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Prolongation of Off-Cycle Interval by Finasteride Is Not Associated with Survival Improvement in Intermittent Androgen Deprivation Therapy in LNCaP Tumor Model

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Abstract

BACKGROUND—We have previously reported that finasteride administration in intermittent androgen deprivation therapy (IADT) can improve survival of nude mice bearing LNCaP xenograft tumors when the duration of off-cycle in IADT was fixed. A recent retrospective study showed that addition of finasteride doubled the duration of the off-cycle, without changing progression to castration resistance. In view of the above difference, we attempted to investigate the relationship of 5α-reductase inhibition with the off-cycle interval and overall survival in a murine model.

METHODS—Subcutaneous LNCaP tumors were established in nude mice (Balb/C-Nu). After the tumors reached a size of 0.5 cm in diameter, the mice were castrated and followed up for 2 weeks after which they were randomized to continuous androgen deprivation (CAD), CAD plus finasteride, IADT, and IADT plus finasteride. The off-cycle was discontinued when the tumor volume was doubled. Subsequent cycles were carried out similarly.

RESULTS—Use of finasteride during the off-cycle of IADT doubled the first off-cycle duration. However, prolongation of the off-cycle by finasteride did not translate into an increase in overall survival.

CONCLUSIONS—The survival advantage of IADT+F over IADT that we previously reported was lost when the off-cycle prolongation by finasteride was allowed. Maximum possible lengthening of the off-cycle by 5α-reductase inhibition is not associated with survival improvement in this animal model.

Keywords

prostate cancer; intermittent androgen deprivation therapy; LNCaP; 5α-reductase inhibitors

INTRODUCTION

Androgen deprivation therapy (ADT) is the mainstay of managing advanced prostate cancer. However, ADT is complicated by economic concerns [1,2], side effects [3], and the almost inevitable progression to castration recurrent disease [4]; this has led to efforts to improve

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ADT. Intermittent androgen deprivation therapy (IADT) has been proposed as an alternative in some patients. The patients are cycled between androgen suppression (on-cycle) and treatment free periods (off-cycle), according to biochemical, largely based on serum PSA, and clinical criteria that vary across protocols [5].

IADT can potentially improve upon standard ADT in all the spheres- prognosis, side effects, and economics. Intermittent, rather than continuous, androgen deprivation could promote the expansion of hormone sensitive cells during the off-cycle that would help sustain androgen dependence for longer periods [6–8], and potentially improve the prognosis. The expected improvement in quality of life is due to testosterone recovery during the off-cycle, which alleviates many of the side effects [9,10], and the reduced costs due to the treatment free periods when the expensive androgen inactivating drugs are not prescribed. Currently available literature suggest that in clinical settings, IADT is safe and has a better side effect profile than continuous ADT, while the treatment efficacy issue is not clear [11].

We have previously identified several androgen response genes that are regulated differentially by testosterone and dihydrotestosterone (DHT) during androgen induced regrowth of the regressed rat ventral prostate after castration [12]. Some of the genes whose expression is preferentially enhanced by testosterone have growth suppressive properties [13–15]. The off-cycle of IADT is similar to the regrowth phase of the regressed normal prostate in that androgen deprived prostate epithelial cells are re-exposed to androgens. Thus, inhibition of steroid 5α-reductase (SRD5A) during this phase could generate a testosterone rich, DHT poor environment and upregulate growth suppressive androgen response genes and potentially improve outcomes. We then showed that the addition of finasteride, an SRD5A2-specific inhibitor, to the off-cycle of IADT can improve survival over standard IADT in an LNCaP xenograft tumor model [16]. It is important to note that the durations of the off-cycles in the 2 groups were kept the same, at 7–10 days, in the study.

Since androgen recovery during the off-cycle improves quality of life in patients treated with IADT, efforts have been directed towards identifying factors that can prolong the off-cycle. Currently, there is an ongoing clinical trial (NCT00283803) looking at the possibility of extending the duration of the off-cycle, by using exisulind, a sulindac derivative which promotes apoptosis. In a retrospective review of data from 101 patients, Scholz et al [17] reported that the addition of finasteride during the off-cycle doubled the duration of the off-cycle from a median of 15 months to a median of 31 months, based upon serum PSA criteria. However, there was no effect on progression to castration recurrent disease. The authors concluded that off-cycle SRD5A inhibition could provide a means to increase the off-cycle duration, and possibly improve the quality of life.

The above literature suggests that off-cycle inhibition of SRD5A can potentially increase the duration of the off-cycle and/or improve survival over standard IADT under certain conditions. In view of the above, we attempted to better define the relation between off-cycle SRD5A inhibition, off-cycle duration, and overall survival using a murine LNCaP xenograft tumor model.

