermore, our studies showed the significance tumor growth retardation by finasteride plus IAS in the first cycle. These observations argue that finasteride plus one cycle of IAS should significantly prolong the life of hosts with LNCaP tumors. The cause of mouse death will be categorized into tumor overgrowth, tumor ulceration, unknown, and circumstances requiring euthanasia. The result will provide further evidence for the benefits of finasteride administration in intermittent androgen ablation therapy.

In order to study the effect of finasteride plus IAS on host survival, it is necessary for us to shift the manpower and resources from doing Shionogi mouse tumor model.

Task 3: Determine the effect of finasteride on the expression of androgen-response genes in LNCaP tumors during intermittent androgen ablation therapy (Month 24-36).

a. Collect LNCaP tumor specimens and serum samples from nude mice.

b. Determine the expression of androgen-response genes, adrenomedullin, calreticulin and PSA, in LNCaP tumors.

c. Analysis of the collected data and prepare the final report for the proposal.

N/A.

Key Research Accomplishments:

Our proposed research requires the ability to deliver appropriate doses of finasteride and T over a prolonged period in nude mice. In the 1st year of the funding period, we have encountered difficulties because the commercially available slow releasing pellets did not work in our system. After multiple tests, we resolved these critical technical problems, which allowed us, in the 2nd year of the funding period, to demonstrate that finasteride administration significantly enhances the efficacy of intermittent androgen ablation therapy in LNCaP xenograft tumor model.

1. We demonstrated that finasteride given during the ‘off-cycle’ of intermittent androgen ablation significantly limits tumor growth in the LNCaP xenograft model. The use of IAS plus finasteride resulted in decreased tumor growth compared to standard continuous androgen ablation. We are not aware of any previous report showing that parental LNCaP tumor size is reduced by hormonal manipulation in relation to castration. If our further studies showing that IAS plus finasteride enhances survival of animal hosts with LNCaP tumors, we would like to argue that a prospective, randomized, blinded clinical trial should be initiated to adequately evaluate this treatment regimen in humans.
Reportable Outcomes:
None.

Conclusions:
Our studies with androgen-sensitive LNCaP human prostate tumor xenografts in nude mice showed significant tumor growth retardation by finasteride plus IAS in the first cycle. Our finding indicates that finasteride plus IAS should prolong the life of nude mice bearing LNCaP tumors. Also, this finding suggests that finasteride administration should enhance the efficacy of IAS on patients with prostate cancer.

References:


Appendices: None.