MATERIALS AND METHODS

Xenograft Tumor Implantation

Early passage LNCaP cells were obtained from American Type Culture Collection (ATCC) and maintained in RPMI 1640 media supplemented with 10% FBS plus penicilin, streptomycin and glutamine. Cells underwent 4–8 passages prior to mouse inoculation. Approximately 10^6 (1 million) LNCaP cells suspended in 250 μl media were mixed with 250 μl Matrigel (Becton Dickinson labware, Bedford, MA) and then inoculated

subcutaneously in the flank region of 6~8 weeks old male athymic mice (BALB/c strain, Charles River Laboratory, Montreal, PQ, Canada) via a 25-gauge needle. All animal experiments were approved by the Institutional Animal Care Use Committee. Tumors were thereafter measured twice weekly, they typically exhibited visible tumor growth 8~12 weeks following inoculation. Tumor volume was calculated by the formula: (length × width^2)/2 [18]. Animals were randomized when the tumors reached 0.5cm in diameter, equivalent to a volume of 0.0625 cu cm. The tumor take rate in our experiments was approximately 75%. The observation of tumor volumes were carried twice weekly until tumor overgrowth (>2cm in diameter), tumor ulceration or severe tumor-related morbidity required euthanasia as per the institute's guidelines.

Pellet Construction

The testosterone and finasteride pellets were made as previously described. Briefly, approximately 7.5 mg of testosterone (Sigma Chemical, St. Louis, MO) was tightly packed into a silicone tube with an inner diameter of 1.58 mm and outer diameter of 3.18mm (Helix Medical, Carpenteria, CA). The ends were plugged with wood sticks and sealed with a silicone adhesive (Dow Corning, Midland, MI). Following air drying overnight, the pellets were then sterilized with 70% ethanol for 10 min and stored in a light-free environment. The finasteride pellets were made similarly, except that the silicone tubing for finasteride implants had a 1.47mm inner and 1.96mm outer diameter (Helix Medical, Carpenteria, CA). Approximately 15mg of finasteride (gift from Merck, Rahway, NJ) was filled into the tubing.

Treatment Protocol and Measurement of Tumor Growth and Animal Survival

The experimental design is illustrated in Figure 1. After the tumors reached a diameter of 0.5 cm, equivalent to a tumor volume of 0.0625 cu cm, the mice were randomized into four groups: (1) Testes Intact Controls (no intervention), (2) Castration only, (3) Finasteride only, and (4) Castration plus finasteride (Castration+F). Trans-scrotal castration of the mouse was performed under isoflurane anesthesia with proper aseptic precautions. Silicone pellets of finasteride were implanted subcutaneously in the flank contralateral to the tumor on randomization. All the mice in the castration only group were considered to be in the "on-cycle". Two weeks after castration, the tumors that remained sensitive to androgen deprivation—as measured by continued arrest or decline in tumor volume as compared to before castration—were randomly assigned to castration only (simulating continuous androgen deprivation, CAD), Intermittent Androgen Deprivation Therapy (IADT; testosterone implants only) or Intermittent Androgen Deprivation Therapy plus off-cycle finasteride (IADT+F, testosterone and finasteride implants). The mice were followed up and the pellets were extracted when the tumors grew to 0.7 cm in diameter (or a tumor volume of 0.1715 cu cm- an approximate doubling of tumor volume). This constituted the completion of one full cycle of IADT—1 on-cycle + 1 off-cycle. Subsequent cycles were carried out similarly. For hormonal determinations, a few mice were sacrificed 7 days after pellet implantation, and blood was collected via terminal cardiac puncture. Determination of serum PSA levels was done in blood samples collected at sacrifice as well as at castration and pellet implantation through tail vein incisions. To study the effect of various androgen manipulations on survival of tumor-bearing animals, the mice were kept alive until they required mandatory euthanasia, in compliance with IACUC guidelines at our institution, due to tumor overburden (size >2cm) or severe tumor-related morbidity. Thus, in our study survival is not defined by death of the animal but by specific institutional guidelines.

Determination of Serum PSA, Testosterone (T), DHT and Estradiol Levels

Blood samples were centrifuged at 2500 rpm in a clinical centrifuge for 5 minutes at room temperature to collect serum, which was stored at −80°C until measurement. PSA levels

were measured using a sandwich enzyme immunoassay kit (CAN-tPSA-4300, Diagnostics Biochem Canada Inc., Ontario, Canada) with a lower limit of detection of 0.1ng/ml. Hormone levels were measured using DSL-4000 *ACTIVE* Testosterone, DSL-9600 *ACTIVE* Dihydrotestosterone and DSL-4400 Estradiol Radioimmunoassay Kits from Diagnostic Systems Laboratories, Inc. (Webster, TX). The lower limits of detection of DSL-4000, DSL-9600 and DSL-4400 are 0.08ng/ml, 4pg/ml, and 4.7pg/ml, respectively. All measurements were performed according to the manufacture's protocols.

Statistical Analysis

GraphPad Prism 4.0 (GraphPad Software, Inc) and SPSS 15.0 (SPSS Inc, Chicago, IL) were used for statistical analysis and MS Excel 2003 was used for graphical composition. Tumor volume and hormonal levels were compared by independent sample t-test. Survival analysis was evaluated using Kaplan-Meier curves and log rank tests. All data were expressed as the Means ± SEM of the samples examined, and values of $P \leq 0.05$ were considered statistically significant.

RESULTS

Androgen Sensitivity of LNCaP Tumors

The studies on the effect of finasteride on IADT require androgen-sensitive LNCaP xenograft tumors. We determined the androgen responsiveness of LNCaP tumors based on their growth response to castration and only mice bearing androgen-sensitive tumors were randomized for further studies. We excluded castration resistant tumors, which is the preferred new term for androgen-refractory, androgen-independent, or hormone-independent tumors. Testes-intact and finasteride only controls were followed up concurrently. Figure 2a depicts that castration inhibited tumor growth whereas the tumors in testes-intact mice displayed a 5-fold increase in volume over 2 weeks (Fig. 2a), indicating that LNCaP tumors in this study were androgen-sensitive. Figure 2a also shows that finasteride treatment alone did not affect the growth rate of LNCaP xenograft tumors in testis-intact mice. The replacement of testosterone, with or without finasteride, increased serum PSA levels in castrated tumor-bearing nude mice, further supporting that the LNCaP tumors responded to androgen manipulation (Fig. 2b).

Finasteride Administration Prolonged 1st Off-Cycle Duration

To address whether finasteride could prolong the off-cycle interval, mice bearing androgen-sensitive LNCaP xenograft tumors were randomized 2 weeks after castration into IADT and IADT+F groups. In this experiment, the first off-cycle spanned from when the tumors were 0.5 cm in diameter to when they grew to 0.7 cm in diameter. The mean duration of the first off-cycle in IADT mice was 8.75 days, and this was doubled to 17.56 days in mice that were treated with IADT+F (Fig. 3). The result indicates that the tumor growth rate was slower in the IADT+F group as compared to the IADT alone during the first off-cycle.

Prolongation of Off-Cycle Interval by Finasteride Was Not Associated with Survival Improvement

To determine whether prolongation of off-cycle interval by finasteride would lead to prolonged survival of the animal host, we continued the animal treatment protocol as described in Figure 1 until the mice had to be sacrificed due to tumor overburden, tumor ulceration or severe tumor-related morbidity. As shown in Figure 4, there was no significant survival difference between the IADT group and the IADT+F group, with a median survival at 125 and 110 days (p= 0.7184) for IADT and IADT+F group, respectively. Thus, the treatment protocol we used in this study did not yield prolonged survival for animals treated

with IADT+F. Also, there was no significant difference among the castration group, castration plus finasteride group and the IADT groups. In contrast, the median survival in testes-intact mice and mice treated only with finasteride was 72 days each. This was significantly less than the survival in mice that were castrated and underwent further hormonal treatment (p=0.0475, logrank test). Thus, androgen deprivation therapy, regardless of continuous or intermittent, was able to increase survival in the LNCaP model.

It is important to note that by the end of the second ON cycle approximately 70% and 40% of the mice in the IADT+F and IADT groups, respectively, became castration-resistant. Thus, by the beginning of the second off-cycle most of the tumors receiving finasteride had castration-resistant growth. Hence, based on the On and Off cycling criteria, the second off-cycle duration was shorter in IADT+F group; accounting for the comparable survival in both groups in spite of the first Off cycle prolongation in the IADT+F group.

Hormonal Measurements

We have previously shown that our system of testosterone and finasteride pellet implantation works satisfactorily in the rat and mouse models, as seen by their effects on wet weights of the ventral prostate and seminal vesicle after pellet implantation. Testosterone pellet induced prostate regrwoth and finasteride pellet partially inhibited the regrowth [12,16]. We examined the serum T and DHT levels to gauge the effect of 5alpha-reductase inhibition (Fig. 5). There was a modest but statistically significant decline in serum DHT levels from the IADT group to the IADT+F group (119 pg/ml Vs 96.5 pg/ml, p=0.0015) while the elevation in serum testosterone levels upon addition of finasteride was not statistically significant (2.025 ng/ml in IADT Vs 2.373 ng/ml in IADT+F group, p=0.54). These small changes in serum hormone levels reflect a clear trend of inhibition of SDR5A enzyme yet the magnitude of the response is lower than expected, which could be due to technical difficulties of the assay. There was a significant increase in serum testosterone levels in testis-intact mice that were implanted with finasteride pellets for 7 days (0.509 ng/ml in finasteride group versus 0.181 ng/ml in control group, p=0.004, Fig 5c). The above data indicate that testosterone conversion to DHT was partially inhibited by finasteride in our experiments.

Testosterone can be converted to estradiol, which could influence prostate cancer growth. Thus, we also measured serum estradiol level as a control. The estradiol in serum samples were undetectable or extremely low and there were no differences in the serum estradiol levels between the treatment groups (data not shown), indicating that estradiol was unlikely mediating the effect of finasteride on LNCaP tumor regrowth in our experiment.

DISCUSSION

Intermittent androgen deprivation therapy (IADT) is a widely used treatment for patients with metastatic androgen-sensitive prostate cancer, often combined with the use of 5α-reductase inhibitor, finasteride or dutasteride. The potential of using 5α-reductase inhibitor to improve IADT has not been explored extensively and consequences of using finasteride or dutasteride are not clear. In a clinical setting when serum PSA level is used as the trigger point for switching off-cycle to on-cycle in IADT, finasteride administration in IADT significantly prolonged the off-cycle interval. However, the off-cycle prolongation by finasteride did not affect progression of prostate cancer to castration recurrence [17]. Our previous study reported that off-cycle maintenance by finasteride in IADT can prolong the animal host survival in LNCaP prostate cancer xenograft model, when the off-cycle interval was not changed. The above observations together led to the hypothesis that prolongation of off-cycle interval by finasteride can offset the survival benefit achieved by finasteride

maintenance in IADT when the off-cycle interval is kept constant. The animal studies described here support this hypothesis.

Using LNCaP xenograft tumor model, this study showed that finasteride administration can prolong the off-cycle interval in IADT, which is consistent with the results from clinical studies. In both patients and animal models, finasteride maintenance doubled the duration of the off-cycle. Serum PSA level was used as the trigger point for ending the off-cycle and resuming the on-cycle in patients undergoing IADT. On the other hand, increase in tumor volume to a predetermined size was used as the trigger point for switching off-cycle to on-cycle in the LNCaP tumor model in this study. Despite these differences, both studies had similar extent of off-cycle prolongation by finasteride, about 2-fold. According to Scholz and colleagues [17], finasteride maintenance in the clinical setting did not affect the progression of prostate cancer to castration recurrence while the off-cycles were prolonged. Similarly, we did not observe survival benefit of finasteride administration in IADT in the animal model, when off-cycle prolongation was allowed in the treatment protocol (Fig. 4a,b). Our finding and the observation of Scholz and colleagues together argue that off-cycle prolongation by finasteride does not retard prostate cancer progression to castration recurrence or result in prolongation of survival.

This study showed that finasteride inhibited the regrowth of LNCaP xenograft tumors in castrated mice upon androgen replacement but not the growth of LNCaP tumors naïve to androgen manipulation in testes-intact animals (Fig. 2a and 3), which is consistent with our previous finding [16]. The off-cycle prolongation by finasteride in IADT should be associated with the inhibition of LNCaP tumor regrowth by finasteride. Since finasteride also prolonged the off-cycle in patients, it is also likely to inhibit prostate tumor regrowth during the off-cycle in patients. The exact mechanism by which finasteride inhibits the regrowth of regressed prostate tumor but not the androgen deprivation naïve prostate tumors is not clear and remains to be investigated. One possible mechanism may be that testosterone is more potent than DHT in the induction of growth inhibitory/tumor suppressive androgen-responsive genes during the regrowth of a regressed prostate but not in the intact prostate [12]. This potential explanation is consistent with our finding that finasteride did not affect LNCaP tumor growth rate in testes-intact mice.

The duration of off-cycle in IADT has the potential to affect survival. In theory, the off-cycle interval can go two extremes, very short or very long. IADT with very short off-cycle may not be very different from the continuous ADT and patients may not have quality of life improvement during the off-cycle. On the other hand, excessive prolongation of off-cycle may weaken the therapeutic effect of androgen deprivation during the on-cycle. Future studies will be needed to determine the optimal off-cycle interval associated with survival benefits. Our studies here indicated that off-cycle prolongation by finasteride was not associated with survival improvement in an animal model. However, our previous studies showed that finasteride in IADT prolonged host survival when the off-cycle prolongation was not allowed [16]. These observations together argue that prolonged off-cycle duration may have eliminated the potential survival benefit of finasteride administration in IADT in the LNCaP model.

In summary, our studies showed that prolongation of off-cycle interval by finasteride in IADT was not associated with survival benefits in LNCaP xenograft tumor model. The prolongation of off-cycle interval may offset the survival improvement by finasteride when the off-cycle interval was not changed. Future clinical studies will be needed to determine whether 5α-reductase inhibition during IADT can prolong the survival of patients when off-cycle interval prolongation is not permitted.

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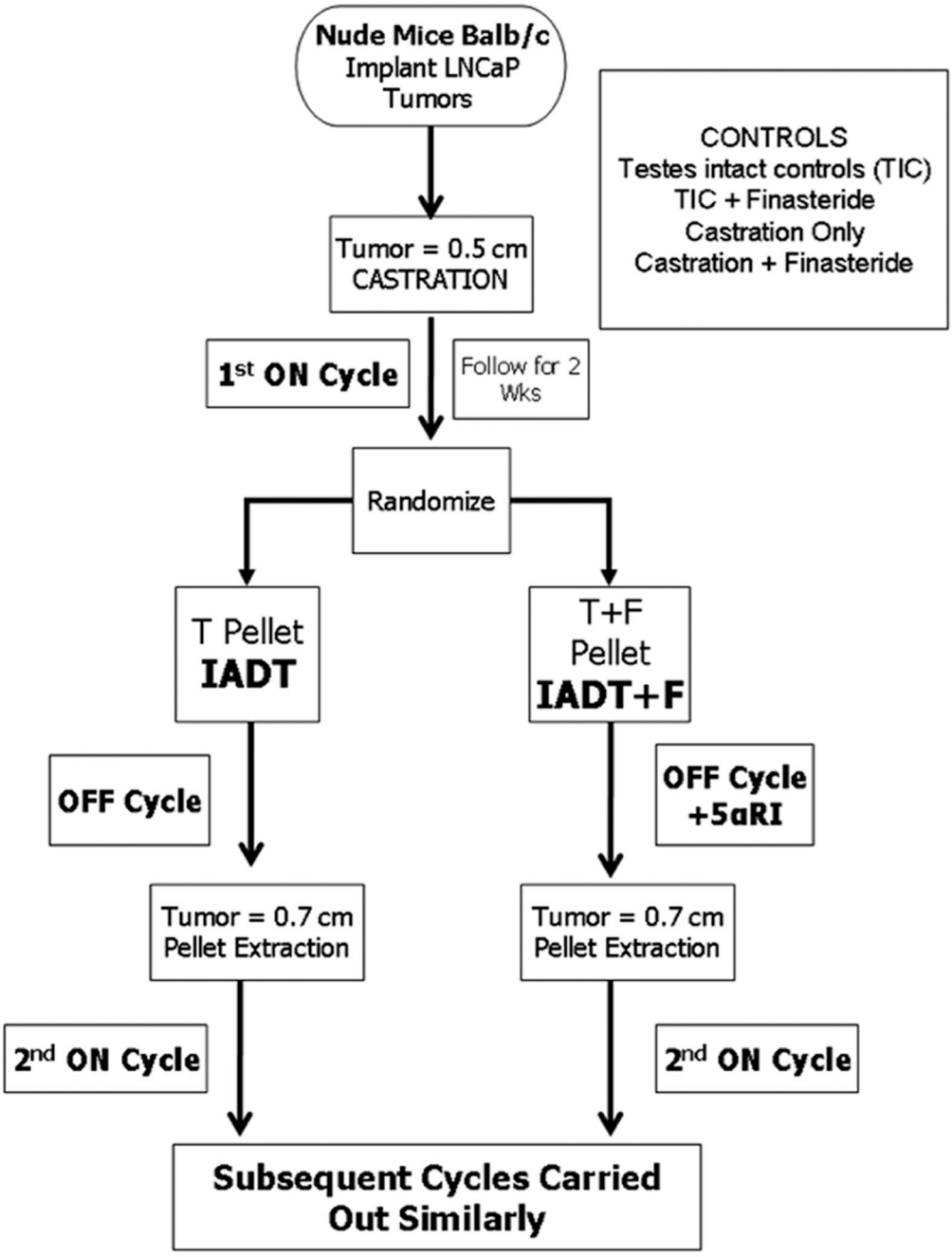

Fig 1.

Flowchart depicting the experimental design. Tumor bearing nude mice were castrated and followed up for 2 weeks before being implanted with testosterone (T) pellets or T plus finasteride (F) pellets. T implantation mimicked intermittent androgen deprivation therapy (IADT), while T+F implantation mimicked IADT + off-cycle 5alpha- reductase inhibition. The pellets were extracted after the tumors grew to 0.7 cm (0.1715 cu cm), and the cycle was repeated. Testes-intact and castrated mice, with or without finasteride implantation, were kept as controls.

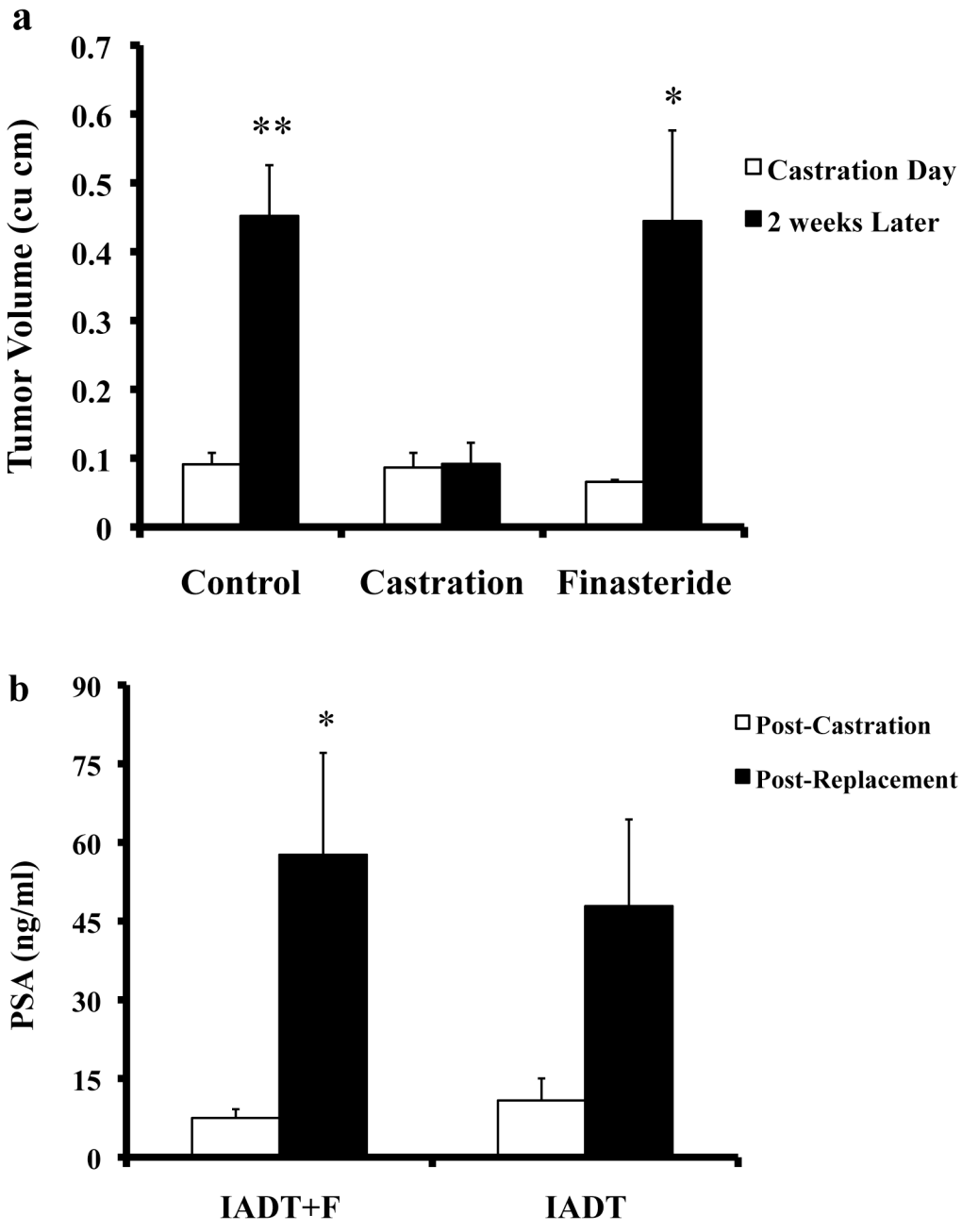

Fig 2.

a: Tumor bearing nude mice were castrated (middle panel), treated with finasteride (right panel) or were followed up without intervention (control, left panel). Castration led to an arrest of tumor growth, and after 2 weeks, the mean tumor volume (0.0915 cu cm) remained similar to that at the time of castration (0.0862 cu cm, p= 0.905). Over the same period, testes intact controls showed a 5 fold increase in tumor volume, from 0.091 cu cm to 0.451 cu cm (**p< 0.01) and the mice treated with finasteride only increased their tumor volumes from 0.0625 cu cm to 0.444 cu cm (* p<0.05). Error bars depict 1 SEM, and the paired t test was used for p value calculation. **(b)** Tumor bearing mice were followed up for 2 weeks after castration and implanted with either a testosterone pellet (Intermittent androgen

deprivation, IADT) or testosterone + finasteride pellets (IADT+F). 7 days after pellet implantation, both groups showed increases in the serum PSA levels, from 7.47 ng/ml to 57.67 ng/ml in the IADT+F group (* $p<0.05$) and from 10.81 ng/ml to 47.88 ng/ml in the IADT group ($p=0.086$). Error bars depict 1 SEM, and the paired t test was used for p value calculation.

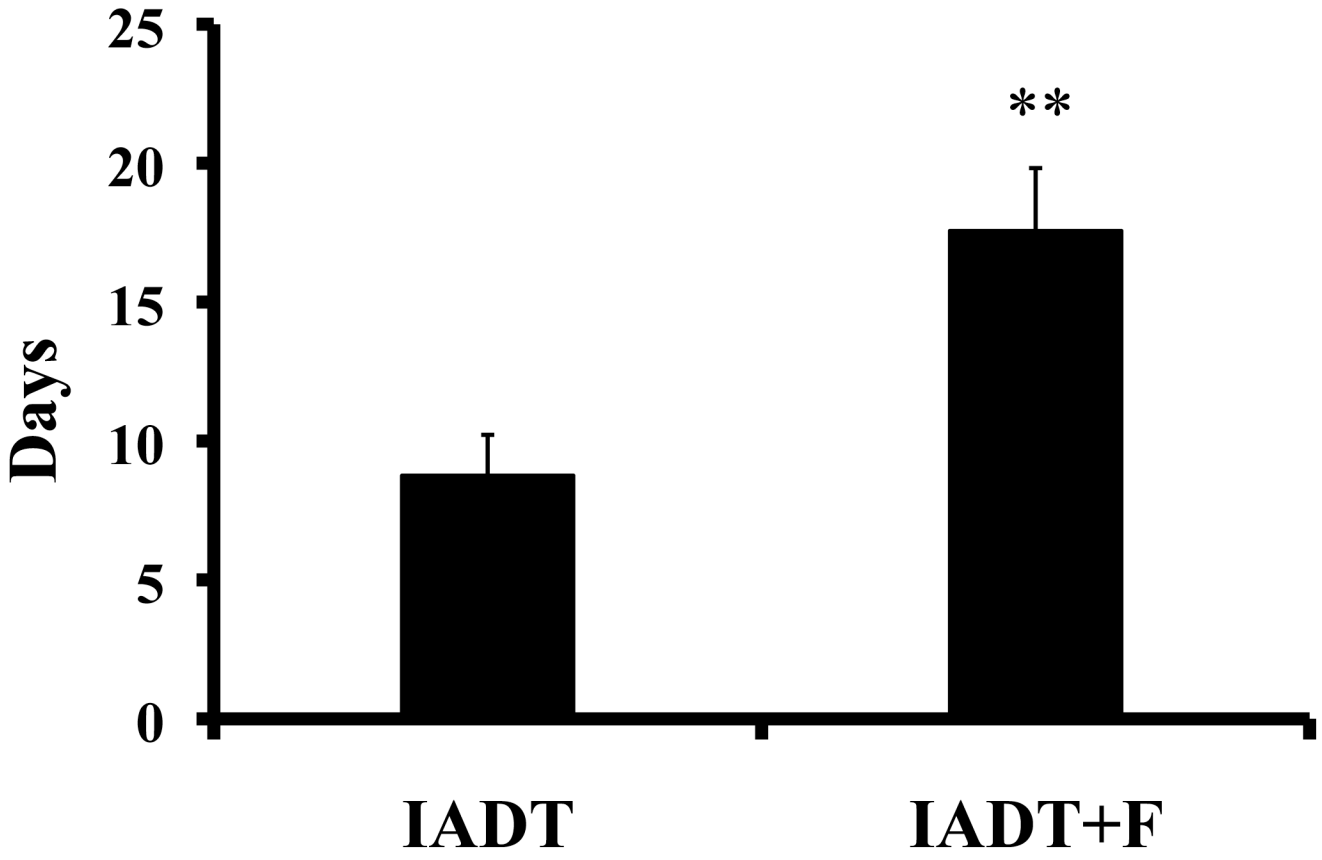

Fig 3.

Effect of off-cycle finasteride on the duration of the 1st off-cycle. Tumor bearing mice were castrated when the tumors reached a size of 0.5 cm in diameter, and considered to be in on-cycle. Two weeks after castration, mice were re exposed to androgens (off-cycle). They were implanted with either a testosterone pellet (Intermittent androgen deprivation, IADT) or testosterone + finasteride pellets (IADT+F). Tumor sizes were measured every third day, and the pellets were extracted when the tumor diameter increased from 0.5 cm to 0.7 cm. Since the individual tumors were not all exactly 0.5 cm in diameter at pellet implantation, an approximate increase of 40% in the size was considered an appropriate time at which to extract the pellets and end the off-cycle. The addition of finasteride during the off-cycle slowed tumor growth, and doubled the mean off-cycle duration from 8.75 days in the IADT group, to 17.56 days in the IADT+F group (** $p<0.01$, independent samples t test). Error bars indicate 1 SEM

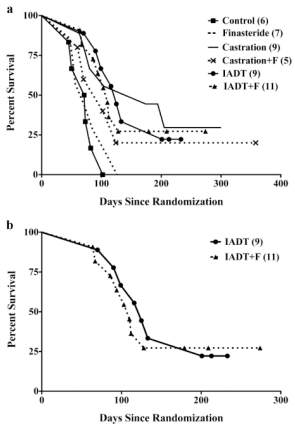

Fig 4.

a: Kaplan-Meier survival curves by experimental groups. Controls- testes intact mice without any intervention; Finasteride- mice implanted only with finasteride pellets; IADT- intermittent androgen deprivation; IADT+F- intermittent androgen deprivation + off-cycle finasteride. The median survivals for controls and finastreide only groups were 72 days each, and they were significantly less than the 4 groups that were castrated with or without other intervention (* $p<0.05$, logrank test). There was no survival difference among the other groups. ($p=0.8498$, logrank tests). (**b)** Comparison of the survival of IADT with IADT +F. The median survival in IADT group was 125 days, and it was 10 days in the IADT+F group ($p=0.7184$, logrank test). Note that the same sets of mice were used for the survival as well the off-cycle duration analyses depicted in Fig 3.

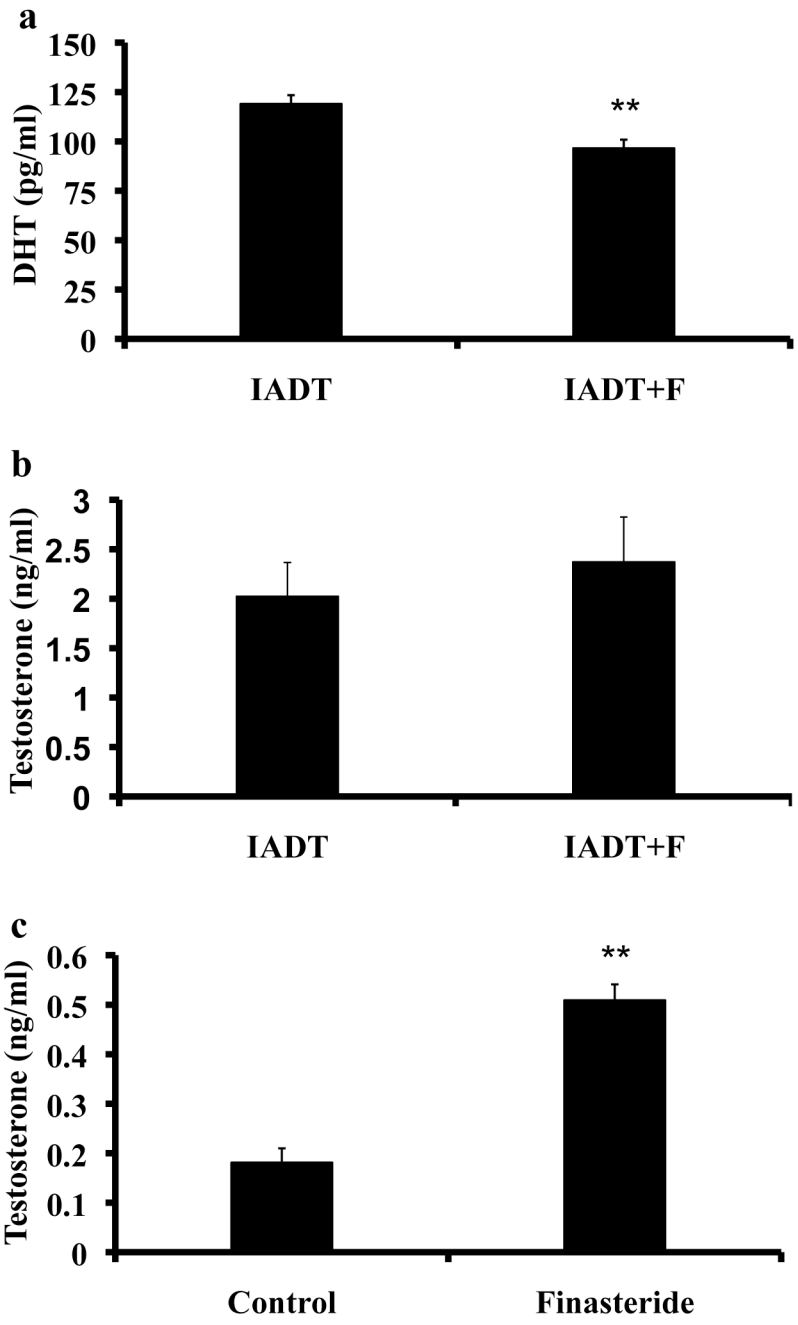

Fig 5.

a: Serum DHT levels 7 days after implantation of testosterone (IADT) or testosterone + finasteride (IADT+F) pellets (** $p<0.01$). (**b)** Serum testosterone levels in the same mice as in 5a ($p= 0.54$). (**c)** Serum testosterone levels in controls and mice treated with finasteride for 7 days (** $p<0.01$). Error bars indicate 1 SEM, and independent samples t test was used to calculate the p value.